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Atmospheric pressure ionisation multiple mass spectrometric analysis of pesticides

Daniela Baglio, Dimitrios Kotzias, Bo Richter Larsen*

Environment Institute, Joint Research Centre, European Commission, I-21020 Ispra (VA), Italy

Abstract

Liquid chromatography–multiple mass spectrometry (LC–MS^n) has been investigated for analysis of polar pesticides in water using an ion-trap instrument and atmospheric pressure ionisation. Carbamate, triazine and phenylurea pesticides were best ionised as positive ions with atmospheric pressure chemical ionisation, while phenoxy acid herbicides, nitrophenols and bentazone yielded stronger signals as negative ions with pneumatically assisted electrospray. The ion fragmentation processes and pathways were studied in detail by MS, MS^2, MS^3 and MS^4. All compounds were observed as their protonised or deprotonised molecular ions by MS and in the successive fragmentation by MS^n the structures of typical (diagnostic) product ions were tentatively identified for each class of pesticide. Phenylureas yield an ion at m/z 72 by MS^2, corresponding to $O{=}C{=}N^+(CH_3)_2$. Carbamates produce $[M+H\text{-}CONCH_3]^+$ fragments by MS^2 from neutral loss of methylisocyanate. Characteristic fragmentation pathways for triazine pesticides are $[M+H]^+ \rightarrow m/z$ 174$\rightarrow m/z$ 146$\rightarrow m/z$ 110 and $[M+H]^+ \rightarrow m/z$ 174$\rightarrow m/z$ 132$\rightarrow m/z$ 104 by MS–MS^2–MS^3–MS^4 from cleavage of lateral chains in the triazine ring followed by ring opening. Phenoxy acid herbicides produce peculiar fragments by MS^2 from loss of the acidic group possibly as the corresponding lactone. Nitrophenols are subject to loss of both $^{\cdot}OH$ radical and NO groups thereby forming the correspondent phenols and quinones. The performance of the method with respect to quantitation compares favourably with traditional methods. With the ion-trap run in a time scheduled single ion monitoring mode, typical limits of detection (LODs) are in the low pg range and the repeatability standard deviations are between 3 and 15%. Assuming extraction of 1-l water samples and 1 ml final volumes the injection of 50-μl aliquots corresponds to LODs well below the requirement for the European Union water directive (EC/80/778).

Keywords: Mass spectrometry; Pesticides

1. Introduction

The large potential in coupling the resolving power of liquid chromatography with mass spectrometry (LC–MS) has been developed into a robust analytical technique over the last few years. Today, LC–MS is a full-fledged analytical alternative where conventional gas chromatography (GC)–MS methods are not adequate due to high polarity, low volatility and thermal instability of the analytes [1].

With the recent development of atmospheric pressure ion sources [atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI)] and ion-trap multiple mass spectrometry (MS^n) it has become technically and economically feasible for many laboratories to analyse unknown polar compounds with LC–MS in a similar way to GC–MS run in the full scan mode.

*Corresponding author. Tel.: +39-332-785-453; fax: +39-332-785-704.

E-mail address: bo.larsen@jrc.it (B.R. Larsen)

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Although the atmospheric pressure ionisation (API) processes are not yet fully understood, it is known that liquid-phase chemistry plays a key role in the ion formation of ESI [2], whereas, APCI involves gas-phase chemistry [3]. Both APCI and ESI are soft ionisation techniques which yield the quasi-molecular ions, $[M+H]^+$ and $[M-H]^-$ in the positive and negative detection mode, respectively. Structural information on analytes can be obtained in single MS instruments by collision-induced dissociation (CID) with a suitable adjustment of the electrical field that exists in the intermediate-pressure desolvation chamber between the ionisation source and the mass analyser region (front-end CID). However, this technique is often unreliable and can suffer from a significant loss in sensitivity [4–7]. Alternatively, structurally significant ions can be obtained by triple-quadrupole MS in which the first quadrupole is used for selection of precursor ions, the second quadrupole is used for CID with e.g., argon as collision gas, and the third quadrupole is scanned for product ions. The high cost for triple-quadrupole instruments is the main disadvantage of this technique. With the recent introduction of the less expensive ion-trap (MS^n) mass spectrometer, CID can be obtained directly in the trap through resonant excitation followed by collisions with helium buffer gas atoms [8]. The present paper investigates the performance of an ion-trap for LC–MS analysis of pesticides in water.

With an ion-trap the MS–MS process can be repeated a number of times and should thus be the ideal tool for the investigation of fragmentation processes and pathways. This potential has been convincingly demonstrated by Kölloker et al. [9] in a detailed MS^n investigation of carbonyl derivatives of 2,4-dinitrophenylhydrazine, in which aldehydes could be differentiated from ketones, straight-chain from branched structures, and unsaturated from aromatic carbonyls. Until now, similar studies for pesticides have not been published.

The most common and best documented approach to yield fragment ions is electron impact ionisation (EI). This technique has been coupled with high-performance liquid chromatography (HPLC) in the particle beam (PB) interface. Molecular fragmentation can be standardised in EI by keeping the source conditions constant (e.g., 70 eV, 200°C, 10^{-3} atm; 1 atm=101 325 Pa) and highly reproducible fragmentation spectra can be obtained and stored in spectral libraries. However, the detection limits that can be reached with PB-MS are insufficient for environmental analysis at trace level. ESI and APCI offer much more sensitivity. Yet, molecular fragmentation is not easily standardised either by front-end CID or by tandem MS, simply because the amount of energy imparted into the analyte molecule cannot be controlled as accurately as in EI. Furthermore, as opposed to EI, which yields odd-electron molecular ions (OE), ESI and APCI produce even-electron molecular ions (EE). The decomposition processes and pathways of the latter are far from fully understood until now [10].

Modern pesticides and their degradation products are easily analysed by LC–API-MS [11–14]. From the vast number of pesticides in present use, we have focused the attention on phenylurea herbicides [15], triazines [16], carbamates [17], chlorinated phenoxy-acetic acids [18,19], and nitrophenols [20]. Since these compounds are readily soluble in water, their runoff into rivers and lakes pose several problems for the supply of clean drinking water. In the present work, the use of LC–MS^n is investigated for the analysis of acidic and neutral pesticides on the European Union Priority List [21]. The mass spectrometer was run with both the APCI and ESI ion source using an infusion inlet to speed-up the acquisition of MS^n fragmentograms. Negative as well as positive product ion fragmentograms were recorded in order to derive fragmentation processes and pathways. These include simple bond cleavages, cleavage with hydrogen transfer rearrangement and skeletal rearrangements including ring openings.

