Recent Applications in LC–MS: Environmental Analysis

Pat Sandra,[1] José Nogueira,[2] Tom Sandra[1] and Frank David,[1]
[1]Research Institute for Chromatography, Kortrijk, Belgium
[2]University of Lisbon, Portugal.

Introduction

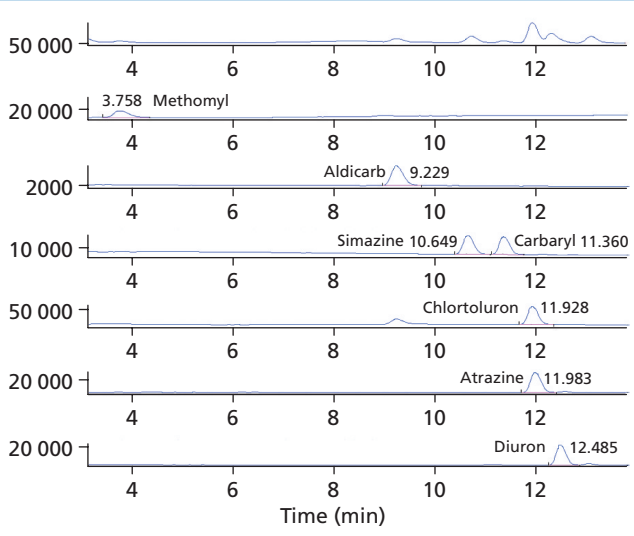
LC–MS is becoming an essential tool for environmental analysis. Environmental laboratories dealing with the analysis of large numbers of samples are increasingly realizing that, notwithstanding the relatively high price of LC–MS instrumentation, the technique has a lot to offer in terms of productivity, ruggedness, ease-of-use, accuracy and precision. As an example, the analysis of *N*-methyl carbamates is nowadays commonly performed by LC–fluorescence detection after postcolumn reaction and derivatization. In many routine laboratories, the technical staff are not highly specialized in separation sciences and to operate the postcolumn device properly, the help of a service engineer is often required. State-of-the-art LC–MS instrumentation is very user-friendly and the productivity for carbamate analysis is much higher. Moreover, the high selectivity of the mass spectrometer often allows simplified sample preparation.

The most frequently analysed substances are pesticides that are not amenable to capillary GC–MS analysis, or only after a derivatization step. Analyses diversify in single component analysis, in group type analysis and, recently, in multiresidue determinations. Each type of analysis will be discussed.

In addition, LC–MS plays a key role in the development of new analytical strategies for pollutants of high priority in the European Community, namely the endocrine disrupting chemicals (EDCs). EDCs are synthetic or naturally occurring chemicals that interfere with endocrine functions. The list of EDCs comprises female sex hormones and synthetic steroids (contraceptives), alkylphenolic compounds (detergents), polychlorinated biphenyls (PCBs), dioxins, polybrominated organic substances (flame retardants), phthalates and bisphenol A (polymers), organotin compounds (antifouling agents, biocides), phytoestrogens etc. Many examples of the application of LC–MS to the analysis of EDCs can be found in the recent chromatographic and environmental literature.[1,2] Other environmentally relevant areas are the analysis of explosives and azo dyes, and the studies on pollutant-DNA adducts etc.

Before the presentation of several applications, an important question should be addressed. What do we need for environmental analysis: an LC–MS (single quadrupole) or an LC–MS^n (ion trap or triple quadrupole)? This question is indeed very often forwarded by laboratory managers and an endless controversial discussion can be the result.

Figure 1: Pesticide multiresidue SPE–LC–MS analysis.



LC–MS plays a key role in the development of new analytical strategies for pollutants of high priority in the European Community, namely the endocrine disrupting chemicals.

Selection between LC–MS and LC–MS^n

'Selectivity' from a chromatographic, spectroscopic and sample preparation point of view is the key word in the discussion on LC–MS or LC–MS^n.

In LC–MS with a single quadrupole, primary ions can be fragmented by collision-induced dissociation (CID). This means, however, that an ion originating from a single compound must be fragmented. When coelution occurs, the resulting multiple-component spectrum is definitely useless when the creation and/or use of an LC–MS library is aimed at. Chromatographic selectivity is thus all important.

In LC–MS^n with an ion trap (or a triple quadrupole), an ion out of a bundle of ions eventually originating from coeluting peaks can be isolated (trapped) and then fragmented. The high MS selectivity largely compensates for the lack of chromatographic resolving power.

