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# Determination of acidic herbicides using liquid chromatography with pneumatically assisted electrospray ionization mass spectrometric and tandem mass spectrometric detection

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**Abstract**

Liquid chromatography–pneumatically assisted electrospray mass spectrometry with negative ionization has been used for the determination of acidic herbicides in ground water. Eighteen pesticides or pesticide degradation products belonging to several different groups of acidic herbicides (phenoxy acids, sulfonylureas, phenols, etc.) were covered in the study. Optimization of electrospray inlet conditions is described as well as results from investigations of the linearity of the detector response. Conditions for tandem mass spectrometry (MS–MS) detection of characteristic daughter ions formed by collision-induced dissociation (CID) of the parent ion are described and a comparison of obtainable instrument detection limits by single MS and MS–MS was made. Detection limits using MS in the selected ion monitoring (SIM) mode were generally in the order of 1 μg/l or below, whereas detection limits were three–four times higher using MS–MS detection. A principle of analysis is proposed based on single quadrupole MS as a method for quantitative determination followed by verification of positive findings by CID MS–MS. Application of the method for detecting acidic herbicides residues in a ''real-world'' ground water sample is demonstrated. © 1998 Elsevier Science B.V.

*Keywords:* Mass spectrometry; Pesticides; Phenoxy acids; Sulfonylureas; Phenols

## 1. Introduction

Contamination of ground water with pesticides from agriculture is still a problem of primary concern. Especially in countries, like Denmark, where the supply of drinking water is almost totally based on the use of ground water, pesticides have become an important part of ground water monitoring programmes.

Polar pesticides are the most likely to leach to ground water and are, therefore, the pesticides of primary interest in ground water monitoring. Analytical methods based on liquid chromatography are often preferred for the analysis of polar pesticides and, in particular, methods using liquid chromatography–mass spectrometry (LC–MS) have been used increasingly during the last few years [1].

LC–MS methods are very attractive because the mass spectrometric detection usually offers the possibility of achieving a high sensitivity together with a high degree of selectivity. High sensitivity of the analytical method is necessary for use in ground water monitoring. A European Union directive [2] limits the content of individual pesticides in drinking

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water to 0.1 μg/l, which means that methods preferably should have detection limits that are about one tenth of this limit or lower, viz. 0.01 μg/l or less. A high degree of detection selectivity is advantageous, because it reduces the possibility of false positive findings.

A number of different LC–MS interfaces (particle beam, thermospray, atmospheric pressure ionization) have been used for the determination of polar pesticides [1], however, during the last few years, atmospheric pressure ionization (API) techniques, high flow pneumatically assisted electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have become the more popular interfacing techniques. Both of these API techniques are soft ionization methods that predominantly give rise to the protonated $(M+H)^+$ or deprotonated $(M-H)^-$ molecular ions in positive- or negative mode, respectively. Several papers have been published [1,3–6] demonstrating the applicability of APCI-MS for the determination of polar pesticides, primarily basic or neutral compounds, in positive ion mode, but only very few [4,5,7] examples of the use of APCI-MS in the negative mode have been published. Lacorte and Barceló [4] as well as Slobodnik et al. [5] have compared negative and positive mode APCI for the determination of various pesticides and found generally much less sensitivity in the negative ion mode.

Acidic herbicides are compounds that are most suited for negative ion mode LC–MS because of their acidic properties. Acidic herbicides in environmental waters have been successfully analyzed using negative ion mode LC–ESI-MS [8,9]. Preliminary investigations carried out in our laboratory (unpublished results) also demonstrated much better sensitivity for acidic herbicides using ESI than APCI.

A very important aspect of performing residue analysis at the low concentrations relevant to environmental waters is to assure a high degree of confidence in the identification of the compounds, to avoid false positives. The MS fragmentation pattern is a powerful tool for obtaining such confidence in compound identification. Crescenzi et al. [9] have demonstrated that fragmentation of acidic herbicides can be achieved using single quadrupole ESI-MS by increasing the pre-analyzer extraction (skimmer cone) voltage. Using tandem mass spectrometric detection, selective fragmentation of the initially formed deprotonated molecular ion is achieved by collision-induced dissociation in the collision cell between the first and second quadrupole.

Pesticide active ingredients belonging to the sulfonyl urea type of acidic herbicide are compounds that are being used increasingly over recent years. Volmer et al. [10] have demonstrated that sulfonylureas can be determined in aqueous environmental samples by positive mode LC–ESI-MS. However, being acidic compounds, the possibility of detecting sulfonylureas as deprotonated molecular ions in negative ion mode ESI-MS could also be expected. Winnik et al. [11] have demonstrated the detection of sulfonylureas by negative ion mode desorption chemical ionization MS, but, to our knowledge, the application of negative ion mode ESI-MS for the detection of sulfonylureas has not been published.

The object of this study was to further investigate the possibility of using LC–ESI-MS for the determination of acidic herbicides, including representative sulfonylurea compounds, in ground water. Furthermore, another object of this study was to establish suitable conditions for performing MS–MS analysis using collision-induced dissociation (CID) of the deprotonated molecular ion.

