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Enhanced Selectivity in the Determination of Triazines in Environmental Samples by Benchtop CGC-MS-MS

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Summary

Some triazines and metabolites have been analyzed in water and soil samples, at the 0.1 and 10 ppb level, respectively, by CGC-MS and CGC-MS-MS using an ion trap detector (ITD). The sample preparation consisted of SPE on LiChrolut EN cartridges for water samples, and of ultrasonic treatment followed by SPE for soil samples. Recoveries were in the order of 90%. The advantage of MS-MS compared to MS operation of the ITD, is the enhanced selectivity resulting in more precise determinations. The metabolites of atrazine and metribuzin have also been studied. Desethylatrazine and desamino-metribuzin are the main metabolites.

1 Introduction

Triazine herbicides are among the most widely used pesticides in agriculture. Because of their relatively high water solubility, triazine and metabolite residues are frequently detected in environmental samples, *e.g.* drinking-, ground-, surface-waters, and soil, and in food products. They therefore constitute a high potential risk for human beings and animals.

The analysis of triazines and metabolites is routinely performed by HPLC [1–10], capillary GC [1,10–12], or SFC [10,13,14]. For sample preparation, traditional extraction methods such as liquid-liquid and solid-liquid extraction are being increasingly replaced by solid phase extraction (SPE) on octadecyl silica or polymeric material. Based on SPE, several on-line pesticide monitoring HPLC systems have recently been introduced [5–8] in which the pesticide residues are enriched by SPE followed by on-line mobile phase desorption and analysis on an ODS column. Detection is normally performed by UV or UV/DAD. The combination with MS has also been described [9], but to our knowledge, this approach is not really applied in routine analysis. In environmental laboratories, LC-MS systems are indeed considered much less user friendly compared to their capillary GC-MS counterparts. Recently, on-line SPE-SFC-UV/DAD has been applied to the analysis of pesticide residues in water samples offering, in comparison to LC, shorter analysis times and more selectivity because of the less polar and tunable nature of the desorption fluid [13,14]. SFC chromatograms, in general, are much “cleaner” than LC chromatograms because polar and ionic solutes such as humic acids, detergents, *etc.* are absent. Nevertheless, the main problem, both in SPE-HPLC-UV/DAD and in SPE-SFC-UV/DAD, is the low specificity of UV detection. Al-

though solute identification and peak purity evaluation can be performed by UV/DAD, interferences with matrix compounds are possible which can lead to false positive results, and this mainly in the sense of overestimation of the concentration [10]. Calibration is of utmost importance here, especially for heavily contaminated or complex environmental samples. Considering the above mentioned shortcomings, as a rule of thumb capillary GC-MS is applied whenever possible for pesticide analysis in our laboratories. A large number of pesticides are indeed found to be amenable to capillary GC analysis [1,11,12,15]. In fact, using state-of-the-art capillary GC, only phenylurea and ionic species, *e.g.* chlormequat, cannot be properly analyzed by GC because of their thermolability or involatility

The most important features of mass spectroscopic detection are its high sensitivity and compound specificity. On the one hand, the identities of the solutes can be elucidated by comparing their mass spectra with library spectra (MS operated in the full scan mode); on the other hand by selecting specific ions for integration (IM or ion monitoring mode), accurate quantification is obtained. Concerning the sensitivity, there is a lot of controversy between the performances of bench top quadrupole mass spectrometers, and ion trap detectors. Both systems have advantages and disadvantages and it is not the aim of this contribution to discuss these aspects, because the selectivity difference between ITD MS and ITD MS-MS is highlighted. In ion monitoring, detectabilities in the order of 1 pg can be reached but spectral information is lost. Upon analyzing real samples, interferences due to coeluting matrix compounds often occur, causing overestimation of the quantities of environmental target compounds. This is especially true nowadays, because the tendency is to simplify the sample preparation step, *i.e.* by applying solid phase extraction, in which the risk of enriching matrix compounds as well is much higher than with the more tedious sample preparation methods such as liquid-liquid extraction followed by silica and florisil clean-up, *etc.* More and more, for quantification of priority pollutants, recording full scan spectra is advised and, in some countries, even required. Ion trap detectors in general offer higher sensitivities in the full scan mode than quadrupole mass spectrometers, *i.e.* 10 and 100 pg, respectively, but here also, when analyzing real samples, the value of the spectral information is often limited by interferences of matrix ions.