An illustration is given in Figure 1. A seven component mixture in a multiresidue experiment (carbamate, phenylurea and triazine pesticides) was spiked in water at the 0.05 ppb level per compound, extracted by solid-phase extraction (SPE, see further) and analysed on a single quadrupole LC–MS combination. Methomyl, aldicarb, simazine, carbaryl and diuron are sufficiently separated to create fragments on a single quadrupole MS by CID. Fragmentation can be required for confirmatory reasons or to create pesticide libraries (see further). Chlortoluron and atrazine are chromatographically unresolved and quadrupole-CID results in a double spectrum. Quantification can be performed by ion extraction or ion monitoring but obtaining a net spectrum for confirmatory reasons or to apply an LC–MS library is impossible. With an MS^n set-up ion 213 (chlortoluron) can be trapped and ion 216 (atrazine) filtered out before fragmentation or vice versa.

Important to note is that MS-CID and MS–MS spectra are qualitatively very similar for peaks corresponding to a single solute as illustrated in Figure 2 for the analysis of carbendazim at a 80 V fragmentor voltage in both instances.

Next to the chromatographic and spectroscopic selectivity, the sample matrix and sample preparation procedure should be included. The cleaner the sample is (tap water versus waste water) and the more selective is the sample preparation step, the less important are both other selectivities.

In our experience two instances can be distinguished in environmental analysis; these being relatively clean samples (tap water, ground water, surface water, etc.) for which analyses both a single MS and MS^n will provide good data, and dirty samples (waste water, soil, sediment, etc.) for which MS^n is definitely recommended when a sample preparation procedure is applied that is universal rather than selective.

Considerations on Sample Preparation

In the framework of this contribution it is impossible to discuss sample preparation for LC–MS analysis in detail but some general trends are summarized. For aqueous samples, SPE is nowadays the method of choice. SPE can be performed off-line manually, semi-automated (e.g., the ASPEC from Gilson) or on-line (the Prospekt from Spark Holland, the Turbo LC from Cohesive Technologies etc.). For solid samples, sonication and especially accelerated solvent extraction (ASE) are increasingly replacing Soxhlet extraction. Further clean-up by SPE is mostly required for solid samples.

Applications

Some typical applications of LC–MS in environmental analysis are presented. The procedures are relatively simple and highly reproducible. However, care should be taken on matrix-induced ion suppression. This effect can be minimized by good sample preparation, good chromatographic separation and optimizing the MS-operating conditions as discussed in reference 3.

Single Component Analysis: Determination of Amitrol

The determination of glyphosate (and its metabolite AMPA) is probably the best known single component analysis. LC–MS with $C^{13}N^{15}$-glyphosate as internal standard is an accurate and sensitive method (see reference 4 and applications on the websites of the companies offering LC–MS instrumentation). Another component for which a sensitive analytical method is required is the non-selective systemic herbicide amitrol. From an analytical point of view amitrol creates two problems. First, its low molecular weight (MW 84) does not give a very specific ion in ESI-MS and second, the solute is highly soluble in water creating problems with enrichment with high recovery out of an aqueous matrix. Both problems could be solved by in-situ

Figure 2: (a) MS-CID and (b) MS–MS at 80 V for carbendazim.