The European Union (EU) Directive (80/778/EC) states that individual pesticide concentration must not exceed the 0.1 μg/l level in water intended for human consumption. In order to achieve this detection limit, a quantitative analytical method for both acidic and neutral pesticides has been developed based on HPLC–APCI-ion-trap-MS and HPLC–ESI-ion-trap-MS with single ion monitoring (SIM) of quasi-molecular ions. The performance of this method is compared to published methods in which quadrupole instruments were interfaced with HPLC.

2. Experimental

2.1. Analytes and sample preparation

The pure analytes were purchased from Dr. Ehrenstorfer (Angsburg, Germany). Two different stock solutions containing the following pesticides: (1) metamitron, carbofuran, monuron, simazine, atrazine, isoproturon, diuron, phenmedipham, terbuthylazine and pendimethalin; (2) dicamba, bentazone, 2,4-D, bromoxynil, dinitrio-ortho-cresol (DNOC), 4-chloro-2-methylphenoxyacetic acid (MCPA), ioxynil, dichlorprop, mecoprop and dinoseb were prepared by dissolving 10 mg of each compound in 40 ml of methanol (gradient grade, Fluka). Standard solutions were stored at 5°C in the dark. Samples for the external calibration and MS^n studies were prepared in a mixture of methanol–Milli-Q water (Millipore) (1:1) by spiking with the appropriate amount of mother solution. The concentration range in the calibration studies varied from 5 to 500 μg/l.

2.2. Analysis

The LC–API-MS^n analyses were performed with a Thermo Separation gradient pump HPLC system coupled to a Finnigan MAT LCQ ion-trap mass spectrometer. The MS^n spectra were acquired using the infusion technique at 3 and 25 μl/min for ESI and APCI, respectively. The two pesticide mixtures were injected separately. Full scan MS spectra (50–450 m/z) were first recorded and the quasi-molecular ion of each compound was identified. Next, MS^2 spectra were recorded by isolating the quasi-molecular ion in the ion-trap followed by CID. The energy required in this process varied between 10 and 20% of the total available collision energy. This process was repeated up to four times (MS^4) by successive isolation of one of the generated ions (product ions) followed by CID to give MS^n.

For the analysis of neutral pesticides, an APCI source operating in the positive mode was employed. The following optimised parameter values were obtained: APCI vaporiser temperature 450°C; capillary temperature 150°C; source voltage 6 kV; capillary voltage +25 V; source current 5 μA; sheath gas (N_2) flow 80 (arbitrary units); auxiliary gas (He) flow 10 (arbitrary units).

For the analysis of acidic pesticides, a pneumatically assisted ESI source operating in the negative mode was used. The following optimised values were obtained: capillary temperature 265°C; spray voltage 4.2 kV; source current 100 μA; capillary voltage −24 V; sheath gas (N_2) flow 80 (arbitrary units); auxiliary gas (He) flow 9 (arbitrary units). The performance of the two methods with respect to quantitation was tested.

For the LC separation of carbamate, triazine and phenylurea pesticides, a mixture of Milli-Q water–methanol was used as mobile phase at a constant flow-rate of 1.0 ml/min. The HPLC system was equipped with a thermostatted (20±0.1°C), 25 cm× 4.6 mm I.D. column packed with a 5 μm Alltima C_{18} reversed-phase material (Alltech, Milan, Italy). The gradient was programmed from 50 to 80% methanol in 30 min and then up to 95% methanol in 15 min. The HPLC system was interfaced to the ion-trap through the APCI source operating in the positive mode. Mass spectra collected in full scan mode were obtained by scanning over the range from 50 to 350 m/z in 1 s. Time scheduled SIM conditions were as follows: LC time 0.00–8.67 min, m/z 203; LC time 8.67–14.57 min, m/z 199, 202 and 222; LC time 14.57–17.79 min, m/z 207 and 216; LC time 17.79–21.69 min, m/z 233 and 301; LC time 21.70–35.68 min, m/z 230; LC time 35.68–45.00 min, m/z 282. Total data acquisition time was 45 min.

Acidic pesticides were eluted with the same gradient program but using Milli-Q water acidified with acetic acid (1000:1) in place of neutral water at a constant flow-rate of 0.8 ml/min. The outlet of the HPLC system was split (3:1) to the ESI interface operating in the negative mode. Full scan mass spectra were obtained scanning the range from 50 to 350 m/z in 1 s. Time scheduled SIM conditions were as follows: LC time 0.00–10.39 min, m/z 219; LC time 10.39–14.32 min, m/z 239; LC time 14.32–19.85 min, m/z 197, 199, 219 and 278; LC time 19.85–24.43 min, m/z 213, 233 and 370; LC time 24.43–35.00 min, m/z 239. Total data acquisition time was 35 min.

3. Results and discussion

3.1. Qualitative results

Only scarce data is available on the structure of ions produced by MS^n of pesticides. To date, only the class of organophosphorus pesticides (OPPs) has been studied in detail by Itoh et al. [17]. These authors have proposed fragmentation schemes deriving from LC–APCI-MS using front-end CID. By employing both negative and positive detection modes according to the chemical properties of the pesticides, mass spectra were obtained and compared to mass spectra from GC–EI-MS and GC–CI-MS analysis. It was shown that, although the mass spectra obtained by APCI give a simpler pattern than those obtained with EI, the mass spectra were distinct enough to identify unknown peaks in the LC chromatogram. Owing to the high specificity of this method, the authors concluded that rapid analysis of known and unknown OPPs can be performed by LC–APCI-MS.

In the following we discuss in detail the obtained MS^n spectra of the investigated polar pesticides with the purpose of identifying typical (diagnostic) ions, fragmentation processes and dissociation pathways.