Compared to standard MS operation modes (full scan or ion monitoring), MS-MS on the other hand offers higher selectivity which can be exploited to identify and quantify compounds at low concentration levels in complex matrices with higher precision. The background of the sample matrix is drastically reduced by excluding all ions, except the selected parent ion or window of parent ions. Only the parent ion(s) is fragmented into characteristic product ions by collision induced dissociation. MS-MS operation involves three steps: ion isolation, collision induced dissociation and analysis of the product ions. Triple quadrupole instruments have been developed for the purpose of MS-MS, but the recent introduction of inexpensive bench top ion trap detectors with MS-MS capabilities [16–20] has opened the technique for routine application in environmental laboratories. The ion trap detector could be transformed into a MS-MS system by using a well defined time programmed RF scan on the ring electrode with additional waveforms applied to the end cap electrodes.

The capabilities of ion trap MS-MS, in comparison to ion trap MS, have been studied for the elucidation and quantification of atrazine and its metabolites desethyl- and desisopropyl-atrazine, simazine, and metribuzin, in water and soil samples spiked at 0.1 and 10 ppb, respectively. The formation of metabolites of atrazine and metribuzin has been followed over a three week period.

2 Experimental

2.31 Materials

The triazines, atrazine, simazine, and metribuzin, and the atrazine metabolites, desethylatrazine and desisopropylatrazine were obtained from Dr. Ehrenstorfer, Germany. The structures, molecular weights and most abundant ion in electron impact MS are given in **Figure 1**.

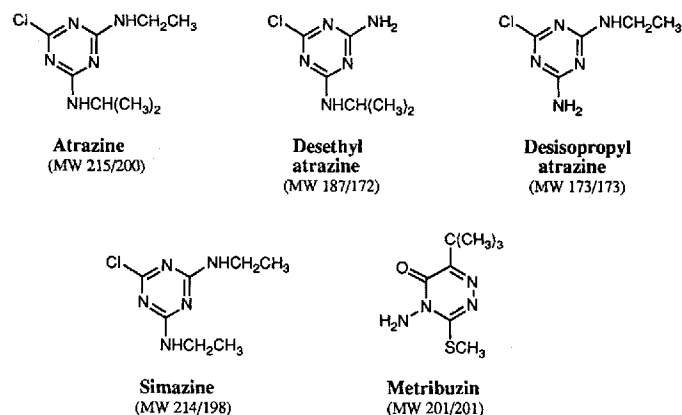


Figure 1. Structures of the pesticides studied with molecular weight and most abundant ion in EI-MS.

The following samples were prepared: (a) HPLC grade water (Labsan, Ireland) spiked with the five pesticides at the 100 ng per liter level (0.1 ppb); (b) rain water collected in April and spiked as (a); (c) water collected from the river Leie, Kortrijk, Belgium, in April; (d) a soil sample collected in April and spiked with the five pesticides at the 10 µg per kilogram level (10 ppb). The samples a, b, and d were analyzed before spiking and were

not contaminated by the pesticides studied in the levels 5 ppt for the water samples and 0.1 ppb for the soil sample. For studies on the metabolism, two additional samples were prepared: (e) rain water spiked at the 100 ppb level with atrazine and metribuzin, and (f) soil spiked at the 1 ppm level with atrazine and metribuzin. The samples were analyzed immediately after preparation and after three weeks. They were stored at ambient temperatures in clear open bottles and, by placing them outside the laboratory, they were exposed to air and sunlight.

2.2 Sample Preparation

The water samples were prepared by solid phase extraction (SPE) on LiChrolut EN 200 mg cartridges (Merck, Germany). 250 ml sample was passed through the cartridges at a flow of 10 ml/min. After drying for 15 min under vacuum, the compounds were eluted with 5 ml ethyl acetate. The extract was concentrated in a gentle stream of nitrogen and adjusted to a final volume of 1 ml with hexane (Pestigrade, Labsan, Ireland).