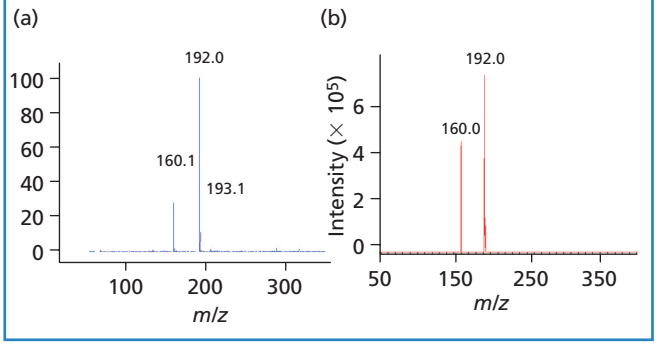
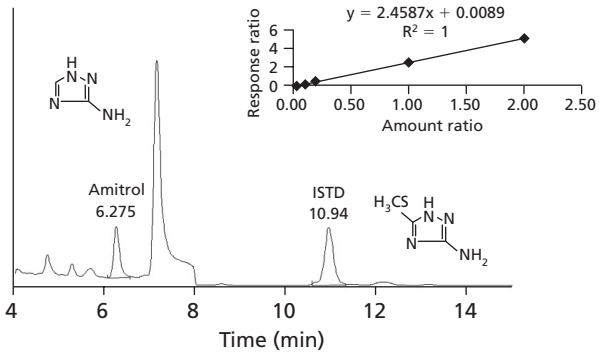
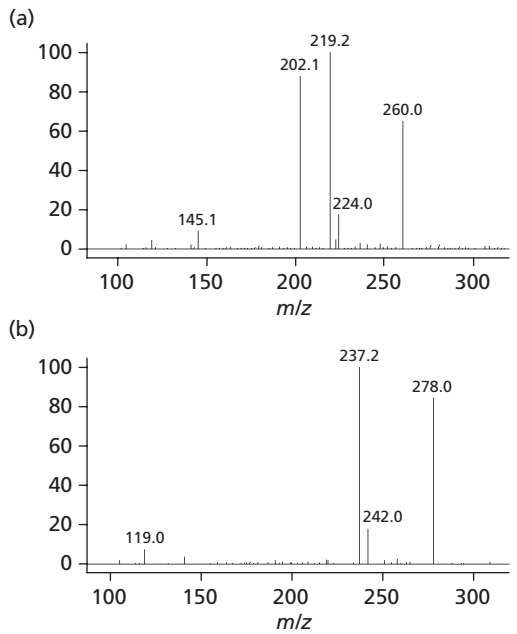


Figure 3: SPE–LC–MS analysis of amitrol at the 0.1 ppb level and calibration graph from 0.02 to 1 ppb.



derivatization with *n*-hexylchloroformate. The derivative has a specific mass at 213 *m/z* $(M+H)^+$ and hydrophobicity (*n*-hexyl) is introduced in amitrol to allow SPE-enrichment on octadecylsilica (ODS). A labelled standard as in the situation of glyphosate is not commercially available and a solute with similar chemical characteristics was selected, namely 3-amino-5-methylthio-1H-1,2,4-triazole, as internal standard (IS). The standard is added to the water sample before derivatization and the molecular weight of the IS derivative is *m/z* 259. The complete sample preparation is as follows. To a 50 mL water sample, an appropiate amount of internal standard is added (typically 1 µg/L or 1 ppb). 2.5 mL of the mixture water:ethanol:pyridine (60:32:8) is then added followed by 200 µL *n*-hexylchloroformate. After vortex treatment for 30 s, SPE on an ODS cartridge is performed. After conditioning the sorbent, the total 50 mL sample is enriched, the cartridge is washed with water, vacuum dried and the solutes are eluted with 3 mL acetonitrile:methanol (1:1). The solvent is evaporated and the residue retaken in 200 µL water. An aliquot, typically 50 µL, is injected on an ODS column with a gradient between 10 mM ammonium acetate/methanol to methanol. Figure 3 shows a typical LC–APCI-positive mode-MS chromatogram of a surface water spiked at the 0.1 ppb level together with a calibration graph between 0.02 and 1 ppb. Ion 213 (amitrol) was monitored between 4 to 8 min (gain 10) and ion 259 (IS) between 8 and 12 min (gain 1).

Figure 4: Flow-injection MS spectra for (a) carbaryl and (b) oxamyl.