## 2. Experimental

### *2.1. Chemicals*

Methanol, gradient grade, and acetonitrile, LC grade, were purchased from Merck (Darmstadt, Germany). Acetic acid (100%), of analytical grade, was from Merck and propylene glycol, of analytical grade, was from Fluka (Buchs, Switzerland). All chemicals were used as received. The water was deionized water that was subsequently purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The eighteen pesticides or pesticide degradation products used as standards were benazolin, bentazone, bromoxynil, chlorsulfuron, 2,4-D, dicamba, 2,4-dichlorophenol, dichlorprop, dinoseb, DNOC, flamprop, fluazifop, ioxynil, MCPA, mecoprop, metsulfuron-methyl, thifensulfuron-methyl and triasulfuron. All pesticide standards were PESTANAL

grade and were purchased from Riedel-de Haën (Seelze, Germany). The purity of all standards was a minimum of 99%.

Stock solutions (1000 mg/l) of individual pesticide standards were prepared by dissolution in acetonitrile. A mixed stock solution (10 mg/l of each compound) containing all eighteen standards was prepared from stock solutions of individual pesticide standards by mixing and diluting with acetonitrile. Stock solutions were stored at −21°C and were stable for at least three months. Calibration standards (10–100 μg/l of each compound) were prepared by appropriate dilution of the mixed stock solution with methanol–water (10:90, v/v).

Isotopically labeled 2,4-D (ring-$^{13}C_6$), from Cambridge Isotope Laboratories (Woburn, MA, USA), was used as the internal standard. A stock solution (100 mg/l) was prepared by dissolution in acetonitrile. A 50-μg/l internal standard solution, used for addition to samples, was prepared from the stock solution by dilution with methanol–water, (10:90, v/v).

### 2.2. Apparatus

The LC–MS system consisted of a Waters (Milford, MA, USA) 600 MS solvent delivery system, a Waters 717 autosampler and a Finnigan MAT (San Jose, CA, USA) TSQ 700 quadrupole mass spectrometer equipped with a Finnigan MAT standard ESI ionisation source. The LC–MS system was connected to a Digital DECstation 5000/125 computer (Maynard, MA, USA) with Finnigan software used for instrument control and data acquisition.

The HPLC column was a Hypersil-BDS $C_{18}$, 5 μm, 250×2.0 mm I.D. from Shandon HPLC (Cheshire, UK).

The solid-phase extraction (SPE) cartridges used for sample clean-up were Porapak Rdx, 500 mg, 6 ml cartridges from Waters. A twelve-cartridge capacity vacuum manifold equipped with 60 ml reservoirs was used for SPE.

### 2.3. Chromatographic conditions

Gradient HPLC was performed with a binary gradient composed of LC solvent A (methanol–water–acetic acid, 90:810:1, v/v) and LC solvent B (methanol–acetic acid, 900:1, v/v) according to the following programme: a linear gradient from 100% A to 50% A from 0 to 3 min; a linear gradient from 50% A to 0% A from 3 to 30 min; maintaining 0% A from 30 to 33 min; returning linearly to 100% A from 33 to 36 min and maintaining 100% A from 36 to 45 min. The flow-rate of the mobile phase was 0.2 ml/min and 50 μl of sample/standard solution were injected into the HPLC system.

For injections made under flow injection analysis conditions (without LC column), a 50:50 mixture of LC solvents A and B was used.

### 2.4. Mass spectrometric analysis

ESI-MS detection was performed with the following ESI inlet conditions: sheath and auxiliary gas, nitrogen; sheath gas pressure, 65 p.s.i. (450 kPa); auxiliary gas, 5 flowmeter units (~ca. 2 l/min); capillary temperature, 250°C; spray voltage, 5 kV.

Mass analysis was performed as selected ion monitoring (SIM) in negative ion mode. Time-scheduled SIM conditions were as follows: LC time 0–16.0 min, $m/z$ 219; LC time 16.0–19.0 min, $m/z$ 197, 198, 239, 242, 356, 380, 386 and 400; LC time 19.0–22.0 min, $m/z$ 197, 199, 219, 225, 276, 356, 370 and 380; LC time 22.0–27.0 min, $m/z$ 161, 199, 213, 233, 320, 326 and 370; LC time 27.0–40.0 min, $m/z$ 239. (Identification of compounds with respect to detection $m/z$, see Table 1). Mass-to-charge window ±0.3 $m/z$ units; the dwell time was 0.5 s for each selected $m/z$. The total data acquisition time was 40.0 min. For ESI-MS experiments performed under full scan conditions, the scan range was 50–450 $m/z$ and the scan time was 2 s.

All MS–MS experiments were performed using argon as the collision gas at a collision cell pressure of 1.0 mTorr and with collision energies ranging between 10 and 30 eV. Mass analysis was performed in MS–MS product ion mode with the first quadrupole locked on the $m/z$ value corresponding to the deprotonated molecular ion of the target compound and with the second quadrupole either locked on a characteristic product ion $m/z$ [selected reaction monitoring mode (SRM)] or scanning from $m/z$ 50 to ca. 50 amu above the molecular mass of the target compound (product ion scan mode).

### 2.5. Sample handling and preparation procedure

A 1.00-ml volume of internal standard solution was added to samples (1 l) of ground water and the pH was adjusted to pH 4.5 using 6.0 ml of 100% acetic acid followed by 5.0 ml of 25% sodium hydroxide. The samples were filtered through GF/C glass fiber filters (Whatman, Maidstone, UK) and the filters were washed with 5 ml of methanol. A filtered sample was applied to the SPE column, which had been conditioned by flushing it with 10 ml of acetonitrile, 10 ml of methanol and 20 ml of water at a moderate flow-rate (ca. 2–4 ml/min). The filtered sample was applied to the column at a flow-rate of 20 ml/min. The SPE column was washed with 20 ml of water and was air-dried by continued suction for 20 min.