Extraction of the soil samples was performed by ultrasonic treatment of 1 g with 10 ml acetone during 30 min. The extract was centrifuged and the supernatant concentrated under nitrogen. The residue was taken up in 100 ml water and the water sample was extracted by SPE as discussed.

2.3 Chromatographic Analysis

Analyses were performed on a Varian 3400Cx gas chromatograph equipped with splitless injection and a Saturn 4 ion trap detector. 1 µl samples were injected splitless at a temperature of 250 °C with a splitless time of 60 s on a DB-5MS column 30 mL × 0.25 mm i.d. × 0.25 µm film thickness (J&W, USA). The oven temperature was programmed from 50 °C (1 min) to 270 °C at 10 °/min. The transfer line was set a 280 °C and the ITD settings were as follows: mass range 50–250 *m/z*, scan time 1 s, Fil/Mul delay 5 min, CID mode for MS-MS non resonant, CID time 20 ms, CID amplitude 30 V. Two modes of operation have been applied: full scan electron impact MS and MS-MS. For MS-MS operation, a parent ion or a window of ions was selected. Since the software only allows the selection of one parent ion (or one window of ions) at a given time, a time program was used with three segments each recording a different parent ion for the solutes eluting in the indicated window. The GC-MS-MS segment program is presented in **Table 1**.

Table 1. CGC-MS-MS segment program.

Segment (min)	Time (min)	Parent ion	Mass window	Pesticide	MS-MS ions
1	0–15.4	173	2	desisopropyl-atrazine	173, 158, 145
1				desethyl-atrazine	172, 159, 145
2	15.4–16.6	201	2	simazine	201, 200, 186, 173, 138
				atrazine	200, 172, 138
3	16.6–25.0	198	2	metribuzin	198, 110

3 Results and Discussion

In the first instance, the blank water sample spiked with 0.1 ppb of the pesticides (sample a), which corresponds to the threshold level for drinking water set by the European Community [21] was analyzed by conventional CGC-MS with the ion trap detector operated in the full scan mode. Using a 250 ml sample for SPE enrichment and concentration of the extract to 1 ml, the final concentration was 25 pg/ μ l for each solute. From this solution, 1 μ l was injected in the splitless mode. The resulting chromatogram is shown in **Figure 2**.

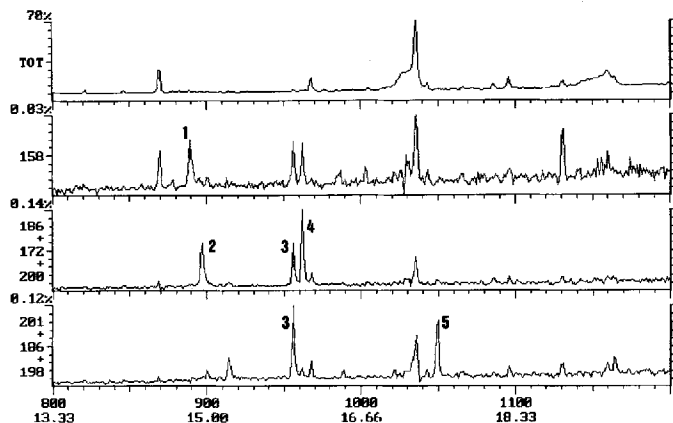


Figure 2. CGC-MS analysis of spiked (0.1 ppb) blank water sample. Peaks 1. desisopropylatrazine, 2. desethylatrazine, 3. simazine, 4. atrazine, 5. metribuzin.

At the 25 pg concentration level, the compounds are difficult to detect in the total ion chromatogram (top trace). Using extracted ion chromatograms, on the other hand, all target compounds could easily be found and even identified by automated library search because the recorded spectra were close-fitting the library spectra (first hit). This demonstrates the sensitivity of the ion trap detector in the full scan mode. The recoveries of the herbicides by SPE on LiChrolut EN were calculated by external standardization and ranged from 83 to 97% (**Table 2**), which indicates, as expected [13,22], that the SPE procedure performed well for these triazines and metabolites.