Group Type Analysis

LC–MS is very well suited for the determination of classes of pesticides, such as carbamates, phenylureas, triazines, phenoxy acids, thiocarbamates etc. As an example, the analysis of carbamates in water samples is discussed. *N*-methyl carbamates are a class of compounds derived from carbamic acid and some derivatives are intensively used herbicides in agriculture because of their broad spectrum of activity. The EC directive on drinking water quality (98/83/CE) established a maximum permissible concentration (MAC) of 0.1 ppb for the individual pesticides. Currently methods to monitor carbamates embrace a preconcentration step (liquid–liquid extraction (LLE) or SPE, followed by LC-postcolumn reaction-derivatization and fluorescence detection. This *N*-methyl carbamate dedicated analytical system can easily be replaced by mass spectroscopic detection without derivatization which is much more rugged and versatile and moreover provides spectral evidence of the presence of the solutes. Limit of quantification (LOQ) values of parts-per-trillion (ppt) have been reported.[5] A simple SPE–LC–MS procedure is described. The *N*-methyl carbamates aldicarb, carbaryl, carbofuran, methomyl, oxamyl and pirimicarb were selected as model compounds. Stock solutions of each carbamate having a concentration of 1 ppm were prepared in methanol and stored at 4 °C. Six standard calibration solutions ranging from 1–50 ppb were each prepared daily in deionized water by appropriate dilution of aliquots of the stock solutions. 50 mL water (deionized, tap or surface) samples were spiked in a concentration ranging from 0.03 to 0.30 ppb for each carbamate. SPE was performed using Zorbax C18, 3 mL, 500 mg cartridges (Agilent Technologies, Waldbronn, Germany). The cartridges were conditioned with 3 mL methanol:acetonitrile 50:50 (v/v), 3 mL of methanol and twice with 3 mL deionized water. The complete 50 mL sample was loaded on the cartridge using 0.4 bar pressure and after washing twice with 3 mL deionized water, vacuum was applied for 2 min. The carbamates were eluted with 3 mL methanol:acetonitrile 50:50 (v/v), the eluate was evaporated under a nitrogen stream and the residue retaken by vortex-mixing in 200 µL water for LC–MS analysis. The analyses were performed on a benchtop Agilent 1100 series LC–MSD

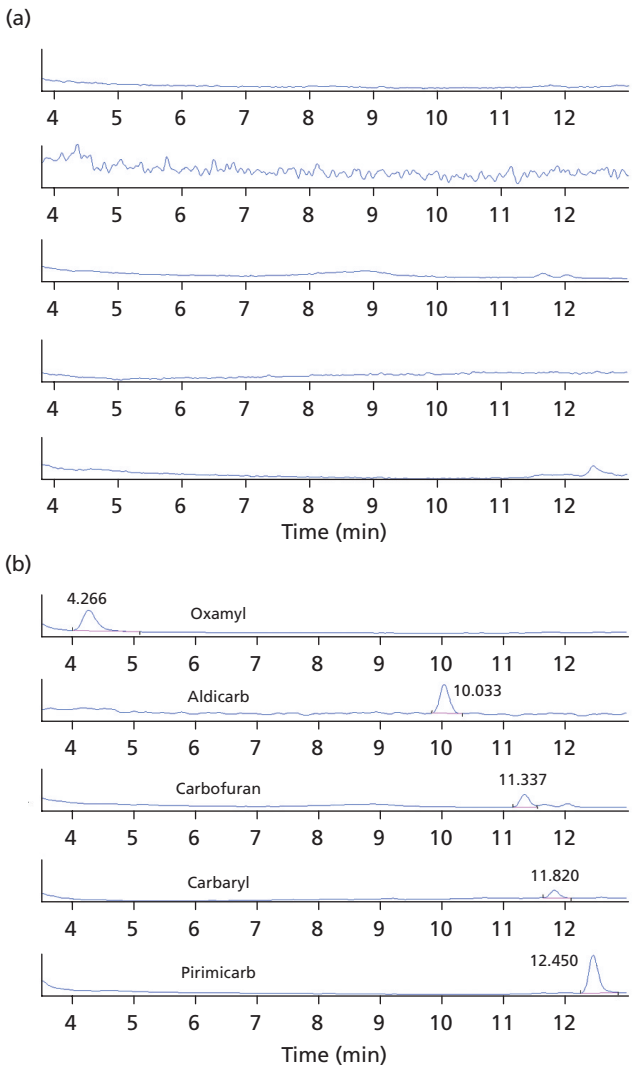
Table 1: Molecular weight (MW), assignation of the ions used in the calibration plots by LC–MSD in SIM mode, correlation coefficients and detection limits obtained for the six carbamates studied.

Compound	MW (Da)	Ion (*m/z*)	Assignation (species)	r^2 (1–50 ppb)[c]	LOD (ppb)[d]
Aldicarb	190	116[a]	$[M\text{-}CH_3NHCOOH\text{-}NH_3+H]^+$	0.9996	0.10
Carbaryl	201	202[b]	$[M+H]^+$	0.9946	0.10
Carbofuran	221	222[a]	$[M+H]^+$	0.9992	0.10
Methomyl	162	163[a]	$[M+H]^+$	0.9999	0.50
Oxamyl	219	278[b]	$[M+Na+2+H_2O]^+$	0.9907	0.10
Pirimicarb	238	239[a]	$[M+H]^+$	0.9990	0.10