Elution of the SPE columns was performed by addition of 5 ml of methanol–acetonitrile (1:1, v/v) without applying a vacuum to the manifold. Following soaking for 2 min, elution was initiated at a flow-rate of 1 ml/min. In addition, 5 ml of methanol–acetonitrile (1:1, v/v) were added to the SPE column and elution was continued. To the pooled eluate was added 50 μl of propylene glycol, and the sample was evaporated under a stream of nitrogen at ca. 40°C and the residue redissolved in 1.00 ml of methanol–water (10/90, v/v).

## 3. Results and discussion

### 3.1. Optimization of ESI inlet conditions

High-flow pneumatically assisted ESI-MS is a soft ionization technique that results in very little fragmentation of the analyte molecule. The ions formed are, in most cases, predominantly the ionized molecule, occasionally together with solvent cluster ions. In the present study, where negative ion mode MS was used, inlet conditions were initially optimized to achieve maximum formation of the deprotonized molecular ion $[M-H]^-$. The different ESI parameters that have been examined include gas flows for formation of and controlling the distribution of the spray (sheath gas and auxiliary gas, respectively), applied electrospray voltage and temperature of the heated capillary, which separates the atmospheric pressure inlet region of the instrument from the low pressure quadrupole analyzer region. The effect of changing the settings for these four inlet parameters has been investigated by injecting a test solution containing seven of the herbicide compounds (2,4-D, bentazone, dichlorprop, dinoseb, DNOC, MCPA and mecoprop, 50 μg/l each) and calculating the signal-to-noise ratio for each compound from the individual SIM chromatograms. When performing the optimization procedure, the settings of one parameter were varied, while the three other parameters were kept constant. For each compound, the parameter setting giving rise to the highest signal-to-noise ratio was set as 100% and responses at other settings were calculated relative to the highest response. Evaluation of the different parameter settings was based on a calculated mean relative response for all seven compounds at each parameter setting. A graphical presentation of the results from this optimization procedure for inlet conditions is shown in Fig. 1.

As is apparent from the figure, optimization of the inlet conditions has a pronounced effect on the formation of the ionized analyte molecule in the electrospray process. On the basis of these results, we have chosen the following conditions, which were used throughout this investigation: Sheath gas pressure, 65 p.s.i.; auxiliary gas, 5 units (~ca. 2.1 l/min); capillary temperature, 250°C and electrospray voltage, 5 kV.

In order to obtain satisfactory chromatographic resolution of the acidic herbicides with respect to peak shape and retention, ion suppression using an acidic LC mobile phase is necessary. We used acetic acid for this purpose, because it is volatile and, as such, is advantageous to use with LC–MS compared to non-volatile acids. Chiron et al. [8] have shown that improved sensitivity of the electrospray MS detection of acidic herbicides could be achieved by the postcolumn addition of tripropylamine. Neutralizing the acidic (formic acid) LC mobile phase by the addition of tripropylamine in equimolar amounts was reported to increase the detection sensitivity by a factor of at least two. We have tried post-column neutralization of the LC mobile phase with tripropylamine too, but, unfortunately, we were unable to achieve any improvement in the detection sensitivity. Thus, we have chosen the more simple