3.1.1. Triazines

In the MS spectra of triazines, the most abundant ions are the quasi-molecular ions $[M+H]^+$ formed during the soft ionisation process inside the APCI interface (Fig. 1 and Table 1). During this process, other ions can be obtained from the association of the target compound with solvent molecules. Typical adduct ions by APCI(+) when methanol is used as eluent are the Van der Waals clusters $[M+nCH_3OH+H]^+$, $[M+nH_2O+H]^+$ and $[M+nCH_3OH+mH_2O+H]^+$. We have observed such adducts with all analytes investigated including aldehydes, ketones, alcohols, epoxides, esters and multifunctional acids [22]. By using other solvents as eluent, similar adduct ions are formed during APCI of triazines, e.g., $[M+CH_3CN+H]^+$ from acetonitrile [23]. Nevertheless, the relative intensities of such clusters are not very high, and they can easily be differentiated from genuine quasi-molecular ions simply by running the ion-trap in SIM ($=MS^2$ with 0% CID energy) mode. Quasi-molecular ions appear with unchanged intensity, while adduct ions disappear completely. In the selection of ions during SIM, a small amount of electrostatic energy is applied (inside the ion-trap) to the selected ion. This energy is far from strong enough to dissociate covalent bonds, but just sufficient to disrupt the weaker bonds prevailing in adduct ions such as e.g., hydrogen bonds.

The MS^2 analysis of triazines with CID above 15% energy leads to fragment ions corresponding to cleavage of the largest lateral chain in the triazine ring. In all cases, the ion at 174 m/z is the base peak. A mechanistic pathway is proposed in Fig. 2A. One hydrogen atom of the methyl group in β-position to the amino group can migrate to the positively charged nitrogen leading to the loss of a disubstituted ethylene molecule. The proposed pathway is in accordance with Stevenson's rule [10] stating that an EE^+ ion upon CID preferentially fragments into a neutral molecule and another EE^+ ion following an internal proton migration. The charge is retained at the atom with the highest proton affinity.

In the further step (MS^3 of the precursor ion at 174 m/z), two different dissociation processes are observed. The major pathway is similar to the one described above and leads to formation of the most intense ion of 146 m/z through the loss of $CH_2=CH_2$ from the N-ethyl lateral chain (Fig. 2B). The other pathway results in ring opening rearrangements and leads to formation of a relatively intensive ion at 132 m/z and a weak ion at 96 m/z (Fig. 2C and D). By MS^3 analysis of the precursor ion at 176 m/z (containing the chlorine isotope ^{37}Cl), it was confirmed that the ions at 146 and 132 m/z retain the chlorine atom, as opposed to the minor ion at 96 m/z.

The spectra derived from MS^4 of the ion at 132 m/z contain only a peak at 104 m/z formed by the loss of $CH_2=CH_2$ from the N-ethyl group as suggested in Fig. 2E. Likewise, from the precursor ion at 146 m/z, the fragment ion at 110 m/z is the only peak in the MS^4 spectra. In Fig. 2F a pathway is proposed through the neutral loss of HCl with a consequent ring opening. MS^4 analysis of the ^{37}Cl containing isotopic form of the precursor ion supports the proposed pathway (loss of $H^{37}Cl$).

In a recent elegant study of the photochemical behaviour of pesticides, Volmer [7] has proposed

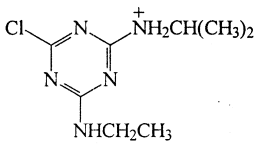
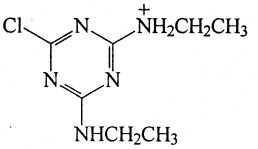
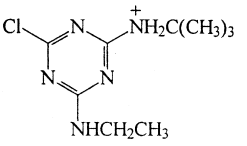
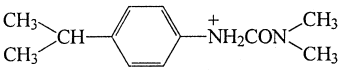
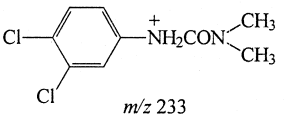
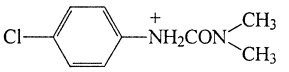
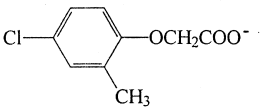
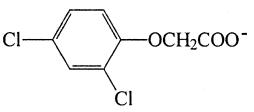
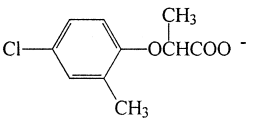
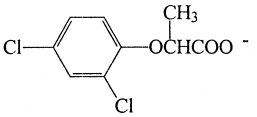
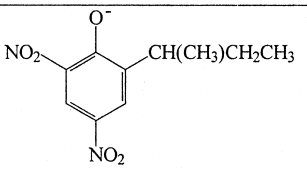
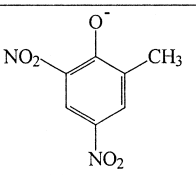
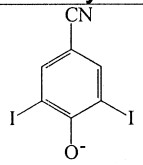
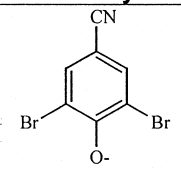
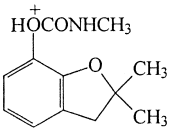
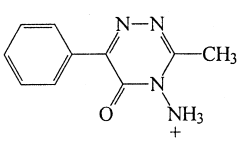
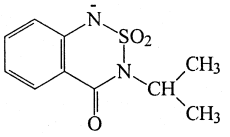
Atrazine	Simazine	Terbuthylazine
 *m/z* 216	 *m/z* 202	 *m/z* 230
Isoproturon	**Diuron**	**Monuron**
 *m/z* 207	 *m/z* 233	 *m/z* 199
MCPA	**2,4-D**	**Mecoprop**
 *m/z* 199	 *m/z* 219	 *m/z* 213
Dichlorprop	**Dinoseb**	**DNOC**
 *m/z* 233	 *m/z* 239	 *m/z* 197
Ioxynil	**Bromoxynil**	**Carbofuran**
 *m/z* 370	 *m/z* 276	 *m/z* 222
Metamitron	**Bentazone**	
 *m/z* 203	 *m/z* 239	

Fig. 1. Structures and quasi-molecular ions for the pesticides studied.

