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Determination of polar pesticides in ground water using liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization

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Abstract

A method is described for determination of pesticides in ground water by liquid chromatography combined with mass spectrometry (LC–MS) using atmospheric pressure chemical ionization (APCI). The study demonstrates the sensitivity of the technique, with detection limits for water samples of about 0.001–0.005 μg/l, corresponding to 50–300 pg injected. Performance data for the method such as recovery, precision and linearity are also reported. It is illustrated that the technique is applicable for many different types of pesticide structures, such as triazines, phenylurea herbicides, acetanilides, organophosphorus insecticides, etc. Twelve different pesticides and pesticide degradation products are included in the study. The optimization of inlet conditions with respect to sheath gas pressure, vaporizer temperature, capillary temperature and corona current is reported.

Applicability of the method for analysis of "real-world" ground water samples is demonstrated.

Keywords: Environmental analysis; Water analysis; Pesticides

1. Introduction

For many years the use of pesticides in agriculture has given rise to concern about the unintended effects on the environment. During the last decade numerous reports on pesticides in ground water have received attention because of the implications for the supply of clean drinking water in the future. Especially in countries mainly relying on ground water for drinking water supply, these findings have caused much concern, e.g. in Denmark, where 99% of the drinking water supply is based on ground water and a consequence has been an increasing need for monitoring ground water quality with respect to content of pesticides. Monitoring for pesticides requires analytical methodology capable of performing determinations at very low concentration levels. A limit of 0.1 μg/l of individual pesticides in drinking water is set by a European Union directive [1], which means that methods used for the monitoring of ground water preferably should exhibit detection limits about one tenth of the limit or lower, viz. 0.01 μg/l or less.

Polar pesticides are those most likely to leach to ground water and are therefore the pesticides primarily to be included in a method applicable for ground water monitoring. A variety of chromatographic methods have been used for determination of

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the more polar pesticides. A review covering both GC and HPLC methods used for the determination of pesticides in water has been published recently [2]. However, since HPLC methods are more suited for polar compounds than are GC methods, which often require derivatisation prior to the analysis or can give problems with thermolabile compounds, HPLC has, in recent years, generally been the preferred method for analysing polar pesticides. HPLC methods are usually based on photometric detection (UV, fluorometric), but HPLC methods using mass spectrometric (MS) detection have also been described for the determination of pesticides in environmental waters [2]. LC–MS methods are especially attractive for their inherent high specificity. A review of the use of LC–MS techniques for polar pesticides has been published very recently by Slobodnik et al. [3], covering both the principles of ionization and applications of the most used LC–MS techniques (thermospray, particle beam and atmospheric pressure ionization). Among these LC–MS interfaces, the authors underlined the potential of the atmospheric pressure ionization (API) for pesticide analysis both in terms of sensitivity and the possibility of achieving structural information, in spite of the rather small number of studies of LC–API–MS published so far. API is a term covering several different principles of ionization [4,5], e.g. electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). APCI, which was introduced only a few years ago, is a soft ionization method, typically generating only ionized molecules $[M+H]^+$ (in positive mode) or $[M-H]^-$ (in negative mode). However, if structural information is desired, some fragmentation can be achieved by pre-analyser collision-induced dissociation (CID), by increasing the voltage difference between two regions of the APCI interface [3].

Very few studies on the application of LC–APCI–MS for pesticide analysis have been published. The potential of the technique for the analysis of some pesticides has been demonstrated [6,7], and its application for the quantitative determination of pesticides has been described by a few groups. Pleasance et al. [8] used LC–APCI–MS for the determination of residues of carbamates in green peppers, Kawasaki et al. applied the technique for determination of organophosphorus [9] and carbamate [10] pesticides in human serum, whereas Doerge and Bajic [11] have determined several groups of pesticides in ground water using LC–APCI–MS.

In this work we have further investigated the APCI technique for the determination of pesticides and we present a LC method using APCI–MS detection for the simultaneous determination of twelve different pesticides or pesticide degradation products in ground water.

