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Review

Trace-level determination of pesticides in water by means of liquid and gas chromatography

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Abstract

The trace-level determination of pesticides and their transformation products (TPs) in water by means of liquid and gas chromatography (LC and GC) is reviewed. Special attention is given to the use of (tandem) mass spectrometry for identification and confirmation purposes. The complementarity of LC- and GC-based techniques and the potential of comprehensive GC×GC are discussed, and also the impressive performance of time-of-flight mass spectrometry. It is also indicated that, in the near future, the TPs rather than the parent compounds should receive most attention—with a better understanding of matrix effects and eluent composition on the ionization efficiency of analytes being urgently required. Finally, the merits of using much shorter LC columns, or even no column at all (flow-injection analysis) in target analysis are shown, and a more cost-efficient and sophisticated strategy for monitoring programmes is briefly introduced.

Keywords: Water analysis; Pesticides

Contents

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1. Introduction

In the past decade, the trace-level determination of organic microcontaminants has been a challenging task for analytical chemists. In this period, much progress has been made in, specifically, the fields of environmental, food and food additive, human and veterinary drug, and agricultural analysis. Several distinct developments and trends can be pointed out, one of these being a greater need for sophistication and procedural integration when performing analyses for either early-warning, monitoring or screening purposes—irrespective of whether detection, identification or confirmation, or quantification is the principal goal. In other words, in the recent past analytical chemists began to realize that the combination of a conventional injection procedure, a single analytical column and a selective detector often does not suffice to recognize and quantify all analytes of interest in a sample. Increasingly, therefore, multidimensional approaches began to make headway to help solve the current analytical problems—one generally felt need being to incorporate the sample preparation in the total analytical procedure. Over the years, two major branches emerged—hyphenation, which is often defined as the on-line combination of a separation technique and a spectroscopic detection device which provides structural information, and coupled-column techniques, a term which does not require further explanation. Combining both approaches in one set-up will further increase the potential—but, unfortunately, also the cost—of the required analytical instrumentation. At the same time, it will enhance the versatility of the developed analytical procedures. Fig. 1, adapted from an earlier drawing by Patrick Arpino indicates some of the more frequently used routes available today in a well equipped laboratory.

While it is true that Fig. 1 does not provide an exhaustive listing of analytical approaches, it will also be clear that the wealth of information implicitly available within this framework, both of a scientific and an applied nature, is such that it cannot easily be dealt with within the context of a conventional-size review. Consequently, a very serious limitation has been made: a single group of analytes of (much current) interest was selected, pesticides, with occasional reference being made to related industrial

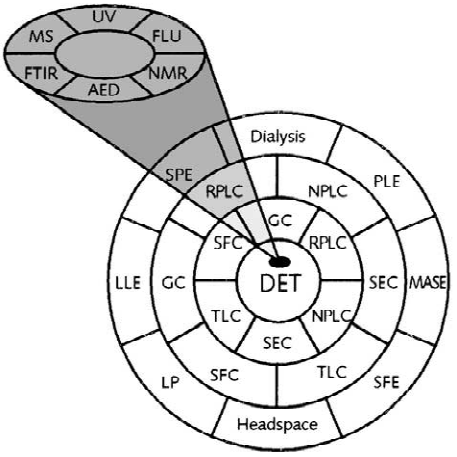


Fig. 1. The versatility of multidimensional analytical procedures.

chemicals and/or pesticide transformation products (TPs), and water analysis was taken as the primary area of application. For recent reviews on closely related topics, the reader is referred to a paper on pharmaceuticals in aqueus environmental samples [1] and a discussion of endocrine disruptors in freshwater sediments [2]. Since it is not our intention to provide an exhaustive overview of the recent literature but, rather, to outline recent developments and alternative strategies and to indicate trends, a fairly wide-ranging pesticide-residue analysis approach rather than a systematic discussion of all analytical procedures that have been—or can be—used, will be the framework of the following text.