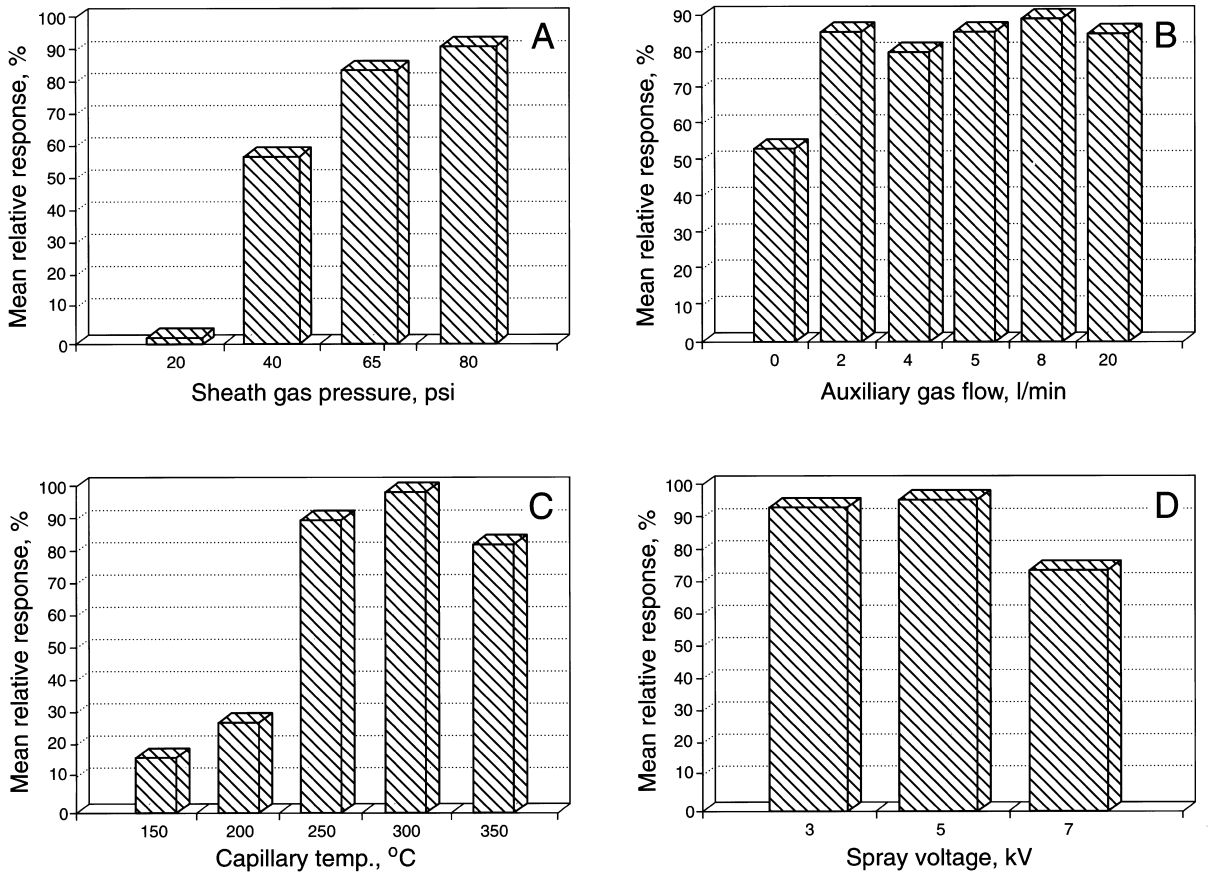


Fig. 1. Optimization of inlet parameters. (A) Sheath gas pressure, (B) auxiliary gas flow-rate, (C) capillary temperature and (D) spray voltage. The relative response was calculated as the signal-to-noise normalised relative to the highest value. The mean for all seven test compounds (see text) was then calculated for each parameter setting as the mean relative response. Fixed settings, except for the target parameter were: Sheath gas pressure, 65 p.s.i.; auxiliary gas flow-rate, 2 l/min; capillary temperature, 250°C; spray voltage, 5 kV.

set-up with the LC column directly interfaced with the ESI inlet of the mass spectrometer.

### *3.2. MS and MS–MS detection*

The highest sensitivity of target compound analysis, such as ground water pesticide monitoring using MS detection, is achieved in the SIM mode. In our case, the lowest detection limits were expected to be achieved by SIM detection of the deprotonated molecules. On the other hand, in order to obtain a high degree of confidence in compound identification, additional spectral information about characteristic fragments would be advantageous, particularly if such spectral information can be obtained while still being able to fulfill the need for low detection limits.

MS–MS using CID is a means of obtaining structurally related spectral information from the initially formed parent ion. We have investigated the possibilities of using this technique to improve the probability of correct identification of acidic herbicides analyzed by ESI-MS. This includes establishing useful conditions for CID of the eighteen acidic herbicides covered by our study as well as comparing instrumental detection limits found using MS and MS–MS in order to quantify the ‘‘cost’’, i.e. the loss in sensitivity for obtaining spectral information.