[a]Base peak; [b]Relative abundance higher than 80%; [c]SIM mode, n=6; [d]S/N=3

equipped with a 100 mm L × 2.1 mm i.d., 5 µm ODS Hypersil column (Agilent Technologies). A gradient was applied between 90% ammonium acetate (10 mM):10% methanol and 10% ammonium acetate (10 mM):90% methanol. The flow-rate was 0.25 mL/min, the analyses were performed at 25 °C and the injection volume was 50 µL with 200 µL/min draw speed. Atmospheric pressure electrospray ionization (APESI) was performed in the positive mode under the following conditions: gain 1, N_2 drying gas flow-rate 12 L/min at 350 °C, nebulizer pressure 35 psig, quadrupole temperature 100 °C and capillary voltage 4000 V. Flow-injection analysis (10 mL) was performed on individual carbamate solutions (10 ppm) to obtain the best spectral data in function of the collision-induced dissociation (CID or fragmentor) voltage. 60 V provided the best results. From the full-scan spectra showing protonated, ammoniated or sodiated ions, selective ions for each carbamate species were chosen for ion monitoring. Figure 4 shows the spectra of carbaryl and oxamyl under the applied conditions and Table 1 gives the ions selected for the quantitative measurements.

Figure 5: Direct injection of drinking water for carbamate analysis. (a) Drinking water and (b) drinking water spiked at 0.3 ppb.



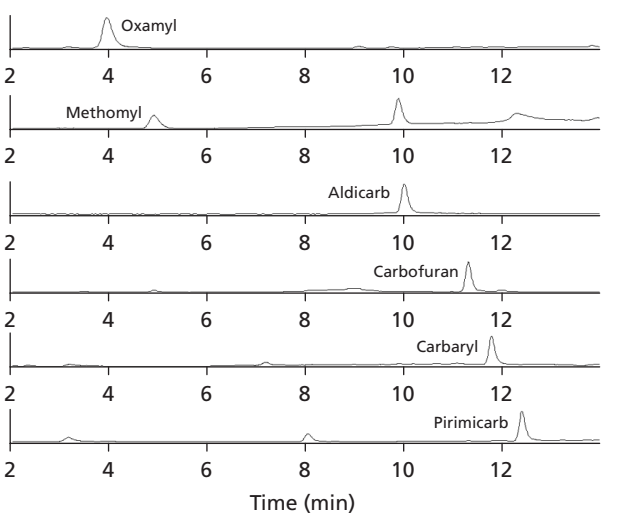
Six-point calibration plots (n = 6, RSD% < 6%)) in the SIM mode were made using standard aqueous mixtures having 1, 2, 5, 10, 25 and 50 ppb and excellent correlation coefficients were obtained (Table 1). The limits of detection (LODs) were 0.1 ppb for all carbamates with the exception of methomyl for which 0.5 ppb was measured. Considering these LODs, it is possible to analyse the carbamates without sample enrichment. Figure 5(a) shows the direct analysis of drinking water (100 µL injection, gain at 5) and spiked with the carbamates, with the exception of methomyl, at the 0.3 ppb level (Figure 5(b)). For those selected carbamates the EU directive can thus be reached without sample preparation.

Nevertheless, application of the SPE procedure is so simple that it is normally performed for tap water and is a must for other aqueous samples. Because the enrichment factor is 250 (from 50 mL to 0.2 mL) LODs drop to the low ppt level. The analysis of a surface water spiked at the 30 ppt level is shown in Figure 6.

The average SPE recovery for n = 10 and the RSD% of the whole procedure at the 30 ppt level are listed in Table 2. In conclusion, the SPE–LC–MS methodology exhibited good robustness, accuracy and precision for ultra-trace analysis of carbamates from water samples down to the ppt level.