Table 2. Recovery of pesticides measured with different techniques.

Sample Technique Pesticide	Blank water GC-MS	Rain water GC-MS	Rain water GC-MS-MS
desisopropylatrazine	93	193	98
desethylatrazine	88	138	110
simazine	83	154	105
atrazine	92	163	99
metribuzin	97	127	115

Full scan CGC-MS, however, could not be successfully applied for the soil sample, spiked at the 10 ppb level (sample d). After extraction (1 g) and concentration (to 1 ml), the injected amount was now 10 pg per compound. Part of the chromatogram and the extracted ion (m/z 200) chromatogram for atrazine are shown in **Figure 3**. More background is detected in this sample and atrazine could not be traced in the total ion chromatogram. In the extracted ion chromatogram, atrazine could be detected with a

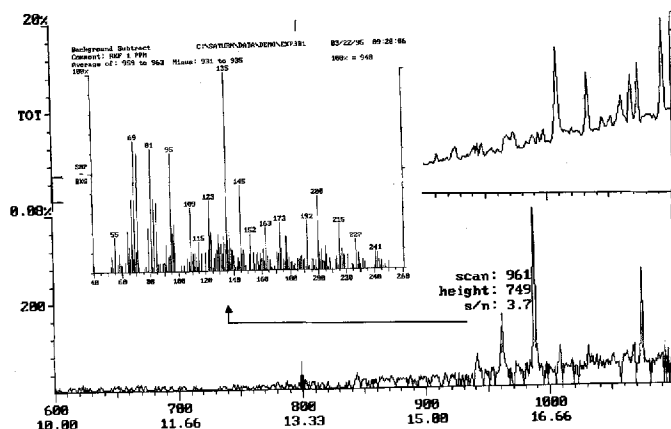


Figure 3. CGC-MS analysis of atrazine in spiked (10 ppb) soil sample. Insert: recorded spectrum.

signal-to-noise ratio of 3.7. The background subtracted spectrum for the atrazine peak is given in the insert of **Figure 3**. The characteristic ions (215, 200, 173) are present but the spectrum is overloaded with matrix ions and the solute was not recognized by automated library search. Therefore the possibilities of MS/MS were evaluated for the same sample.

Successful application of MS-MS, requires good selection of a parent ion or ion window and of the collision induced dissociation (CID) voltage. The latter determines the energy for further fragmentation of the isolated parent ion. The optimization of the CID voltage for a given solute is done experimentally as illustrated for atrazine. A 10 ppm standard of atrazine was analysed by CGC-MS-MS selecting as parent ion the molecular ion 215, and a mass window of 2. The CID voltage was first set at 0 V. This means that no fragmentation should occur after isolation of the ion. **Figure 4** shows the analysis and the spectrum (left) of atrazine obtained at 0 V and indeed no fragmentation is observed. The right spectrum in **Figure 4**, gives the spectrum recorded at 30 V and the specific electron impact fragmentation ions of atrazine are detected.

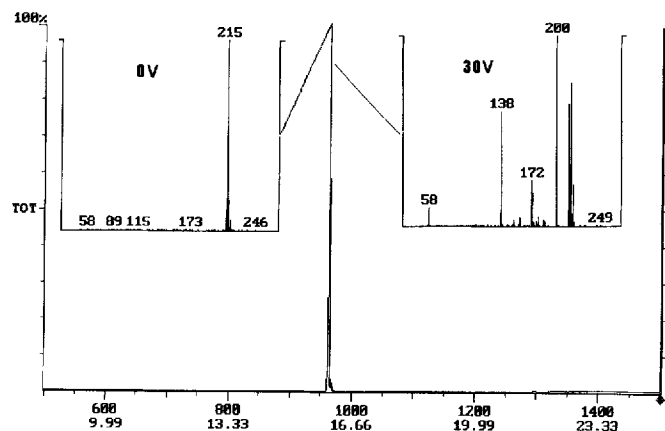


Figure 4. CGC-MS-MS analysis of atrazine (10 ng) at 0 and 30 V CID voltage.