2. Experimental

2.1. Chemicals

Dichloromethane was of HPLC glass-distilled grade, purchased from Rathburn (Walkerburn, UK); 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). Anhydrous sodium sulfate (Merck) was purified by Soxhlet extraction with dichloromethane before use. All other chemicals were used as received. Water was deionized water subsequently purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Tap water from the laboratory was used for performance studies as blanks and for preparation of spiked water samples. The twelve pesticides or pesticide degradation products used as standards were alachlor, atrazine, simazine, terbuthylazine, cyanazine, desethylatrazine, desisopropylatrazine, dimethoate, hexazinone, isoproturon, metamitron and metazachlor. All pesticide standards were of Pestanal grade and were purchased from Riedel de Haën (Seelze, Germany). The purity of desethylatrazine was 97%, whereas the purity of all other standards was min. 99%. Stock solutions (200 or 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 twelve 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 com-

pound) were prepared by appropriate dilution of the mixed stock solution with LC solvent A (see Section 2.3.

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 quadropole tandem mass spectrometer, equipped with a Finnigan MAT standard APCI ionization source and in this study only used in single MS mode. 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 Novapak C_{18}, 4 μm, 150×2.0 mm I.D. from Waters.

2.3. Chromatographic conditions

Gradient HPLC was performed with a binary gradient composed of LC solvent A, methanol–water–acetic acid (100:900:2, v/v) and LC solvent B, methanol–acetic acid (1000:2, v/v) according to the following programme: linear gradient from 100 to 55% A from 0 to 3 min; linear gradient from 55 to 45% A from 3 to 15 min; linear gradient from 45 to 0% A from 15 to 23 min; returning linearly to 100% A from 23 to 25 min and maintaining 100% A from 25 to 31 min. The flow-rate was set to 0.2 ml/min and 50 μl of a sample/standard in solvent A was injected onto the HPLC system.

2.4. Mass spectrometric analysis

A scheme of the APCI inlet is depicted in Fig. 1. The sheath gas participates in the formation of an aerosol from the LC eluent. The auxiliary gas, the use of which is optional, helps to focus the spray towards the capillary, which is the interface between atmospheric pressure and the vacuum manifold. The vaporizer heater flash vaporizes the droplets at temperatures up to 600°C. The corona current is set typically in μA and the necessary corona voltage, typically in kV, is applied.

APCI–MS detection was performed with the following APCI inlet conditions: Sheath gas, nitrogen; sheath gas pressure, 40 p.s.i. (1 p.s.i.=6894.74 Pa); vaporizer and capillary temperature, 500 and 170°C, respectively; corona current, 5 μA.