Table 1
Product ions derived from multiple mass spectrometric analysis of pesticides (relative ion intensity, %, is reported in parentheses)

Compound	MS (m/z)	MS^2 (m/z)	MS^3 (m/z)	MS^4 (m/z)
Atrazine	216	174 (100)	96 (28)	
			132 (100)→	104 (100)
			146 (92)→	110 (100)
Simazine	202	174 (100)	96 (21)	
			132 (100)→	104 (100)
			146 (68)→	110 (100)
Terbuthylazine	230	174 (100)	96 (11)	
			132 (82)→	104 (100)
			146 (100)→	110 (100)
Isoproturon	207	72 (100)		
		165 (53)→	72 (100)	
			120 (44)→	92 (100)
Diuron	233	72 (100)		
		163 (2)		
		176 (80)		
		205 (5)		
		218 (2)		
Monuron	199	72 (100)		
		142 (50)		
		171 (80)		
		184 (40)		
MCPA	199	141 (100)		
2,4-D	219	161 (100)		
Mecoprop	213	141 (100)		
Dichlorprop	233	161 (100)	125 (100)	
Dinoseb	239	194 (55)		
		197 (100)		
		209 (10)→	179 (100)	
		222 (20)→	192 (100)	
DNOC	197	167 (100)→	137 (100)	
		180 (10)		
Ioxynil	370	127 (100)		
		243 (6)		
Bromoxynil	276	79 (100)		
Carbofuran	222	165 (100)	123 (100)→	95 (100)
			137 (8)	
Metamitron	203	175 (100)→	106 (60)	
		186 (5)	134 (11)	
			158 (100)	
Bentazone	239	175 (100)→	132 (100)	
		197 (93)		

structures for the CID fragment ions observed from atrazine using a triple-quadrupole mass spectrometer for LC–ESI-MS–MS analysis. The collision energy obtainable with argon as collision gas with this technique is considerably higher than the energy obtainable with helium as collision gas in an ion-trap. Thus, a higher degree of fragmentation is observed. It is interesting to note the APCI–ion-trap-MS^n system used in the present study matches the performance of the ESI-triple-quadrupole-MS–MS system. Hence, all the fragments observed in the study of Volmer were also observed in the present study except for a minor fragment at 79 m/z, possibly deriving from double cleavage of the triazine ring to form $[H_2N\text{-}C(NH)\text{-}Cl+H]^+$. Moreover, the fragment structures proposed by Volmer can be explained by the mechanistic pathways deduced from the present MS^n analysis. A good example is the major fragment ion at 146 m/z obtained by ESI-triple-quadrupole-MS–MS of atrazine, which may derive from cleavage of the lateral chains in the triazine ring or from cleavage of the shortest side-ring followed by ring opening. This ion was observed by APCI-ion-trap-MS^3 analysis of all triazine pesticide regardless of the type of lateral chains. MS^4 analysis points to the structure with an intact triazine ring according to Fig. 2F, rather than an open structure (further loss of HCl is seen but not of the shortest side-chain).

Since triazine pesticides are easily analysed also by GC–MS, a comparison between the fragmentation patterns deriving from the different techniques is feasible. Vincze and Yinon studied CID mass spectra with GC–ion-trap tandem MS using both EI and CI [24]. Given that the collision energy with these techniques is higher than with the API-MS, a higher degree of fragmentation is obtained. However, it is interesting to observe that the same characteristic ions from the class of triazines reported in the EI and CI spectra can be obtained with APCI-MS^n (in this study), with the only difference that the latter ionisation technique yields a protonated form of the fragments. For example, the ion at 174 m/z in the APCI-MS^n has its corresponding ions at 173 and 172 m/z in the EI-MS^n and CI-MS^2 deriving from the loss of $-C_2H_4$ and $-C_2H_5$. Furthermore, fragments with a positive charge located on a carbon atom (e.g., the ion at 96 m/z) can be found with APCI-MS^n as