2. Pesticide analysis: GC-based procedures

Since the early 1970s, increasing attention has been devoted to the detection and quantification of a wide variety of organic microcontaminants—mainly because of their highly adverse, i.e., toxic, effects in environmental compartments, in food and human breast milk, and for wildlife. Essentially all of the early xenobiotics, such as the polychlorinated biphenyls (PCBs) and polychlorinated triphenyls (PCTs), the organochlorine pesticides and phthalate esters, shared one characteristic: they could be subjected to GC analysis without prior derivatization or another type of analyte conversion. Such analyses, then usually combined with selective, i.e., thermionic

(NPD), electron-capture (ECD) or flame-photometric (FPD) detection, were markedly successful because of the combination of good selectivity and excellent detectability. Therefore, for other classes of compounds of interest such as, e.g., anilines and phenols, suitable derivatization procedures were devised to enable their inclusion in GC-based methods of analysis.

At that time, sample preparation was almost invariably performed off-line, and liquid–liquid extraction (LLE) and liquid partitioning (LP) were the methods of choice. With many types of sample being of a semi-solid or solid nature, this was an excellent approach—and still is a good approach today, even though one should add that the usual limitation to GC-amenable solvents was more realistic for the classes of compounds which, then, were in the limelight than for the much wider range of analytes of interest today. One main disadvantage was that LLE and LP cannot easily be combined on-line, or even at-line, with column chromatography (CC). This is especially true when (partial) solvent evaporation is a main step to ensure sufficient trace enrichment of the analytes and, consequently, adequate detectability. In addition, for most of the period being considered here—and, unfortunately, in many laboratories even today—injection volumes in GC analysis were 0.5–1 μl. Since such volumes were usually taken from 0.5 to 1 ml extracts obtained after evaporation (and redissolution), a rather meagre aliquot of about 0.1% was all that was injected. This, in its turn, caused samples to have to be unduly large to meet the detection criteria, and organic solvent consumption to be large, with volumes of 100–500 ml per analysis not being exceptional. Quite apart from the distinct environmental unfriendliness of the approach, the ''large sample prep'' versus ''small GC run'' mismatch strongly advocated against attempting to combine both modules on-line in a single set-up and, thereby, initiate a major improvement in system efficiency.

More recently, many scientific papers, reviews and books have discussed how the above deficiency was eliminated by the introduction of modern injection techniques based on the use of an on-column or loop-type interface or a programmed temperature vaporizer: the rapid injection of up to 100 μl of an organic solvent and, consequently, of a sample extract, has become a routine procedure called large-volume injection (LVI) [3–7]. With suitable miniaturization on the front, i.e., the sample preparation, end, it is now possible to inject 10–90% of an extract. The two- to three-orders of magnitude improved performance can be used to facilitate the miniaturization, while simultaneously enhancing analyte detectability. The userfriendliness of modern LVI–GC probably is a main cause of the renaissance of GC-based analytical procedures. However, two other aspects should be included. Solid-phase extraction (SPE), to be discussed in some detail in Section 4 below, was for a long time considered to be useful exclusively for LC. With its easy processing of 10–100 ml volumes of aqueous samples, ''larger sample size'' was the answer of the generally much less efficient LC procedures to the sophistication of GC. However, the 100 μl LVI approach now also allows the direct introduction of all analytes preconcentrated on an SPE cartridge, into a capillary GC column, and the superiority of GC over LC has thereby been re-established.

An interesting example of the above is given in Fig. 2 which shows the SPE–GC–MS analysis of 10 ml of surface water, both raw and after spiking with 86 microcontaminants (0.5 μg/l each). From the full-scan trace of the spiked sample, limits of detection (LODs) of 10–40 ng/l can be calculated for a large majority of all target compounds, and the mass traces for the four diagnostic ions of peak No. 11 (benzaldehyde; concentration, 30 ng/l) demonstrate that analyte identification is straightforward. Actually, if an ion-trap MS is used in the MS–MS mode (which, admittedly, implies a limitation to target compounds) instead of a conventional quadrupole instrument, LODs for 10-ml sample volumes can be improved to at least 0.1–1 ng/l. The favourable results discussed in this paragraph can serve to illustrate the impact of the second additional cause which favoured the re-emergence of GC, the excellent performance, userfriendliness and moderate price of modern GC–MS instruments. At the same time, SPE–GC–MS is a first example of a truly multidimensional technique combining, as it does, the coupled-column and hyphenation approach.