Mass analysis was performed as selected ion

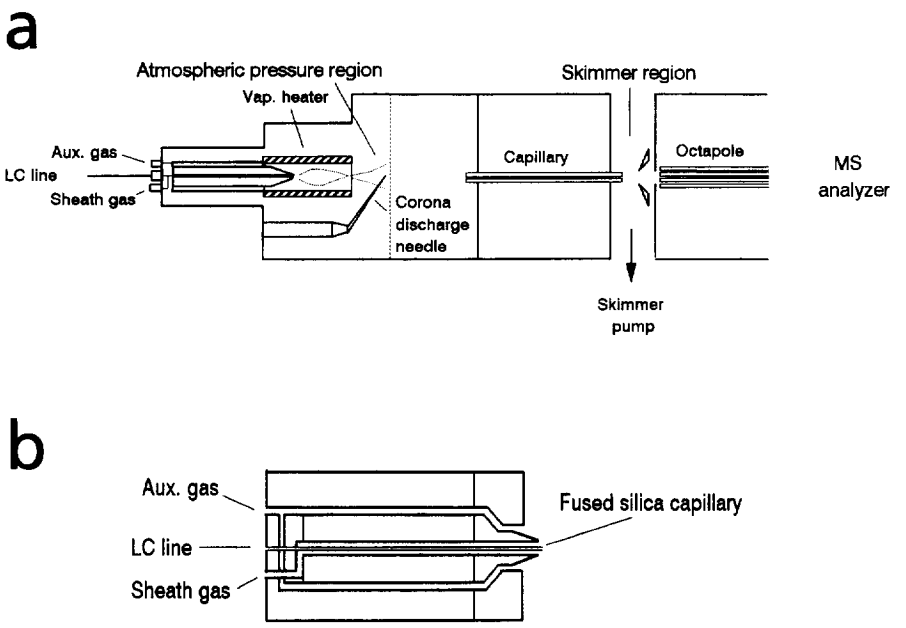

Fig. 1. General scheme of the APCI inlet.

monitoring (SIM) in positive ion mode. Time-scheduled SIM conditions were as follows: LC time 0–15.0 min, m/z 174, 188, 203, and 230; LC time 15.0–19.0 min, m/z 202, 241 and 253; LC time 19.0–23.5 min, m/z 207, 216 and 278; LC time 23.5–32.0 min, m/z 230 and 270. Mass-to-charge window ± 0.3 m/z units; dwell time was 0.5 sec for each selected m/z. Total data acquisition time was 32.0 min.

2.5. Sample handling and preparation procedure

Samples (1 l) of tap water (used as blank samples and for preparing spiked samples for recovery experiments) and ground water were preserved immediately after sampling by addition of 5 ml of 4 *M* hydrochloric acid and 100 ml of dichloromethane. The bottles were shaken thoroughly and stored at 4°C until analysis. Storage time was as short as practically possible, 2–3 weeks or less.

The preserved sample was extracted by stirring (magnetic stirrer) for 30 min. The organic layer was then removed with a pipette and the sample extracted twice more with 100 ml of dichloromethane by stirring for 30 min. The pooled dichloromethane extract was dried with anhydrous sodium sulfate and concentrated to 3–5 ml by rotary evaporation (30°C). The concentrated extract was transferred quantitatively with dichloromethane to a vial (10 ml) containing 50 μl of propylene glycol. Dichloromethane was evaporated under a stream of nitrogen at 37°C and the residue redissolved in 1.00 ml of LC solvent A.

3. Results and discussion

Before optimization, a mass spectrum of each compound was recorded in order to select the most abundant mass-to-charge ratio (m/z) for further studies. For all compounds, the protonated molecule yielded the most abundant signal. APCI is a soft ionization technique giving rise to virtually no fragmentation as reported in previous papers describing APCI [3,4]. This is unambiguously demonstrated to be valid in this study also. In Fig. 2 mass spectra (m/z 120–320) of four of the compounds covered by the present method are depicted. The compounds are isoproturon (M_r 206), atrazine (M_r 215), alachlor (M_r 269) and metazachlor (M_r 277), where M_r is the monoisotopic molecular mass. The spectra consist primarily of the peak corresponding to the protonated molecules. Fig. 2a and Fig. 2b show mass spectra of isoproturon and atrazine with no fragmentation while Fig. 2c and Fig. 2d shows mass spectra of alachlor and metazachlor, where some fragmentation has occurred but only to a limited degree. The signal of the protonated molecule is more than three times higher than the signal of the most abundant fragment ion. For achievement of the best sensitivity, the extraction voltage was kept low to minimise further fragmentation in the preanalyser zone.

3.1. Optimization of inlet conditions

Corona current, sheath gas flow, vaporizer temperature and capillary temperature were optimized. A mixed calibration standard containing all twelve compounds (50 μg/l each) was injected and a signal-to-noise ratio for each compound was calculated from the individual SIM chromatograms. The influence of variations in each parameter setting on the signal-to-noise ratio of individual compounds was determined by repeated injections of the solution, keeping the other three parameters 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 twelve compounds, at each parameter setting. A graphical presentation of the results from the optimization of the capillary temperature is shown in Fig. 3a. The optimum temperature was established to be 170°C.