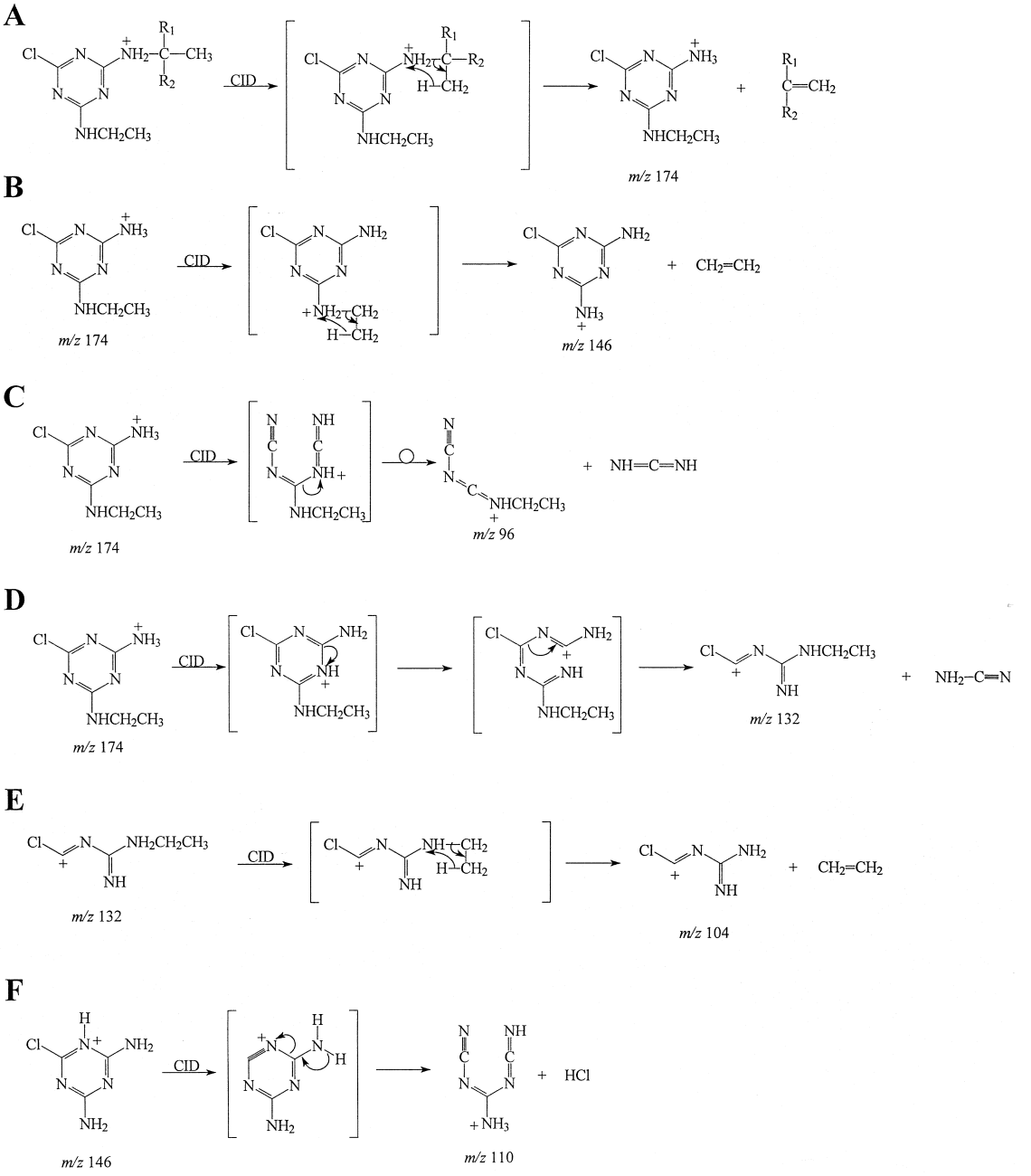


Fig. 2. Fragmentation pathways for triazine herbicides.

well as with EI-MS^n and CI-MS^n. The proposed structure for the ion at 96 m/z in the GC–MS^n study is one of the possible resonant structures. The same structure has been adopted by Volmer [7]. The structure we propose in the present study is another possible, more probable, resonance form.

3.1.2. Phenylureas

The most abundant ions in the MS spectra of phenylurea pesticides are the quasi-molecular ions $[M+H]^+$ (Fig. 1 and Table 1).

In the MS^2 spectra, the most intensive peak is at 72 *m/z*. This fragment ion is characteristic to phenylurea pesticides and has previously [1] been assigned the structure $[OCN(CH_3)_2]^+$, which is stabilised by resonance between three different structures and is formed by CID together with a substituted aniline as a neutral molecule according to Stevenson's rule as shown in Fig. 3A. Besides this diagnostic ion, other fragments are obtained. For isoproturon, the relatively intense ion at 165 *m/z* is tentatively identified as shown in Fig. 3B. The formation pathway is similar to the pathway proposed for cleavage of lateral chains in triazines via migration of a hydrogen atom in the β-position to a positively charged atom. As indicated in Fig. 3B, the positive charge in the protonated isoproturon molecule is only partial (δ+), formed through a dislocation of the π-electrons of the aromatic ring. This formation pathway is also postulated by Yinon and Vincze [25] in their studies on the CID processes of phenylurea pesticides using GC–ion-trap MS–MS. These authors found that the formation of rearrangement product ions vs. cleavage product ions is dependent on the CID voltage and CID time. Since, CID in an ion-trap is typically performed by the application of a low-voltage across the end-cap electrodes at the resonance frequency of the ion of interest, rearrangement processes are favoured. Moreover, the time between collisions is sufficiently long for ions with enough energy to dissociate through low-energy pathways before another activation step occurs. Our results are in accordance with these observations; in fact, as shown in Table 1, the intensity of the product ion at *m/z* 218 for diuron, corresponding to a homolytic cleavage of the $N–CH_3$ bond, is less than that of the product ion at *m/z* 176 deriving from the migration of a methyl group to the nitrogen atom. The same behaviour is observed for monuron, which differentiates from diuron only by a chlorine atom on the benzene ring. Other product ions formed via rearrangement pathways are at *m/z* 171 and *m/z* 205 for monuron and diuron, respectively. They derive from the migration of two hydrogen atoms of the methyl group to the positively charged nitrogen with subsequent loss of ethylene (Fig. 3E).

It is interesting to note that for diuron, loss of Cl_2 occurs with formation of a triple bond inside the ring. Since this structure is highly unstable, the intensity of this product ion is very low (2%) (Fig. 3G).

The MS^3 spectrum of the precursor ion at 165 *m/z* contains the characteristic ion at 72 *m/z*, $[OCN(CH_3)_2]^+$ with a relative abundance of 100%. Also a relatively intensive ion at 120 *m/z* is seen, explained by the loss of dimethylamine, $NH(CH_3)_2$ to form a protonated benzylisocyanate ion as depicted in Fig. 3C. When this ion is subjected to fragmentation (MS^4) a loss of CO occurs followed by formation of a carbocation stabilised by resonance (Fig. 3D).

3.1.3. Phenoxyacids

2,4-D, MCPA, dichlorprop and mecorprop have the same skeletal structure. The differences lie in the substituent in the 2-position of the ring (methyl or chlorine) and in the carbon in the β-position to the carboxylic function (hydrogen or methyl). Therefore, their fragmentation behaviour in the ion-trap is quite similar.

The MS spectra show the quasi-molecular ion $[M-H]^-$ as base peak (Fig. 1 and Table 1). Besides this, also adduct ions are observed, the most intensive ones coming from the acetate in the eluent, $[M+CH_3COO]^-$. By ESI(−) analysis of multifunctional carboxylic acids, we have previously observed similar adduct ions [22]. They are easily recognised and rather than posing problems, they can be helpful in differentiating between steric isomers and may enhance the ESI response of some compounds [22].

The MS^2 spectra of the quasi-molecular ions of phenoxyacid pesticides show only one product ion corresponding to *m/z* 141 for mecoprop and MCPA, and *m/z* 161 for 2,4-D and dichlorprop. Fragment ions at *m/z* 141 and *m/z* 161 have also been observed from phenoxyacids by ESI-triple-quadrupole-MS–MS with argon as collision gas [26]. As a pathway for these fragment ions, we propose a cleavage of the carboxylic group thereby forming the corresponding phenolate ions and a lactone as a neutral loss (Fig. 4A). In the MS^3 analysis of the dichlorinated phenolate ions from 2,4-D and dichlorprop, ring opening occurs from neutral loss of HCl. This process is probably a single-step reaction but may be perceived as a two-step reaction as