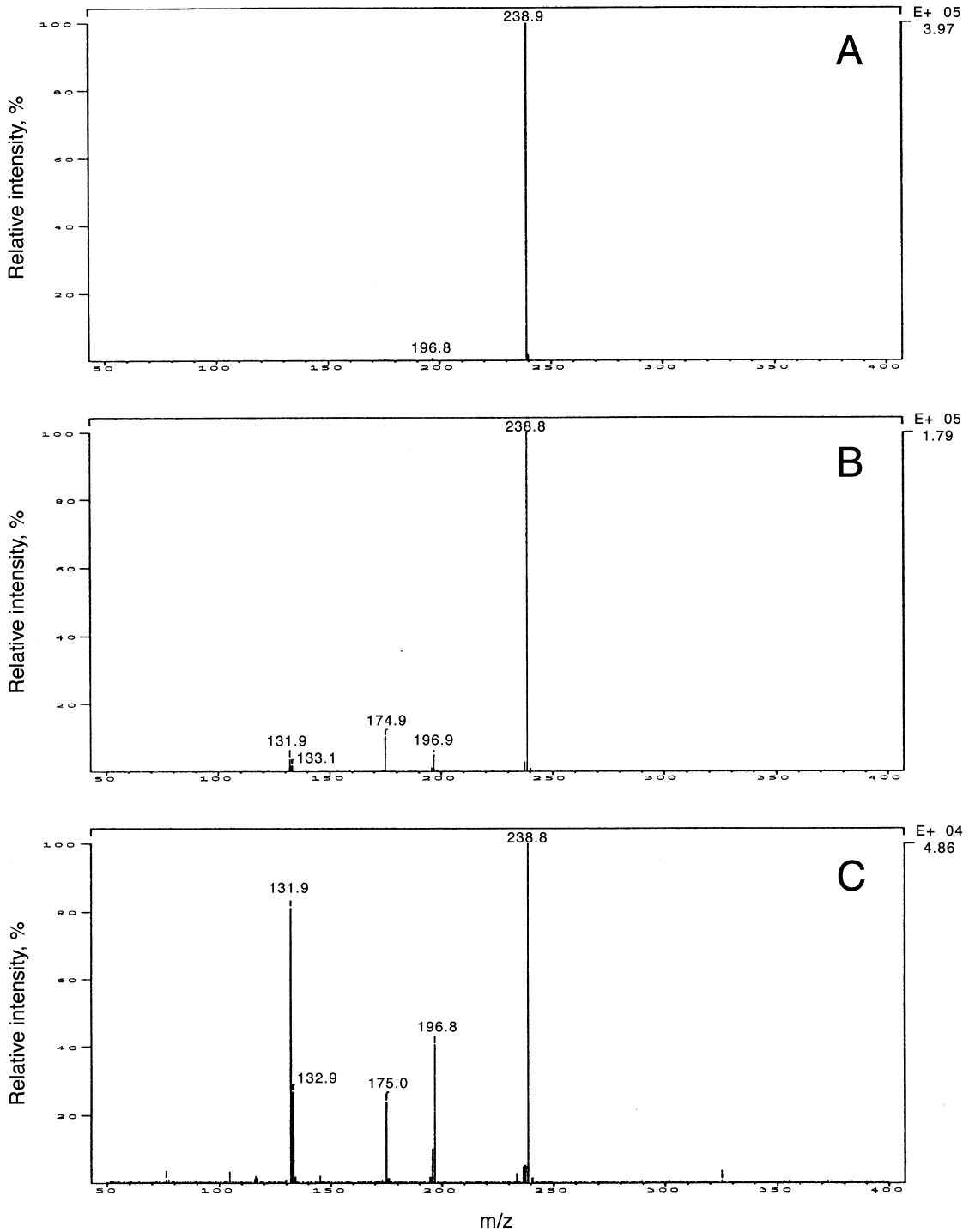


Fig. 2. Full scan (50–400 amu) product ion spectra of bentazone from ESI-MS–MS analysis at different collision cell offset voltages. (A) 10 V, (B) 20 V and (C) 30 V offset.

Table 1
ESI-MS–MS[a] data on product ion spectra for pesticides or pesticide degradation products obtained at three different collision energies

| Compound | MW[b] | Parent ion | Collision energy (eV) | Product ions (% rel. abundance) |
|---|---|---|---|---|
| 2,4-D | 220 | 219 | 10 | 219 (100), 161 (70) |
| | | | 20 | 219 (5), 161 (100) |
| | | | 30 | 161 (100), 125 (12) |
| 2,4-Dichlorophenol | 162 | 161 | 10 | 161 (100) |
| | | | 20 | 161 (100) |
| | | | 30 | 161 (100) |
| Benazolin | 243 | 242 | 10 | 242 (20), 198 (100), 170 (18) |
| | | | 20 | 198 (8), 184 (5), 170 (100) |
| | | | 30 | 184 (15), 170 (100) |
| Bentazon | 240 | 239 | 10 | 239 (100) |
| | | | 20 | 239 (100), 197 (7), 175 (5) |
| | | | 30 | 239 (100), 197 (42), 175 (25), 132 (90) |
| Bromoxynil | 277 | 276 | 10 | 276 (100) |
| | | | 20 | 276 (100) |
| | | | 30 | 276 (100), 81 (15), 79 (15) |
| Chlorsulfuron | 357 | 356 | 10 | 356 (100), 139 (10) |
| | | | 20 | 356 (8), 139 (100) |
| | | | 30 | 139 (100) |
| Dicamba | 220 | 219 | 10 | 219 (15), 175 (100) |
| | | | 20 | n.d.[c] |
| | | | 30 | n.d. |
| Dichlorprop | 234 | 233 | 10 | 233 (100), 161 (85) |
| | | | 20 | 233 (5), 161 (100), 125 (5) |
| | | | 30 | 161 (100), 125 (10) |
| Dinoseb | 240 | 239 | 10 | 239 (100) |
| | | | 20 | 239 (100), 193 (8) |
| | | | 30 | 239 (100), 222 (8), 207 (10), 193 (65), 163 (10) |
| DNOC | 198 | 197 | 10 | 197 (100) |
| | | | 20 | 197 (100), 180 (10), 167 (8), 137 (10) |
| | | | 30 | 197 (100), 180 (55), 151 (30), 137 (30), 122 (45) |
| Flamprop | 321 | 320 | 10 | 320 (100) |
| | | | 20 | 320 (20), 276 (10), 248 (36), 234 (30), 121 (100) |
| | | | 30 | 320 (5), 248 (35), 234 (15), 170 (8), 121 (100) |
| Fluazifop | 327 | 326 | 10 | 326 (100), 254 (15) |
| | | | 20 | 326 (7), 254 (100) |
| | | | 30 | 326 (5), 254 (100), 226 (10) |
| Ioxynil | 371 | 370 | 10 | 370 (100) |
| | | | 20 | 370 (100), 127 (5) |
| | | | 30 | 370 (100), 243 (10), 127 (25) |
| MCPA | 200 | 199 | 10 | 199 (100), 141 (26) |
| | | | 20 | 199 (5), 155 (5), 141 (100) |
| | | | 30 | 199 (5), 141 (100) |