Even in a brief overview such as this, two more detectors providing structural information should be mentioned, the Fourier transform infrared (FT-IR)

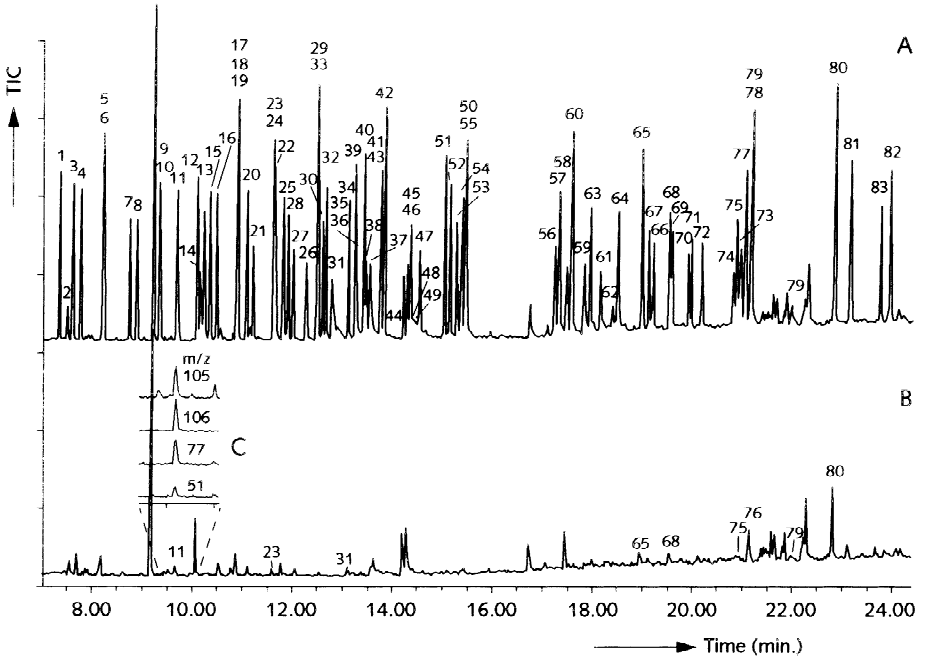


Fig. 2. TIC chromatogram for SPE–GC–MS of 10 ml of river Rhine water, (B) non-spiked and (A) spiked at the 0.5 μg/l level with 86 microcontaminants. A 50-μl volume of methyl acetate was used as presolvent. The insert (C) shows the mass chromatograms of four characteristic masses of benzaldehyde (m/z 51, 77, 105 and 106). The time scale for the mass chromatogram is twice as large as for the TIC chromatogram [3].

and the atomic emission (AED) detection methods. In the recent literature, the latter of these two detectors has been given a prominent place [8–14]. It is now generally appreciated that AED is not as vulnerable as it was once considered to be, that it provides an element-over-carbon selectivity which is frequently much better than that of conventional selective GC detectors and that detectability of many of the more important (hetero) atoms in organic compounds is at the low- or sub-ng level. Excellent wide-ranging studies on GC–AED for pesticide analysis have been published by Stan and co-workers [9–11]. Eisert et al. [12] made a comparative study for several thermolabile pesticides with LC–UV and LC–MS, and GC with FID, NPD, ECD and AED detection. LODs in LC–UV were usually below 1 ng at 220 nm while LC–thermospray (TSP)-MS gave LODs of 1–10 ng (scan) and 60–800 pg (selected ion monitoring, SIM). For GC, LODs usually were <20 pg (FID), <1 pg (NPD), <1 pg (ECD) and <300 pg (AED).

Recently, Brinkman and co-workers [15–17] extensively demonstrated the practicality of SPE–GC–AED and SPE–GC–AED/MS for surface and waste water analysis, and also for the determination of pesticides in food. As regards the latter application, Table 1 shows some interesting results. After recognizing all analyte peaks present above a preset threshold value (a permitted routine because of the validity of the ‘‘universal calibration principle’’) in the various hetero atom channels of interest in AED, identification was completed by recording the mass spectra at the peak apexes. Quantification of the five pesticides (trifluralin, alachlor, bromophos-ethyl, tetrachlorvinphos and azinphos-ethyl in carrot, onion and cauliflower) was done by both MS and AED and was, somewhat surprisingly, slightly better with the latter detection method.