The vaporizer is a heater for flash vaporization of the eluent. Large amounts of solvent are removed and small droplets are formed. Apparently the signal-to-noise ratio declines if the vaporizer temperature is too high. Fig. 3b shows that the optimum temperature was 500°C, which accordingly was chosen for the rest of the study.

Fig. 3c shows the influence of the corona discharge current on the signal-to-noise ratio. The discharge current was varied between 2 and 8 μA.

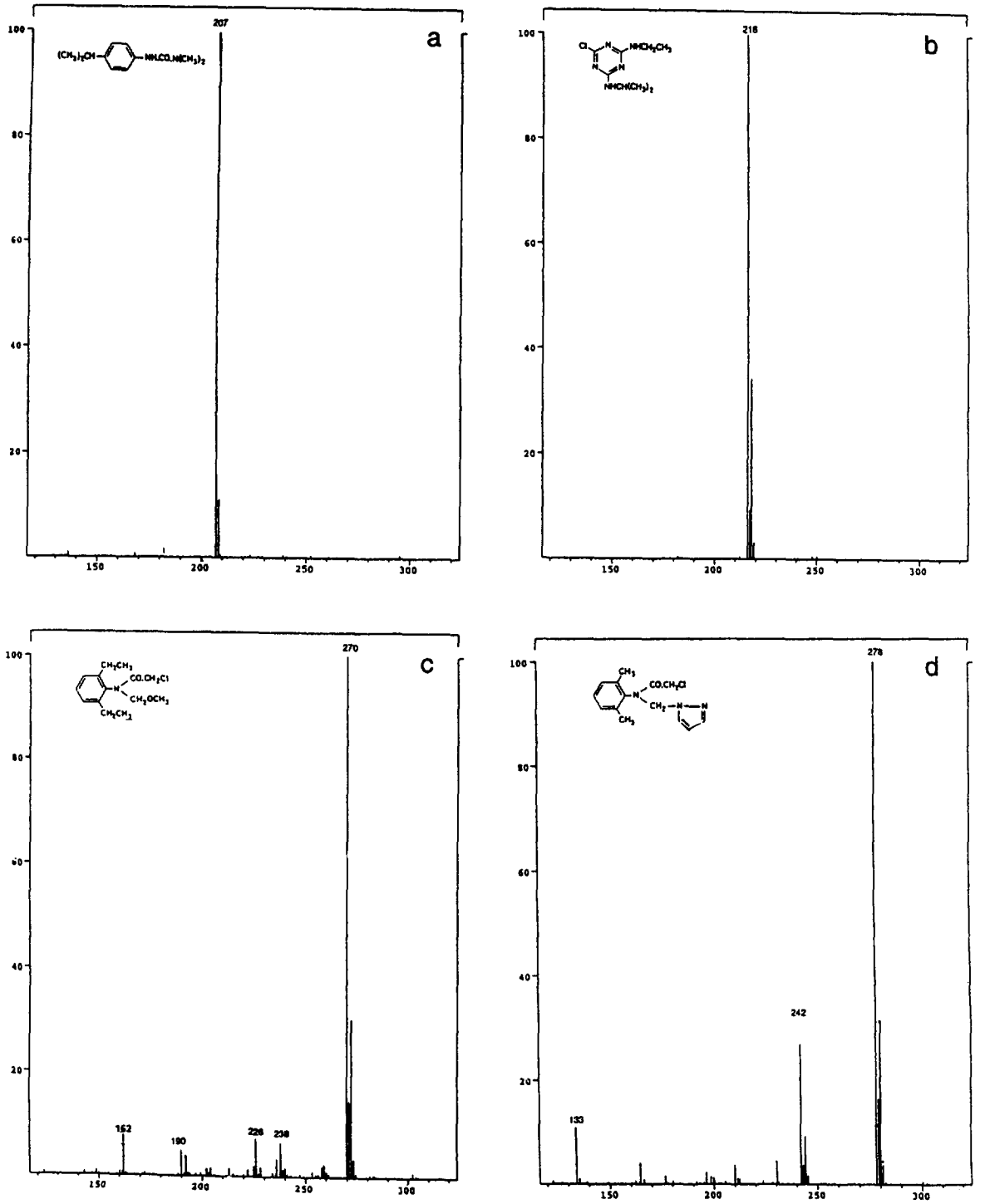

Fig. 2. APCI spectra of four different pesticide compounds. Full scan (m/z 120–320, scan time, 2 s). a = Isoproturon, b = Atrazine, c = Alachlor and d = Metazachlor.