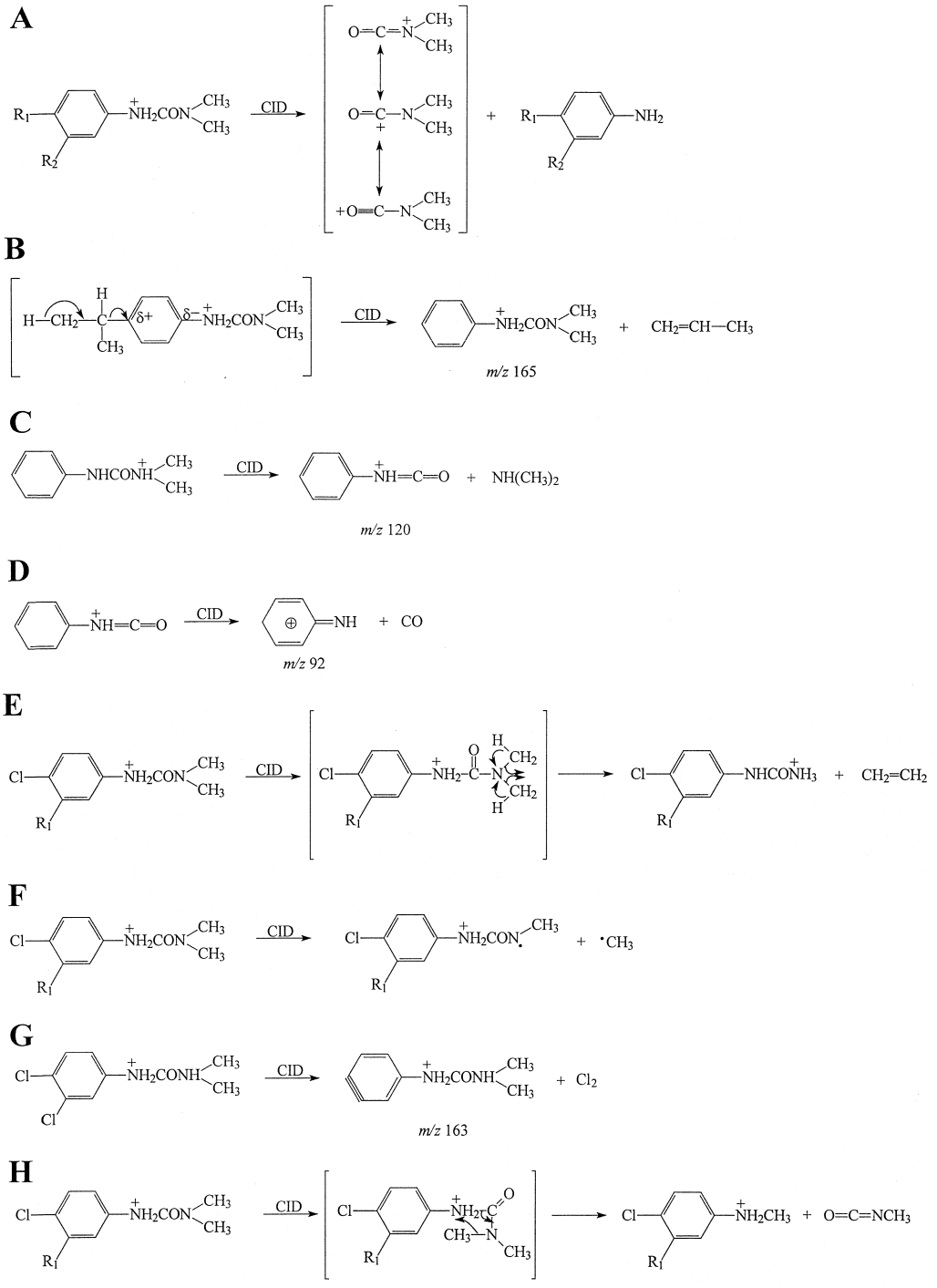


Fig. 3. Fragmentation pathways for phenylurea herbicides.

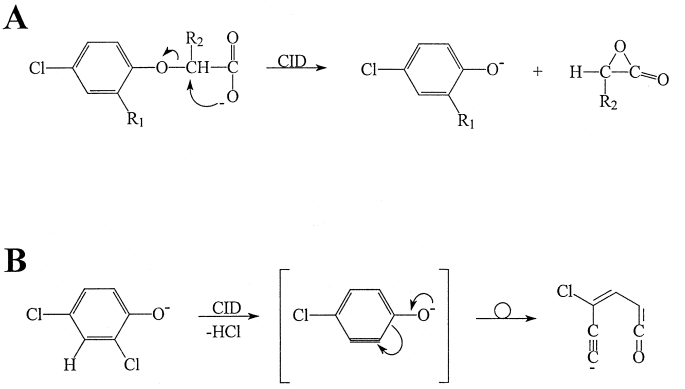


Fig. 4. Fragmentation pathways for phenoxyacetic acid herbicides.

depicted in Fig. 4B. First, the neutral loss of HCl creates a triple bond inside the ring. Since this structure is highly unstable, the ring opens and through a subsequent bond rearrangement a resonance stabilised carbanion is formed.

3.1.4. Nitrophenols

Dinoseb and DNOC give a strong signal as $[M\text{-}H]^-$ by ESI (Fig. 1 and Table 1). The MS^2 spectra show the loss of $NO^{\cdot}$ yielding the corresponding quinone, where the unpaired electron and the negative charge are delocalised in the ring. The fragment ion at $[M\text{-}NO]^{-\cdot}$ is characteristic to nitro compounds as also reported in the literature [9,20]. The mechanism for the formation of $NO^{\cdot}$ is unclear. Probably, the process is a single-step reaction where the electron in the negatively charged oxygen of the nitro group attacks the carbon in the ring, followed by a homolytic cleavage of the C–N bond. This results in the formation of the radical $NO^{\cdot}$ and, after structure rearrangement, of a negatively charged quinone radical. The $NO^{\cdot}$ loss is also observed in the MS^3 spectra of the ion at m/z 209 and 167 for dinoseb and DNOC, respectively (Table 1). In this case, the resulted product ions at m/z 179 and m/z 137 retain only the negative charge in accordance with the nitrogen rule for even-electron ions [10].