The same approach, eventually after some minor modifications, can also be used to monitor other pesticide classes in aqueous media. As an example, Figure 7 shows the analysis of some phenoxyacid herbicides spiked in surface water at the 0.1 ppb level. For the SPE procedure it was mandatory to adjust the pH of water with phosphoric acid to 2 in order to protonate the acids giving them sufficient retention on an ODS cartridge. Only 10 mL water was applied on the cartridge and elution was performed with methanol:methyl-*t*.butylether in the ratio 10:90 (v/v). The residue was redissolved in 0.5 mL water:methanol (60:40). The analysis was performed on a LC–MSD equipped with a 100 mm L × 2.1 mm i.d., 5 µm Hypersil ODS column (Agilent Technologies) in the isocratic mode with 70% ammonium acetate (10 mM):30% methanol. The flow-rate was 0.4 mL/min, the analyses were performed at 30 °C and the injection volume was 50 µL. Atmospheric

Figure 6: SPE–LC–MS analysis of carbamates in water at 0.03 ppb.



pressure chemical ionization (APCI) was performed in the negative mode under the following conditions: ion monitoring at gain 10 from 3.5–8 min for ions 199 (MCPA) and 219 (2,4-D), and from 8 to 20 min for ions 213 (mecoprop), 233 (dichlorprop) and 253 (2,4,5-T), N_2 drying gas flow-rate 5 L/min at 350 °C, nebulizer pressure 60 psig, vaporizer at 325 °C, capillary voltage at 4000 V and corona current at 25 µA.

Pesticide Multiresidue Analysis

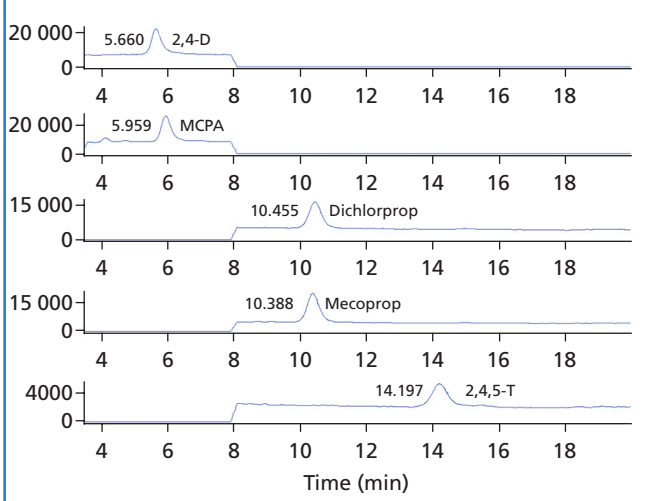
As LC–MS becomes easier to use and readily available in environmental laboratories, the need for pesticide multiresidue methods becomes more and more apparent. A multiresidue method should embrace a simple and versatile sample preparation procedure, fixed LC operating conditions (i.e., column choice and mobile-phase composition), and last but not least the availability of universal searchable LC–MS libraries as they do exist, even in combination with retention time locking, in capillary GC–MS.

We are not yet at the stage where procedures and systems are available for multiresidue screening by LC–MS, but recent developments point out that it is a realistic dream for the near future. As already illustrated with the multiresidue analysis in Figure 1, for the application of only reversed-phase LC on ODS with nearly the same mobile-phase composition for all the different applications shown in this contribution and the versatility of SPE on ODS cartridges, uniform procedures can be worked out. By performing SPE at two pHs (2 and 8) nearly all pesticides (i.e., acids, neutrals and bases can be enriched from aqueous samples). For LC, a gradient between ammonium acetate and methanol or acetonitrile, and pH eventually adjusted with a volatile base or acid, on a high-quality ODS column such as a Zorbax Eclipse C18 (150 mm L, 2.1 mm i.d., 5 or 3 µm particles) will result in the elution of nearly all pesticides under MS-friendly conditions. From the MS-side, most of the pesticides ionize with positive or negative electrospray ionization. Moreover, it has recently been illustrated that using performance-based conditions for different LC–MSD systems (including low, mid and high fragmentation energies), consistent and reproducible spectra can be obtained.[6] A NIST MS search program can be linked to the ChemStation and spectra can be added creating a NIST library. For unknown samples, searches against the NIST library elucidate the pesticides.[7] The quantitative aspect in an LC–MS pesticide multiresidue method is good as illustrated in Figure 8 which shows the calibration graphs for simazine, methomyl and diuron in the 0.02 to 1 ppb range corresponding with the chromatogram shown in Figure 1.