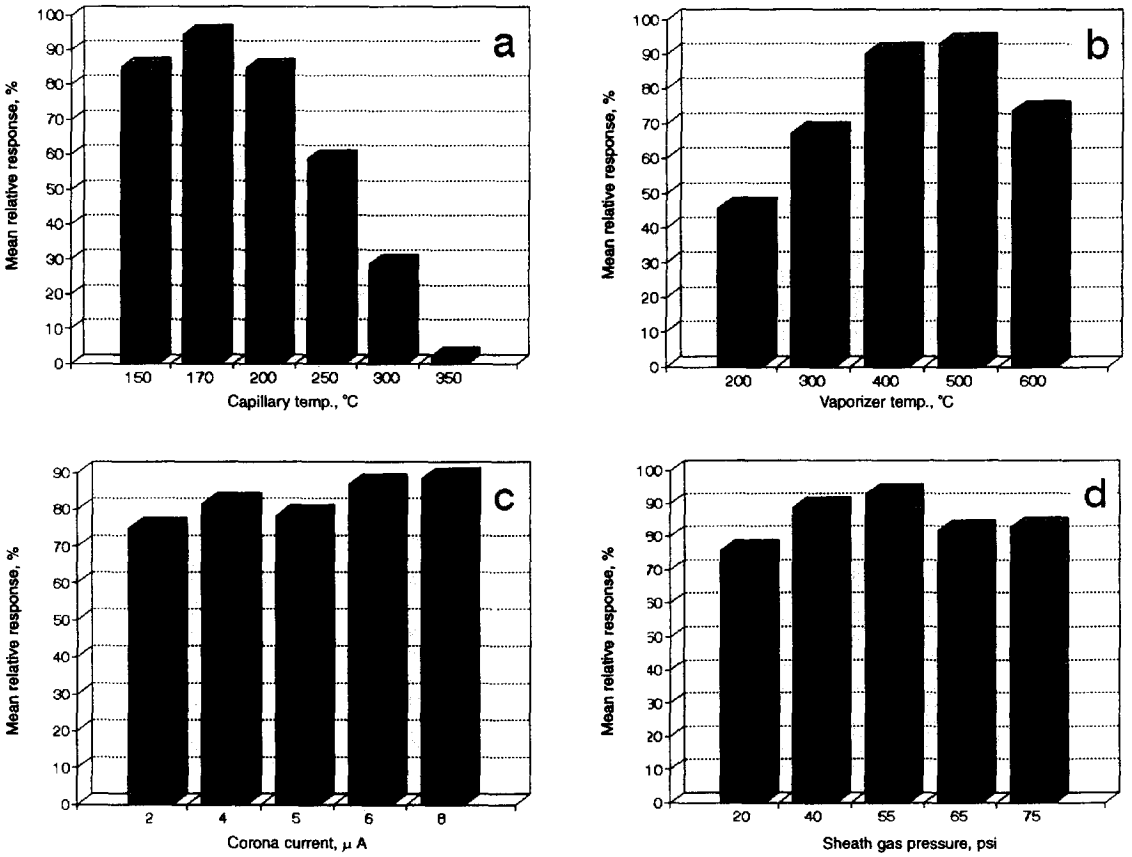

Fig. 3. Optimization of the inlet parameters. (a) Capillary temperature, (b) vaporizer temperature, (c) corona current and (d) sheath gas pressure. The relative response was calculated as the signal-to-noise ratio normalised relative to the highest value. The mean for all twelve compounds was then calculated for each parameter setting as the mean relative response. Fixed settings except for the target parameter were: capillary temperature, 170°C; vaporizer temperature, 500°C; corona current, 5 μA; sheath gas pressure, 65 p.s.i.