Table 1. Continued

| Compound | MW[b] | Parent ion | Collision energy (eV) | Product ions (% rel. abundance) |
|---|---|---|---|---|
| Mecoprop | 214 | 213 | 10 | 213 (100), 141 (40) |
| | | | 20 | 213 (5), 141 (100) |
| | | | 30 | 213 (5), 141 (100) |
| Metsulfuron-Me | 381 | 380 | 10 | 380 (100) |
| | | | 20 | 380 (10), 139 (100) |
| | | | 30 | 139 (100) |
| Thifensulfuron-Me | 387 | 386 | 10 | 386 (100), 220 (10), 139 (10) |
| | | | 20 | 386 (50), 220 (38), 139 (100) |
| | | | 30 | 386 (10), 139 (100) |
| Triasulfuron | 401 | 400 | 10 | 400 (100) |
| | | | 20 | 400 (10), 139 (100) |
| | | | 30 | 139 (100) |

[a] Flow injection analysis conditions; 5 μl injection of standard solution (20 mg/l); first quadrupole set at parent ion ±0.3 $m/z$; collision cell pressure (argon), 1.0 mTorr.
[b] Monoisotopic molecular mass.
[c] No detectable product ions.

### *3.2.1. Conditions for CID MS–MS*

The extent of fragmentation of the initially formed parent ion depends on the collision energy and the collision gas pressure in the collision cell between the first and the second quadrupole of the mass spectrometer. In practice, it is simpler to keep the collision gas pressure constant during MS–MS experiments and control the CID by controlling the collision energy by the applied voltage difference between the first quadrupole and the collision cell. We have collected daughter ion spectra for the eighteen acidic herbicides at collision energies of 10, 20 and 30 eV. A typical example of the effect of increasing the collision energy is shown in Fig. 2, where an increased degree of fragmentation of the bentazone parent ion is observed at higher collision energies.

Table 1 lists data from daughter ion spectra of the eighteen acidic herbicides covered in this study. Mass-to-charge values and intensities (relative to base peak) are listed for a maximum of five (most abundant) ions present at intensities >5% relative to the base peak. The daughter ion spectra are obtained by injecting a 20 mg/l standard solution of each pesticide under flow injection analysis conditions and collecting full scan (50–400 amu) daughter ion spectra.

### *3.2.2. Comparison of instrumental detection limits*

In order to be able to estimate the ''cost'' in terms of detection sensitivity when increased identification probability is requested, a determination of instrumental detection limits was made. In Table 2, detection limits are shown for single MS (full scan and SIM) as well as for CID tandem MS [daughter full scan and selected reaction monitoring (SRM)].

Detection limits were generally five–ten times lower when only selected ions were detected, compared to full scan mode. Similarly, when comparing MS and MS–MS, detection limits were generally three–four times lower using MS than when CID MS–MS was applied. However, for some compounds (e.g. bromoxynil and ioxynil), detection limits using MS–MS were 100–200 times higher, primarily because CID of the parent ions did not fragment into characteristic daughter ions. The compounds apparently underwent complete dissociation, as the only detectable daughter ions were the bromide and iodide ions, respectively.

Instrumental detection limits are listed as concentrations in injected solutions, whereas, to be related to the concentration levels relevant to ground water analysis, it is necessary to incorporate the concentration factor achieved in the sample preparation step. In our sample preparation procedure, a

Table 2
Instrument detection limits (μg/l in injected solution, signal-to-noise ratio=3[a]) of eighteen pesticides or pesticide degradation products obtained by LC–ESI using single MS and MS–MS modes

| Compound | $t_R$ | Single MS | | | MS–MS | | |
|---|---|---|---|---|---|---|---|
| | (min) | Full-scan | SIM | Ion | Full-scan | SRM | Ion (CE[b], eV) |
| 2,4-D | 20.9 | 1 | 0.5 | 219 | 10 | 3 | 161 (20) |
| 2,4-Dichlorphenol | 23.3 | 70 | 10 | 161 | –[c] | –[c] | –[c] |
| Benazolin | 17.0 | 15 | 2 | 242 | 37 | 5 | 170 (20) |
| Bentazon | 17.2 | 0.7 | 0.1 | 239 | 18 | 4 | 132 (30) |
| Bromoxynil | 20.5 | 0.7 | 0.1 | 276 | 33 | 15 | 79 (30) |
| Chlorsulfuron | 19.0 | 4 | 0.4 | 356 | 19 | 7 | 139 (20) |
| Dicamba | 14.5 | 50 | 9 | 219 | 68 | 8 | 175 (10) |
| Dichlorprop | 23.8 | 3 | 0.6 | 233 | 10 | 2 | 161 (20) |
| Dinoseb | 29.2 | 1 | 0.3 | 239 | 14 | 2 | 193 (30) |
| DNOC | 20.7 | 0.8 | 0.1 | 197 | 71 | 23 | 122 (30) |
| Flamprop | 23.5 | 4 | 0.3 | 320 | 19 | 3 | 121 (20) |
| Fluazifop | 23.9 | 3 | 0.4 | 326 | 4 | 3 | 254 (20) |
| Ioxynil | 22.3 | 0.4 | 0.06 | 370 | 100 | 21 | 127 (30) |
| MCPA | 22.0 | 8 | 1 | 199 | 16 | 4 | 141 (20) |
| Mecoprop | 24.6 | 3 | 0.4 | 213 | 11 | 3 | 141 (20) |
| Metsulfuron-methyl | 18.0 | 4 | 0.4 | 380 | 12 | 6 | 139 (20) |
| Thifensulfuron-methyl | 17.5 | 5 | 0.5 | 386 | 23 | 6 | 139 (30) |
| Triasulfuron | 17.2 | 5 | 0.5 | 400 | 23 | 3 | 139 (20) |

[a] Measured on mass chromatogram of most abundant ion (single MS) or daughter ion (MS–MS).
[b] CE=collision energy.
[c] No detectable daughter ions in MS–MS mode.

concentration factor of 1000 was obtained (1 l of ground water concentrated to 1 ml before chromatographic analysis).