The MS^2 spectra contain other product ions corresponding to $[M\text{-}OH]^{-\cdot}$ (m/z 222 and m/z 180 for dinoseb and DNOC, respectively). The loss of an $^{\cdot}OH$ radical has been observed by Astratov et al. [20] in their studies on the identification of pollutants in ammunition hazardous waste sites by discharge thermospray HPLC–MS. In their MS spectra, besides the loss of one or two $NO^{\cdot}$ depending on the number of nitro group present in the molecule, they observed the loss of $^{\cdot}OH$ radical only for a few compounds. Such compounds are characterised by the presence of a hydroxyl group in the *ortho* position to an alkyl or amino moieties. However, neither a structure nor a formation pathway for these product ions was suggested. A possible mechanism is here postulated: first the electron of the negatively charged oxygen attacks the hydrogen atom of the alkyl group in the *ortho* position (methyl for DNOC and 1-methylpropyl for dinoseb). Then, a homolytic cleavage of the C–O bond occurs followed by structure rearrangement.

The product ions at m/z 197 and m/z 194 for dinoseb are formed through the cleavage of the lateral chain yielding the neutral molecules 1-propene and CH_3NO and the correspondent nitrophenolates.

3.1.5. Halogenated phenols

Ioxynil and bromoxynil belong to this class of pesticides with halogen atoms in their structures (Fig. 1 and Table 1). They are analysed as the $[M\text{-}H]^-$ ions in ESI(−). The characteristic fragmentation process is the loss of halogen substituent from the ring. For example, the MS^2 spectrum of ioxynil shows the iodine anion at m/z 127 as base peak and the product ion at m/z 143 corresponding to $[M\text{-}H\text{-}I]^{-\cdot}$. The same fragmentation pattern for bromoxynil is observed where the bromine anion at

m/z 79 represents the highest peak in the MS^2 spectra.

3.1.6. Carbamates

Carbofuran is studied as a representative of the class of carbamates. In the MS spectrum, the quasi-molecular ion at 222 m/z is the most intensive peak (Fig. 1 and Table 1).

The MS^2 spectrum presents only a product ion at 165 m/z deriving from the neutral loss of the $CONCH_3$ group. MS studies of other carbamate pesticides have reported identical product ions [15] and it seems evident that the ion $[M\text{-}CONCH_3+H]^+$ is characteristic for N-methylcarbamate pesticides.

In the further step (MS^3 of the ion at 165 m/z), two product ions are formed. The most abundant is at 123 m/z, resulting from the 5-atom ring opening (Table 1). The structure of the latter ion at 137 m/z maintains the two rings, while two hydrogen atoms migrate to the carbon in the ring giving rise to neutral loss of ethylene.

When the ion at m/z 123 is subjected to fragmentation in MS^4 step, protonated phenol is formed with subsequent CO loss.

Two other compounds not belonging to the above classes were also studied. Table 1 reports the product ions for metamitron and bentazone and their relative intensities. The fragmentation pathway for bentazone follows cleavage of lateral chains as described for, e.g., the triazines. A peculiar mechanism for bentazone and metamitron is the transformation of a six-member ring into a five-member ring probably through an initial ring opening.

3.2. Quantitative results

Calibration curves were determined by external calibration in the concentration range from 5 to 500 μg/l in injected solutions, corresponding to 0.25–25 ng. Standard solutions were injected three times over 3 working days. Regression lines were calculated by averaging the three calibration curves obtained each day. As shown in Tables 2 and 3, the linearity is good for all pesticides with correlation coefficients (r^2) higher than 0.99. It is noteworthy that for bentazone, DNOC, ioxynil and dinoseb convex curves are obtained over the range studied (i.e., two-orders of magnitudes). Other authors have observed this behaviour [19] and probably the phenomenon is related to the ion formation processes of electrospray. However, at present, the mechanism leading to this non-linearity is unknown. Rather than using an arbitrarily chosen non-linear function for calibration for these pesticides, we have elected to determine linear regression lines in a limited concentration range, e.g., from 5 to 50 μg/l.

Since blank samples give no signal in the SIM analysis, the regression lines shown in Tables 2 and

Table 2
HPLC–APCI-MS data: detection limits, linearity and instrument precision (SIM ions were used for quantification)

Compound	Parent ion	Calibration equation ($y=bx+c$)[a]	r^2	Detection limit (pg)		Instrument precision (RSD, %)	
				MDL[b]	ML[c]	Repeatability[d]	Reproducibility[e]
Metamitron	203	$y=189\,173x-83\,987$	0.998	100	320	7.2	8.4
Carbofuran	222	$y=258\,138x+192\,220$	0.995	80	250	5.5	5.0
Monuron	199	$y=58\,547x+304\,705$	0.994	120	370	8.5	17
Simazine	202	$y=153\,097x-121\,277$	0.998	40	130	3.0	2.8
Atrazine	216	$y=252\,187x+100\,790$	0.998	110	350	8.2	6.6
Isoproturon	207	$y=119\,465x+16\,504$	0.995	110	340	7.8	17
Diuron	233	$y=47\,836x-92\,224$	0.997	200	630	14	20
Phenmedipham	301	$y=27\,811x-30\,016$	0.999	150	460	13	13
Terbuthylazine	230	$y=302\,370x+201\,281$	0.999	40	140	3.1	4.5
Pendimethalin	282	$y=174\,271x+194\,976$	0.988	200	640	8.1	26

[a] r^2 is the correlation coefficient, x is the injected concentration in μg/l and y is the peak area.
[b] MDL is the method detection limit defined as 3.14 standard deviation.
[c] ML is the interium minimum level defined as 10 standard deviation.
[d] Repeatability was calculated on the basis of six replicates at 10 μg/l injected within day.
[e] Reproducibility was calculated on the basis of three replicates at 10 μg/l injected on different days.

Table 3
HPLC–ESI-MS data: detection limits, linearity and instrument precision (SIM ions were used for quantification)

Compound	Parent ion	Calibration equation	r^2	Detection limit (pg)		Instrument precision (RSD, %)	
		($y=bx+c$)[a]		MDL[b]	ML[c]	Repeatability[d]	Reproducibility[e]
Dicamba	219	$y=10\,138x-42\,170$	0.999	330	1100	10	3.6
Bentazone	239	$y=129\,855x+257\,790$	0.999	110	340	5.7	3.6
2,4-D	219	$y=21\,564x+20\,575$	0.999	390	1240	7.3	16.
Bromoxynil	276	$y=17\,931x+226\,045$	0.997	90	300	3.9	2.8
DNOC	197	$y=66\,609x+199\,127$	0.998	70	210	6.2	7.1
MCPA	199	$y=29\,573x+43\,408$	0.998	130	420	9.4	19
Ioxynil	370	$y=138\,286x+50\,380$	0.999	60	200	3.7	5.1
Dichlorprop	233	$y=34\,783x+118\,368$	0.998	90	290	8.4	6.7
Mecoprop	213	$y=35\,567x+13\,398$	0.999	100	320	7.6	7.0
Dinoseb	239	$y=231\,520x+582\,784$	0.999	100	320	4.9	4.5

[a] r^2 is the correlation coefficient, x is the injected concentration in μg/l and y is the peak area.
[b] MDL is the method detection limit defined as 3.14 standard deviation.
[c] ML is the interium minimum level defined as 10 standard deviation.
[d] Repeatability was calculated on the basis of six replicates at 10 μg/l injected within day.
[e] Reproducibility was calculated on the basis of three replicates at 10 μg/l injected on different days.