In setting up the MS/MS mode, a careful selection of the CID voltage is thus of utmost importance. The influence of the voltage on the relative abundance of the most important ions for atrazine is shown in **Figure 5**. A too low voltage results in no fragmentation and thus no characteristic product ions while a too high voltage results in too much fragmentation and loss of molecular weight information. From the curves, it could be deduced that 30 V gives for atrazine, a spectrum best fitting with a standard electron impact spectrum. Because of the similar structures of the herbicides studied, 30 V was found to be the optimum value for the other solutes as well.

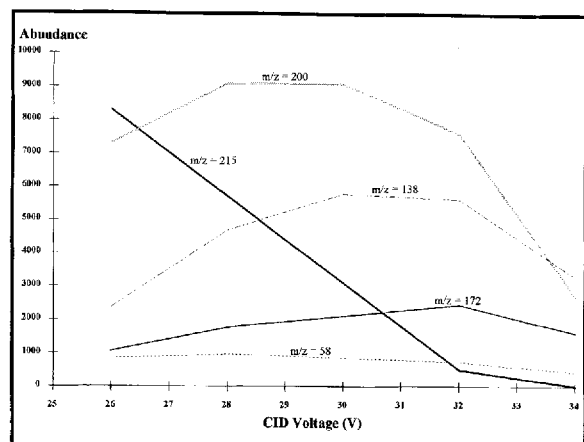


Figure 5. Influence of CID voltage on ion abundance for atrazine.

The MS-MS settings were applied to the soil extract previously analysed by CGC-MS (**Figure 3**) and the resulting chromatogram is shown in **Figure 6**. The background is much lower compared to the CGC-MS analysis and atrazine can be detected with a signal-to-noise ratio of 19 which is approximately 5 times higher in comparison to the MS method. This illustrates the gain in selectivity with MS-MS. Moreover, a compound specific spectrum was obtained matching very well with the library spectrum for atrazine. Positive compound identification was thus possible, even in this complex matrix and at this low level.

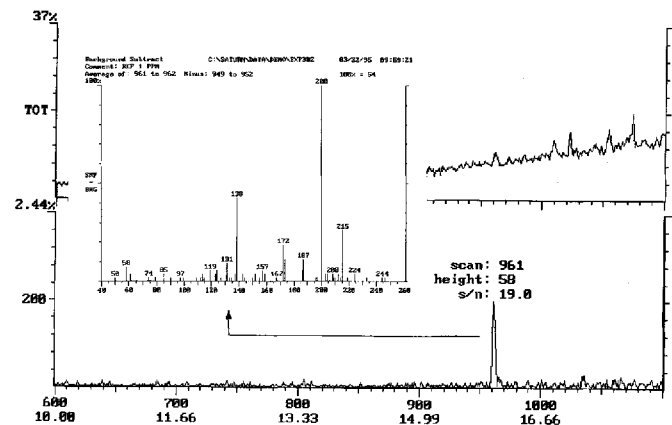


Figure 6. GC-MS-MS analysis of atrazine in spiked (10 ppb) soil sample. Insert: recorded spectrum.

For the simultaneous determination of the five pesticides, different time segments, *i.e.* MS-MS settings, were required. In each segment, the best parent ion and CID voltage for each compound should be selected. This, however, requires very good chroma-

tographic separation of all compounds. Because of the close elution of the solute pairs desisopropylatrazine-desethylatrazine and simazine-atrazine, an ion window was selected for both pairs that contains an ion characteristic for both closely eluting compounds. For the five target compounds, three time segments *i.e.* one for desisopropylatrazine-desethylatrazine, one for simazine-atrazine, and one for metribuzin were applied. The selected parent ions, and the typical product ions are listed in Table 1.

The potential of MS-MS *versus* MS is illustrated with the analyses of the spiked rain water sample (sample b). **Figures 7** and **8** show the CGC-MS and CGC-MS-MS analyses, respectively.