Table 2: Average recoveries and relative standard deviations (RSD) obtained from 10 assays on Zorbax SPE cartridges at the 0.03 ppb level.

n=10	Mean recovery (%)	RSD (%)
Aldicarb	73.7	14.1
Carbaryl	88.4	14.7
Carbofuran	85.3	13.8
Methomyl	92.6	13.4
Oxamyl	86.5	11.1
Pirimicarb	92.3	11.2

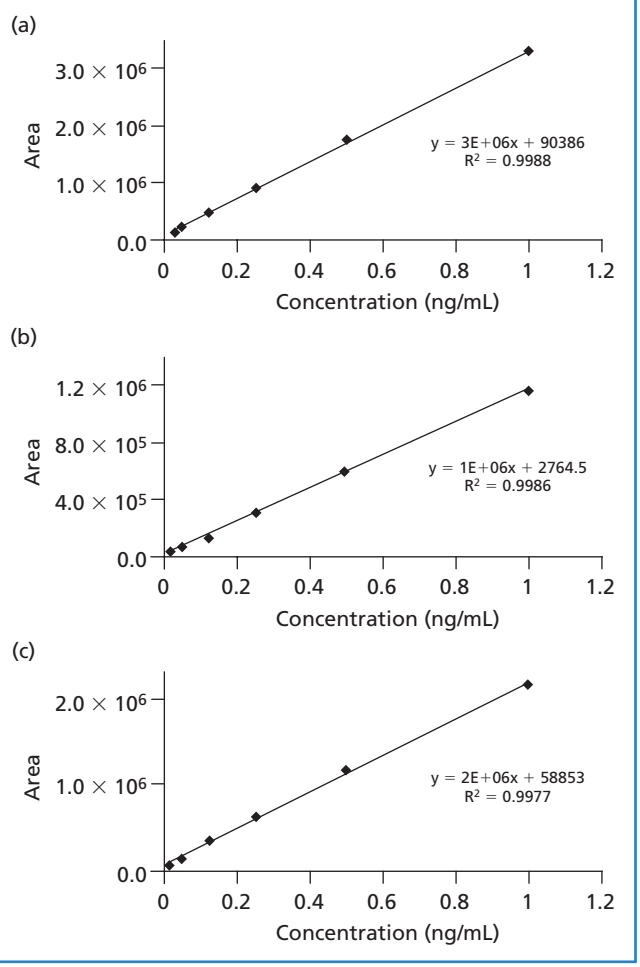
Figure 7: SPE–LC–MS analysis of phenoxy acids in water at the 0.1 ppb level.



Analysis of Explosives in Soil

Determination of organic micropollutants in solid samples, such as soil and sediment can be performed by the LC–MS

Figure 8: Calibration graphs for (a) simazine, (b) methomyl and (c) diuron for SPE–LC–MS (chromatogram in Figure 1).



methods described for water samples after appropriate extraction of the soil by ASE or ultrasonic extraction eventually followed by clean-up using the SPE technology. Nowadays we receive more and more enquiries concerning the determination of explosives in soil samples, and the application of LC–MS is mandatory for the analysis of some explosives. As an illustration, a soil sample extracted by sonication with acetonitrile was analysed by both capillary GC–MS and LC–MS. The capillary GC–MS chromatogram is shown in Figure 9 and the following 'explosive' related compounds could be identified and quantified (ppb level): pentachloronitrobenzene (1), musk xylene (2), 1,5-dinitro-naphthalene (3), musk ketone (4) and 1,8-dinitro-naphthalene (5). The picture was, however, far from complete because only nitro-aromatics can be properly analysed by capillary GC. Figure 10 shows the LC–MS analysis of the same extract diluted 1:1 in 10 mM ammonium acetate. 10 µL was injected on a 15 cm L, 4.6 mm i.d., 5 µm Zorbax SB-C18 column (Agilent Tecnologies) at a flow-rate of 0.7 mL/min of mobile phase 60% ammonium acetate 10 mM:40% methanol. The MS was operated in the ESI negative mode. Figure 10 shows the obtained chromatogram with the recorded spectra and the ion extracted traces. The first peak corresponds with cyclotetramethylene tetranitramine also known as HMX or octogen and the second peak to cyclotrimethylene trinitramine also known as RDX or hexogen, which is one of the main substances in semtex. Concentrations were in the ppm range.

Figure 9: Explosives in soil extract analysed by capillary GC–MS.