However, only minor variations in the signal-to-noise ratios were noted and a 5-μA corona discharge current was maintained throughout the experiments.

The sheath gas flow is of importance for the formation of droplets moving towards the corona needle and the heated capillary. Fig. 3d displays the variation in signal-to-noise ratios if the sheath gas pressure is altered. The sheath gas pressure was altered between 20 and 75 p.s.i. Only minor shifts in sensitivity were observed. A sheath gas pressure equal to 40 p.s.i. was selected as the optimum. Supply of auxiliary gas flow gave no further enhancement of the signal in this application.

3.2. Detection limits, linearity, recovery and precision

Determination of detection limits were performed for tap water spiked with the pesticide compounds. Samples of water (1 l) were spiked with 10 ng of each compound to give concentrations of 0.01 μg/l. The method detection limit (MDL) was calculated as three times the standard deviation (3 S.D.) for six replicate determinations. Table 1 shows MDL's for the twelve compounds in tap water with the SIM *m/z* used for detection and calculations. Detection limits were for all compounds equal to or below 0.006

Table 1
Performance data from determination of detection limits and linearity

Pesticide	SIM ion	D.L. (3 S.D.), μg/l	$y = bx+c$[a]	r^2
Desisopropylatrazine	174	0.002	$y = 9880x-29\,400$	0.9991
Dimethoate	230	0.001	$y = 8900x-12\,300$	0.9998
Metamitron	203	0.002	$y = 8600x-18\,600$	0.9998
Desethylatrazine	188	0.001	$y = 8500x-21\,400$	0.9995
Cyanazine	241	0.004	$y = 13\,000x+28\,500$	0.9989
Simazine	202	0.002	$y = 11\,100x-2800$	0.9996
Hexazinone	253	0.006	$y = 10\,900x-26\,300$	0.9994
Atrazine	216	0.002	$y = 11\,400x-12\,400$	0.9998
Metazachlor	278	0.002	$y = 6000x+2100$	0.9998
Isoproturon	207	0.003	$y = 9800x-3600$	0.9995
Terbuthylazine	230	0.003	$y = 9400x-1100$	0.9995
Alachlor	270	0.004	$y = 4400x+2800$	0.9999

SIM ions were used for quantification. Standard deviation (S.D.) was calculated on the basis of the results from six samples of tap water (1 l) spiked to 0.01 μg/l and the detection limit (D.L.) was calculated as 3 S.D. Linear regression data is calculated from standards injected at concentrations from 5–500 μg/l (seven levels). r^2 is the correlation coefficient.
[a] x = injected concentration in μg/l; y = peak area.

μg/l. More than half of the investigated compounds had detection limits close to 0.001 μg/l. Detection limits in injected amounts were in the range from 50–300 pg. Blank samples (tap water) did not give rise to any interfering peaks or background and carry over from one injection to another was not observed.

Linearity of the chromatographic determination was examined for the concentration range 5–500 μg/l (in injected solution), corresponding to a sample concentration prior to preconcentration of 0.005–0.5 μg/l, which is the relevant range for ground water pollution. Linearity was excellent (correlation coefficient, $r^2>0.999$) for all compounds.

The response factor or slope for the calibration curve varied between 6000 and 13 000 response units, i.e. same order of magnitude for all compounds.

Recoveries were calculated for water samples spiked to a concentration of 0.05 μg/l. Each series included two recovery samples and the pooled mean of 23 series is shown in Table 2, with the relative standard deviation (% R.S.D.) within and between series based on the 23 duplicate recovery samples. Eight of the twelve compounds showed recoveries of about 100%. Compounds with free amino groups (desisopropylatrazine, metamitron, desethylatrazine) gave lower recoveries. However, this might be improved if the acidic extractions of the water samples were succeeded by neutral or basic extractions.