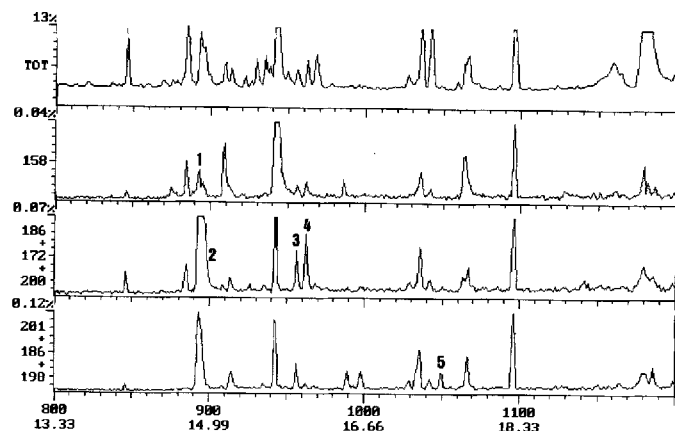


Figure 7. GC-MS analysis of spiked (0.1 ppb) rain water sample. Peaks: see **Figure 2**.

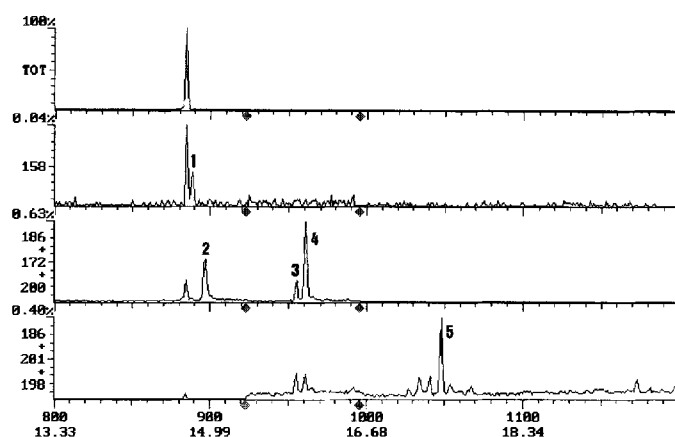


Figure 8. GC-MS-MS analysis of spiked (0.1 ppb) rain water sample. Peaks: see **Figure 2**.

In the CGC-MS chromatograms, the presence of desisopropylatrazine and desethylatrazine is difficult to establish due to the presence of interfering peaks and no selective ion could be found for good integration. Nevertheless, the concentrations in the rain water extract were measured and recoveries, compared to sample a, ranged from 127% for metribuzin to 193% for desisopropylatrazine (**Table 2**). Matrix interferences lead to false positive results, *i.e.* to overestimation of the concentration of pesticides in the sample. This overestimation is not due to the sample

preparation method, since the recoveries for pesticides spiked in blank water were between 83% and 97%. The pesticides can be quantified more accurately with MS-MS due to the increased selectivity. The recoveries calculated ranged from 98 to 115 % (Table 2). In conclusion, for environmental samples the use of CGC-MS-MS allows, without loss in sensitivity, an important improvement in selectivity and thus in identification and quantification capabilities.

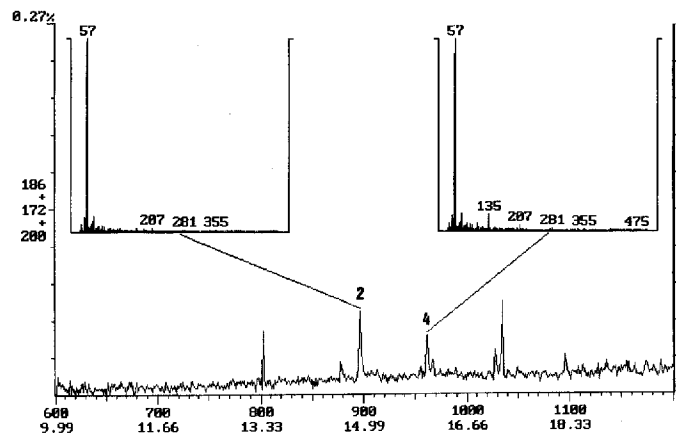


Figure 9. GC-MS analysis of extract of the river Leie, Belgium. Peaks: see Figure 2; inserts: recorded spectra.