### *3.3. Method performance evaluation*

#### *3.3.1. Linearity*

Linearity of the response by LC–ESI-MS was investigated by injecting standard solutions of all eighteen analytes (nine different concentrations in the range 5–500 μg/l) and detection using MS in SIM mode. Satisfactory linearity ($r>0.989$) was found for thirteen of the eighteen compounds, whereas for five compounds, four of the phenols (DNOC, dinoseb, bromoxynil and ioxynil) and bentazone, a non-linear, convex curved relationship was found between the amount of analyte injected and the detection response. No explanation was found for the non-linear relationship of the detector response for these five compounds. The phenomenon is probably related to the electrospray ionization process and the ability of the individual compounds to form deprotonated molecular ions. Nevertheless, a comparison of linearity of the response for these five compounds with and without the post-column addition of tripropylamine, to facilitate ionization of the analytes, showed no difference between the two calibration curves. Calibration curves for representative analytes are shown in Fig. 3.

#### *3.3.2. Method detection limits, precision and recovery*

Determination of method detection limits was performed on tap water spiked with the pesticide compounds. Samples of water (1 l) were spiked with 10 ng (50 ng for 2,4-dichlorophenol and dicamba) of each compound to give concentrations of 0.01 μg/l (0.05 μg/l for 2,4-dichlorophenol and dicamba). The method detection limit (MDL) was calculated as three times the standard deviation for six replicate determinations. Table 3 shows the MDLs for all of the compounds in tap water following determination using single MS detection in SIM mode. MDLs were in the 0.001–0.010 μg/l range, except for the two compounds, 2,4-dichlorophenol and dicamba, which had MDLs of around 0.03 μg/l.

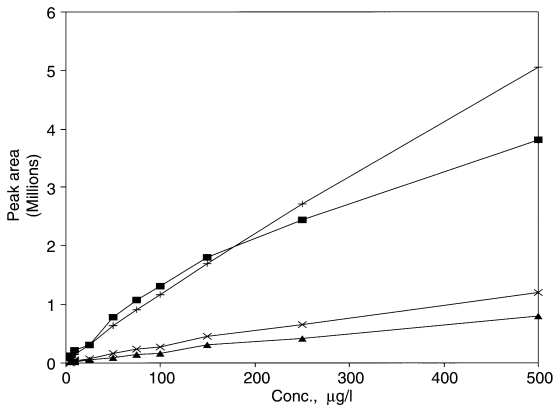


Fig. 3. Representative calibration curves (peak area vs. injected concentration) of four selected analytes from LC–ESI-MS analysis in SIM mode. (■) Bromoxynil, (+) dichlorprop, (×) metsulfuron-methyl and (▲) dicamba.

Method precision and recovery were calculated from a total number of thirteen series of analysis, each containing two recovery samples spiked to a concentration of 0.05 μg/l. Recoveries for each compound, expressed as the overall mean from these thirteen series of analysis, are shown in Table 3. As can be seen from the table, recoveries for some of the compounds, especially the sulfonylurea compounds, are rather low (<40%), whereas recoveries for the phenoxyacid type of compounds are satisfactory (>80%). The SPE was carried out with a general ''in-house'' procedure for pesticide extraction following adjustment of the pH to 4.5, making the procedure applicable to basic/neutral pesticides as well as to acidic pesticides. Performing the SPE at a lower pH might give better recoveries for some of the acidic herbicides covered in this investigation. Repeatability precision, expressed as the mean relative standard deviation, for each compound, was calculated from duplicate determinations of recovery samples and the results are also shown in Table 3. Repeatability precision values were in the range 10–20%, except for the sulfonylurea compounds for which repeatability precisions were in the range 20–30%. The relatively high values for these compounds are most likely a consequence of the low recoveries found for these compounds.

### *3.4. Application to real-world samples*

The potential of the method for the analysis of real-world ground water samples has been demonstrated. In our laboratory, samples of ground water from different sampling sites all over Denmark have been received for pesticide residue analysis as part of an evaluation of a currently running ground water

Table 3
Recoveries at a concentration level of 0.05 μg/l, method detection limits (MDLs) and repeatability precisions

| Compound | Recovery[a] (%) | MDL (μg/l) | Precision (R.S.D.)[a] (%) |
|---|---|---|---|
| 2,4-D | 81 | 0.003 | 11 |
| 2,4-Dichlorphenol | 47 | 0.026 | 17 |
| Benazolin | 44 | 0.008 | 19 |
| Bentazon | 61 | 0.002 | 9 |
| Bromoxynil | 57 | 0.003 | 10 |
| Chlorsulfuron | 35 | 0.004 | 19 |
| Dicamba | 33 | 0.032 | 15 |
| Dichlorprop | 89 | 0.003 | 11 |
| Dinoseb | 60 | 0.007 | 22 |
| DNOC | 51 | 0.003 | 14 |
| Flamprop | 87 | 0.004 | 11 |
| Fluazifop | 93 | 0.004 | 10 |
| Ioxynil | 55 | 0.005 | 13 |
| MCPA | 85 | 0.003 | 14 |
| Mecoprop | 86 | 0.003 | 10 |
| Metsulfuron-methyl | 28 | 0.004 | 28 |
| Thifensulfuron-methyl | 27 | 0.002 | 29 |
| Triasulfuron | 30 | 0.009 | 21 |

[a] Mean of duplicates from thirteen series of analysis.