Instrument precision was investigated by comparing standard deviation of the response from the injection of 50 μg/l calibration standard solutions. A mean R.S.D. was calculated for each compound based on seven different series of analysis comprising a total of 29 injections of the standard (see Table 2). There was generally good instrument precision with relative standard deviations below 10%, with the exception of alachlor which had a R.S.D. of 20%.

3.3. Application of the method

The method has been used in our laboratory for more than one year, as part of an on-going investigation into the contamination of Danish ground water with pesticides. During that period, more than 200 samples of ground water, collected from various areas of the country, have been analysed. In total, the pesticides metamitron, simazine, atrazine, isoproturon and terbuthylazine as well as the two triazine degradation products, desethylatrazine and desisopropylatrazine, have been detected one or more times in concentrations ranging from the detection limit level to 19 μg/l. The pesticides dimethoate, cyanazine, hexazinone, alachlor and metazachlor have not been detected in any of the samples, so far.

Table 2
Recoveries at a concentration level of 0.05 μg/l. Method and instrument precision

Pesticide	Recovery (%)	Precision [R.S.D.(%)]		Instrument precision [R.S.D. (%)]
		Within series	Between series	
Desisopropylatrazine	37	6	37	6
Dimethoate	102	6	14	7
Metamitron	77	9	21	10
Desethylatrazine	75	8	19	7
Cyanazine	82	15	35	7
Simazine	101	6	15	5
Hexazinone	106	7	15	8
Atrazine	100	3	12	4
Metazachlor	107	7	12	5
Isoproturon	107	6	12	6
Terbuthylazine	95	7	15	4
Alachlor	96	10	20	20

Recoveries were mean recoveries from 23 series of analyses, each including two samples of spiked tap water. Precision data were calculated from variations between these duplicate determinations. Instrument precision [R.S.D. (%)] was based on seven series of analyses with 29 injections of 50 μg/l standards.

Typical examples of chromatograms [SIM mass chromatograms and reconstructed ion chromatograms (RIC)] of a spiked sample (0.05 μg/l of each compound) and a "real-world" ground water sample are depicted in Fig. 4. The corresponding contents of atrazine, desethylatrazine, desisopropylatrazine and metamitron in the ground water sample were 0.03, 0.22, 0.02 and 0.005 μg/l, respectively.

Future work with this LC–MS technique will include investigation of the possibilities for incorporation of more polar pesticides and degradation products into the method. Furthermore, we will investigate the possibility of combining the use of APCI with ESI; in preliminary experiments, negative ion mode ESI has demonstrated better detectability for acidic herbicides (e.g. phenoxy acids) than has APCI.

4. Conclusions

This study has demonstrated that LC–MS with APCI is a sensitive and specific method for determination of different types of pesticides in ground water down to the detection limit level (0.001–0.006 μg/l). Inlet parameters for the APCI interface were optimized and it has been shown that for routine purposes fine adjustments of the parameters were of no particular importance since none of the parameters displayed distinct optima.

Instrument linearity and precision were very good in the concentration range relevant to ground water analysis. The mass spectrometric conditions gave very limited or no fragmentation of the compounds, which generally resulted in rather simple mass spectra showing the protonated molecule as the dominant peak in the spectrum. Furthermore, performance of the entire method of analysis covering twelve different pesticides and pesticide degradation products has been evaluated and found satisfactory for the purpose of ground water analysis, with detection limits below one tenth of the 0.1 μg/l limit set by the EU drinking water directive.

The method has been used in our laboratory on hundreds of samples for a period of more than one year and has proven to be robust and suitable for routine applications.