With the same instrumental conditions, a water sample from the river Leie, Belgium was analysed. The GC-MS ion extracted chromatogram is shown in **Figure 9**. Desethylatrazine and atrazine are detected but their presence could not be confirmed as the spectra taken for both signals (see inserts) do not at all correspond with the library spectra for desethylatrazine and atrazine.

The same sample was analyzed with CGC-MS-MS; the chromatogram of which is shown in **Figure 10**. Desethylatrazine and atrazine are detected but their identity can now be confirmed by their specific spectra (see inserts). The concentrations were 0.09 ppb desethylatrazine and 0.08 ppb atrazine. Atrazine is often used in this region and its detection in low levels is typical for this period of the year; the concentration increasing in the months

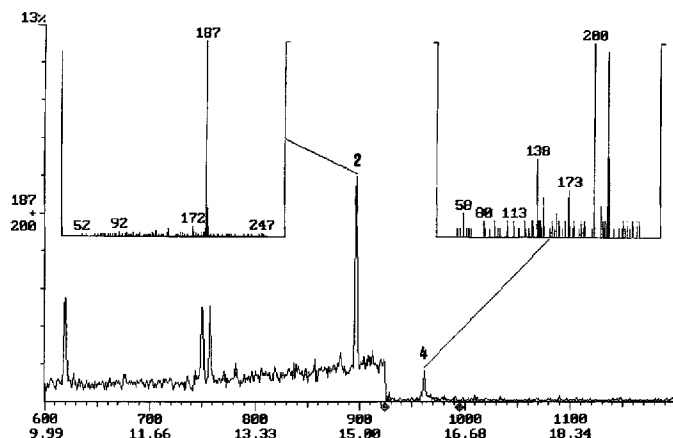


Figure 10. GC-MS-MS analysis of extract of the river Leie, Belgium. Peaks: see Figure 2; inserts: recorded spectra.

May through September. Important to note is the high concentration of desethylatrazine which obviously is the main metabolite of atrazine as the desisopropyl analogue was not detected.

To study the metabolism of atrazine and metribuzin more in detail, a rain water (sample e) and soil (sample f) sample spiked with a high level of atrazine and metribuzin, 100 ppb and 1 ppm, respectively. The analyses were performed immediately after spiking and after three weeks. For atrazine, desethylatrazine was detected as main metabolite. For metribuzin, desamino-metribuzin was found to be the main metabolite. The concentrations of the pesticides and their metabolites were determined by CGC-MS and CGC-MS/MS. The results are summarized in **Table 3**. Atrazine was found to be relatively stable in rain water, but in soil a 40% loss was detected. Only 1 to 2% from this could be ascribed to desethylatrazine. MS and MS-MS gave similar results. For metribuzin, on the other hand, MS-MS gives more accurate results. With MS, the concentrations both in water and soil, are overestimated as the sum of metribuzin and metabolite are larger than 100%, while with MS-MS, the sum metribuzin and metabolite is roughly 100 %.

Table 3. Concentrations (ppb) measured in spiked rain water and soil after 3 weeks.

Technique	Atrazine		Desethylatrazine		Metribuzin		Desamino-metribuzin	
	MS	MS-MS	MS	MS-MS	MS	MS-MS	MS	MS-MS
Rain water								
spiked	100	100			100	100		
measured ^{a)}	99.5	90.4	0.12	0.1	79.3	54.1	152	51.0
Soil								
spiked	1000	1000			1000	1000		
measured ^{a)}	660	640	14	13	980	740	311	204

a) After 3 weeks, external standardization.

4 Conclusion

Compared to GC-MS, applying the ion trap detector in the CGC-MS-MS mode offers enhanced selectivity which results in improved identification and quantification capabilities for low traces of priority pollutants in environmental samples.

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