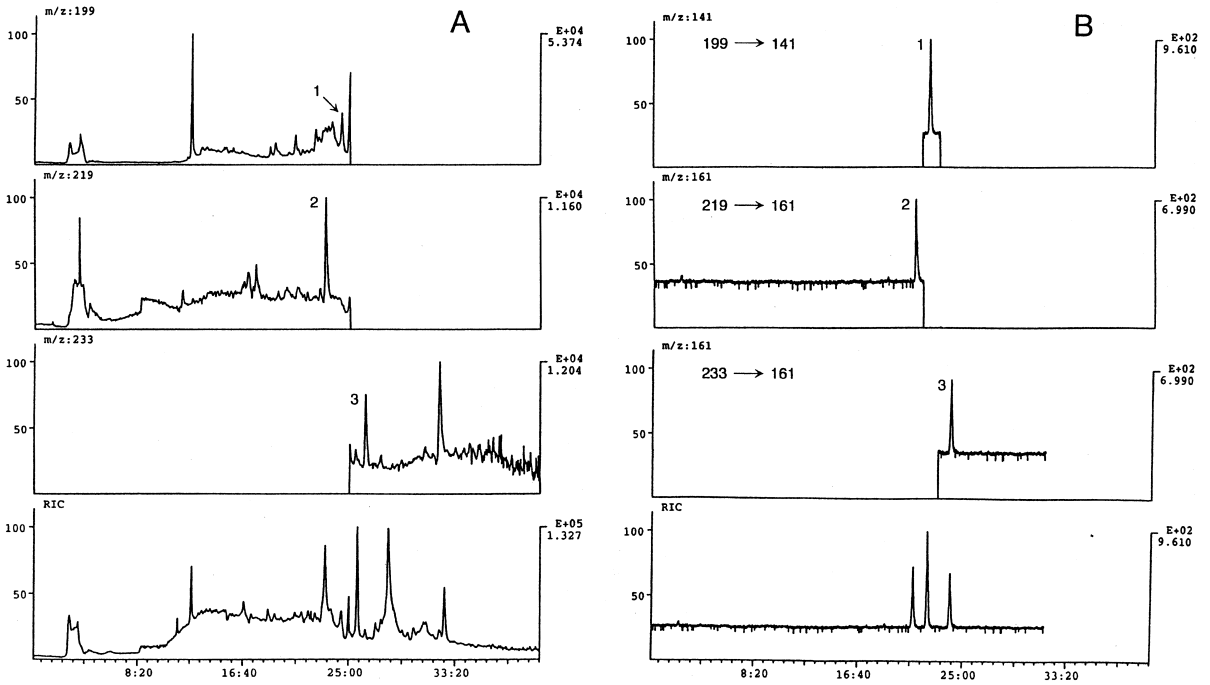


Fig. 4. Typical chromatograms from an LC–ESI-MS analysis of an authentic ground water sample following pre-concentration by solid phase extraction. The sample was analysed using single MS in SIM mode (A) and, subsequently, by MS–MS in SRM mode (B) for verification. Compound identification: MCPA (1), 2,4-D (2) and dichlorprop (3). Experimental conditions are given in Section 2.

monitoring programme. All samples were first analyzed for the eighteen acidic herbicide target compounds using LC with ESI single MS detection in SIM mode. Subsequently, positive findings were verified by a second chromatographic analysis using MS–MS in SRM mode. An example of the results from such analyses is shown in Fig. 4.

The first chromatographic analysis using single MS detection showed (Fig. 4A) peaks with chromatographic retention times and detection masses corresponding to the three phenoxy acids, 2,4-D, dichlorprop and MCPA (mass chromatograms $m/z$ 219, 233 and 199, respectively). In the second chromatographic analysis (Fig. 4B), using MS–MS, these findings were verified by detecting the characteristic product ions ($m/z$ 141 fragment ion of MCPA and $m/z$ 161 fragment ions of 2,4-D and dichlorprop). The concentrations found in the sample were 0.012 μg/l of 2,4-D, 0.006 μg/l of dichlorprop and 0.010 μg/l of MCPA.

## 4. Conclusions

LC using pneumatically assisted electrospray mass spectrometry detection in negative ion mode has been shown to be a highly advantageous technique for the determination of acidic herbicides in ground water. The soft electrospray ionization process results in the formation of the deprotonated molecular ion $[M-H]^-$ of the analytes, which can be determined with maximum sensitivity by SIM mode single MS. This will make the method potentially attractive for target compound analysis, like ground water monitoring studies.

Furthermore, it has been demonstrated that increased confidence in compound identification can be obtained by MS–MS based on detection of product ions formed by CID of the initially formed deprotonated molecular ion. The increased level of confidence obtained by MS–MS detection is, however, accompanied by a decrease in detection sensitivity.

On average, a three-fourfold reduction in detection sensitivity was found when comparing MS with MS–MS detection, with significant variation between the different compounds investigated.

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