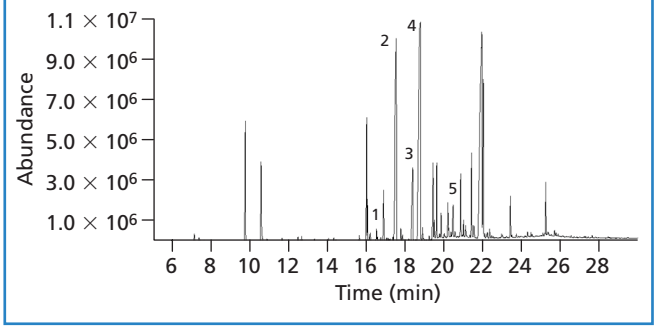
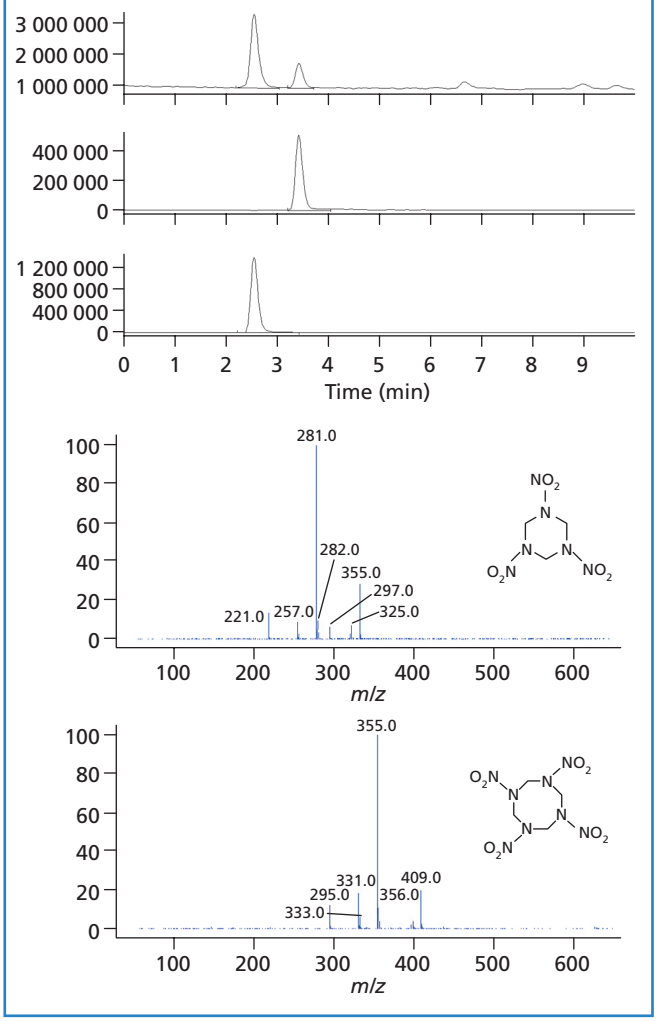


Figure 10: Explosives in soil extract analysed by LC–MS.



Conclusion and Future Trends

LC–MS is a very valuable tool for environmental analysis. The technique will be applied increasingly for routine determinations of pesticide residues. Libraries will be created to facilitate pesticide elucidations in multiresidue methods. In addition, new developments are expected to determine ultratraces of EDCs. This also involves robust and easier sample preparation methods than those currently available.

Acknowledgement

We thank Agilent Technologies for supporting our LC–MS research.

References

1. D. Barcelo, Ed., *Applications of LC–MS in Environmental Chemistry*, (Elsevier Science, Amsterdam, 1996).
2. A. Gentili et al., *Chromatographia*, **56**, 25 (2002).
3. P. Sandra et al., *LC•GC Europe's Guide to LC–MS*, (Dec. supplement 2001), 8–21.
4. R.J. Vreeken et al., *J. Chromatogr. A*, **794**, 187 (1998).
5. N. Wang and W. L. Budde, *Anal. Chem.*, **73**, 997 (2001).
6. J.M. Hough et al., *Anal. Chem.*, **72**, 2265 (2000).
7. C.Kai Meng and P. Zavitsanos, Agilent Application note 5988-5445EN, April 2002, (www.agilent.com).

Pat Sandra is Professor in Separation Sciences at the University of Ghent, Belgium and the University of Stellenbosch, South Africa, and Director of the Research Institute for Chromatography, Kortrijk, Belgium.
José Nogueira is Associated Professor at the University of Lisbon, Portugal and working on the analysis of pollutants in the aquatic environment.
Tom Sandra is an application chemist at the Research Institute for Chromatography, Kortrijk, Belgium.
Frank David is R&D Manager at the Research Institute for Chromatography, Kortrijk, Belgium.