3 are valid only for the concentration range studied. In particular extrapolation to lower concentrations should not be done.

The intra-day precision (i.e., repeatability) was estimated by injecting standard solutions containing all pesticides at 10 μg/l six times during a working day. The inter-day precision (i.e., reproducibility) was evaluated by analysing standard solutions at 10 μg/l three times over three working days. The repeatability and reproducibility for all pesticides were within the range of 3 to 20%.

The sensitivity of the analytical procedure can be evaluated in terms of the method detection limit (MDL) and the interium minimum level (ML) defined as 3.14- and 10-times the standard deviation, respectively [27]. Since these parameters depend on the spiking concentration, a rigorous approach is used. The US Environmental Protection Agency (EPA) guidelines require that the ratio of spiking concentration to estimated MDL should be lower than 5:1. If the ratio is greater, the spiking concentration should be iteratively reduced until the criterion is achieved. According to the above-mentioned criterion, the spiking concentration selected was 10 μg/l (500 pg) for all pesticides. Detection limits were in the range of 30 to 350 pg (expressed as injected amounts), which compares well with detection limits reported for LC–API-triple-quadrupole-MS [16,18,28]. Assuming quantitative extractions of 1 l water samples (e.g., by solid-phase extraction with graphitised carbon black [29]) and final volumes of 1 ml, the injection of 50-μl aliquots corresponds to method detection limits well below the requirement in the EU water directive (80/778/EEC). As an example the SIM chromatogram of extracted tap water spiked at 1 μg/l with neutral pesticides is shown in Fig. 5. This concentration level is 100 times lower than the requirement in the EU water directive and demonstrates the high sensitivity, which can be obtained with LC–API-ion-trap-MS.

This analytical method has been employed for the identification and analysis of photo-transformation products of pesticide-related molecules, both in the presence of humic acids and in river and lake water [30,31]. The product identification was performed by applying the mechanistic schemes derived from this work. The quantification study was done also in presence of 5 mg/l of humic substances with no significant problems deriving from matrix-effect.

4. Conclusion

A LC–API-ion-trap-MS^n method has been developed for the analysis of acidic and neutral pesticides representing all major classes of these compounds.

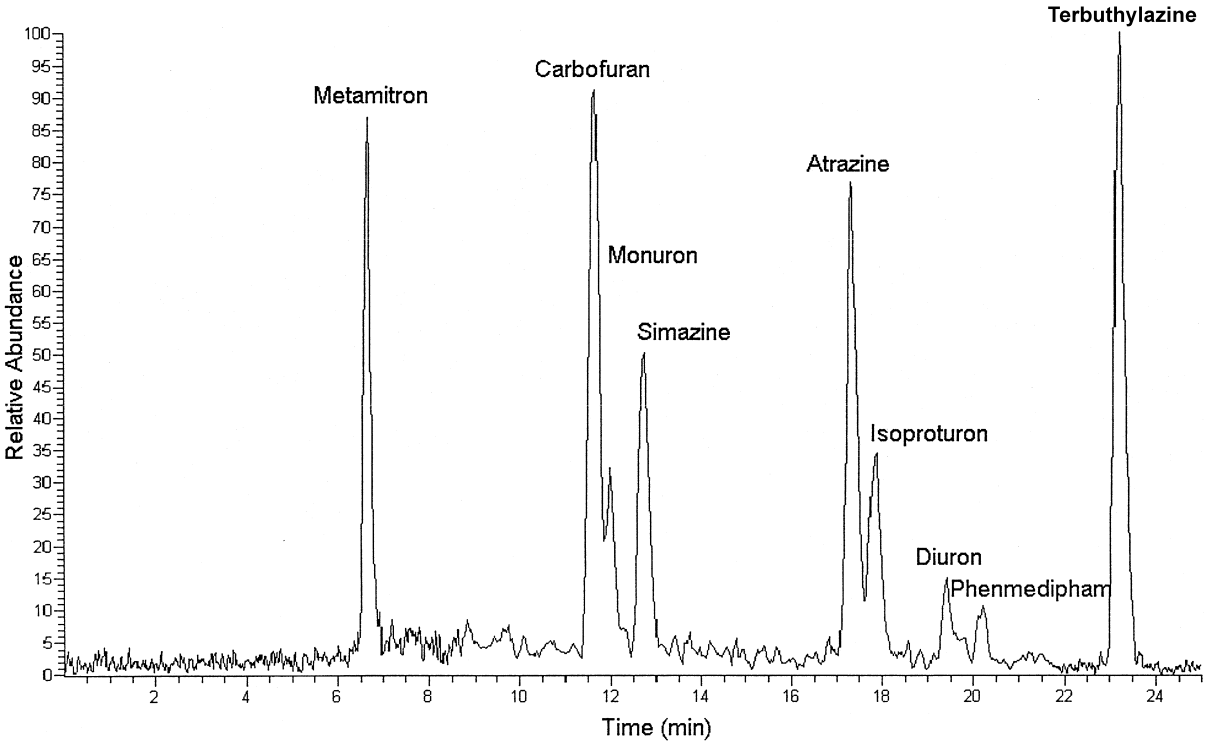


Fig. 5. SIM chromatogram obtained by LC–APCI-MS of a water solution of neutral pesticides spiked at 10 μg/l (injected concentration).

The fragmentation processes and pathways have been studied in detail to facilitate the identification of unknown pesticides. For each class of pesticide diagnostic product ions have been tentatively identified.

The performance of the method with respect to quantitation is satisfactory. Typical detection limits (MDLs) are in the pg range and the repeatability lies between 3 and 10%.

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