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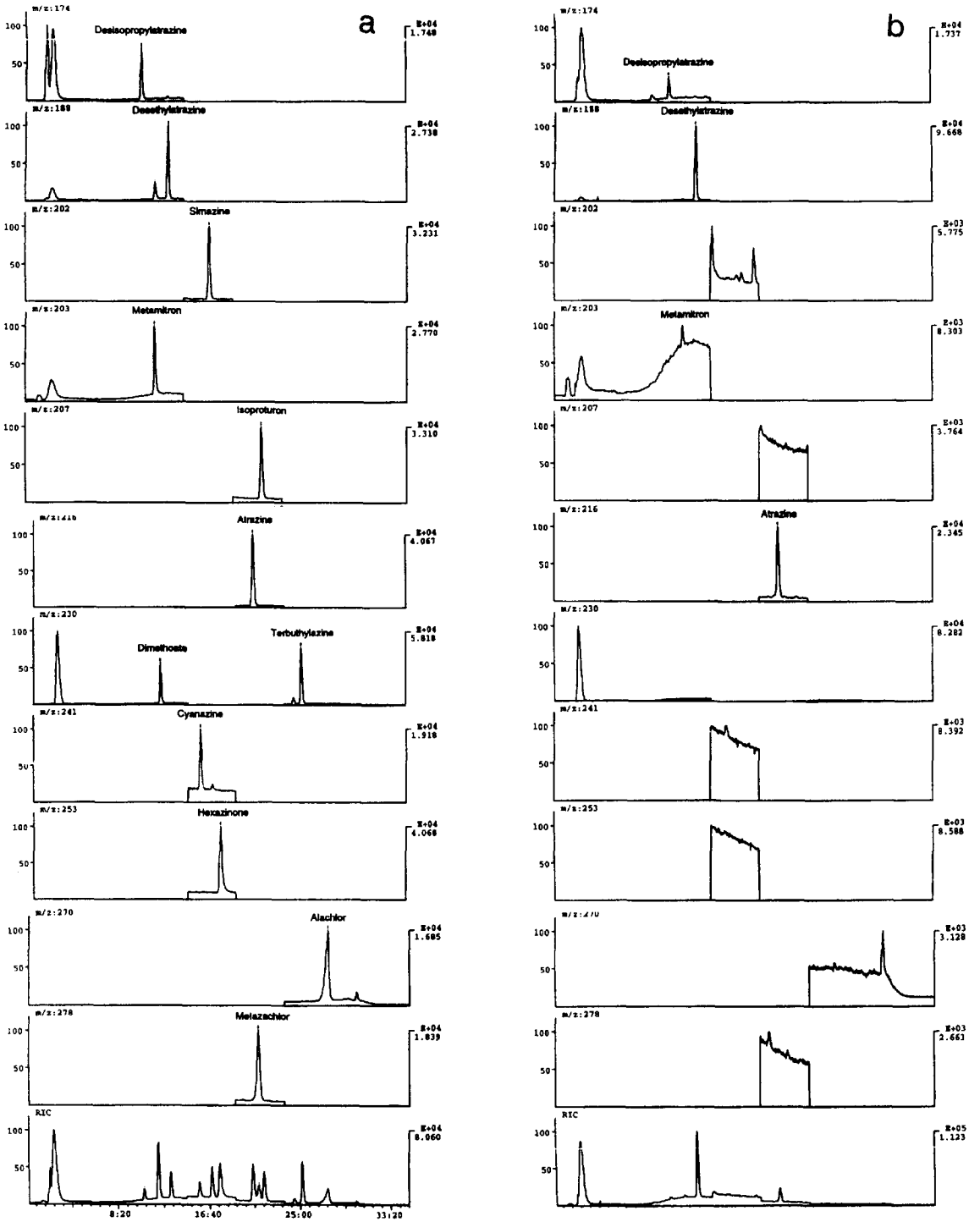

Fig. 4. Typical chromatograms of (a) tap water spiked with all twelve pesticides and pesticide degradation products (0.05 μg/l each) and (b) authentic ground water sample containing atrazine, desethylatrazine, desisopropylatrazine and metamitron (at concentrations of 0.03, 0.22, 0.02 and 0.005 μg/l, respectively). Experimental conditions are given in Section 2.

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