The potential of SPE, or LVI, combined on-line with GC–AED/MS, a typical multidimensional approach, shows up most clearly when tracing all (unknown) compounds containing a certain element—such as, e.g., chlorine because of expected toxicity, or tin because of an anti-fouling agent

Table 1
Comparison of MS and AED detection in quantitative GC analysis of vegetables

Analyte	True value (mg/kg)	Experimental concentration (mg/kg)							
		MS		AED					
		m/z 1	*m/z* 2	Br	Cl	F	N	P	S
Carrot									
Trifuralin	1.24	1.18	1.19			0.98	1.24		
Alachlor	1.31	1.32	1.31		1.35		1.34		
Bromophos-ethyl	1.18	1.19	1.20	1.12	1.07			1.37	1.09
Tetrachlorvinphos	1.24	1.39	1.79		1.18			1.07	
Azinphos-ethyl	1.61	2.00	1.93				1.51	1.87	1.59
Onion									
Trifuralin	1.24	1.53	1.48			1.19	1.24		
Alachlor	1.31	1.31	1.35		1.28		1.31		
Bromophos-ethyl	1.18	1.18	1.11	1.20	1.11			1.27	0.54
Tetrachlorvinphos	1.24	1.30	1.41		1.04			0.95	
Azinphos-ethyl	1.61	1.82	1.79				1.53	1.93	1.38
Cauliflower									
Trifuralin	0.21	0.20	0.20			n.d.	0.20		
Alachlor	0.22	0.22	0.22		0.24		0.20		
Bromophos-ethyl	0.20	0.19	0.20	0.21	0.20			0.16	0.19
Tetrachlorvinphos	0.21	0.22	0.21		0.17			0.10	
Azinphos-ethyl	0.27	0.30	0.30				0.29	0.31	0.25

survey—is the main goal. If more general profiling of organic compounds is the primary aim, i.e., if C, H, and O are the elements of interest, FT-IR rather than AED is, of course, the preferred detection mode. Much of the more innovative work in this area has been performed by Wilkins and his group [18,19] who designed complicated, but elegant, GC as well as multidimensional GC (MDGC) systems with FT-IR plus MS detection. One representative scheme of MDGC–MS/FT-IR is shown in Fig. 3. It was

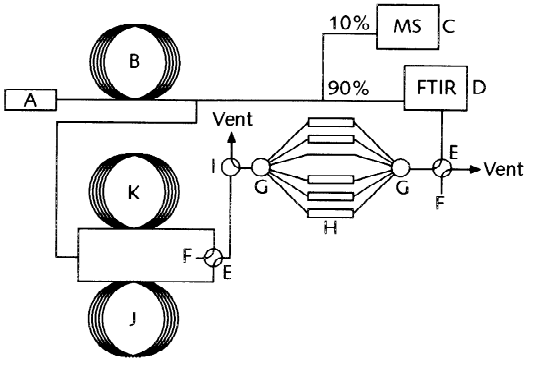


Fig. 3. Schematic set-up of multidimensional GC system with FT-IR and MS detection [20].

successfully used for the analysis of irritants in a variety of soaps. There is no doubt that IR is a powerful detection and identification technique. Nevertheless, its application for trace-level analysis has been limited. This is mainly due to the fact that GC–lightpipe IR is a robust technique but provides insufficient sensitivity, while deposition-based GC-cryotrapping and matrix-isolation IR do give the required sensitivity but cannot be called userfriendly at all. Even tiny amounts of water, or organic solvents, can completely ruin the detection by their crystallization on the trapping material. What can, nevertheless, be achieved is clearly shown in Fig. 4 which features the trace-level analysis of pesticides in surface/harbour water by SPE–GC–cryotrapping FT-IR. With a 20-ml sample, analyte recognition is successful at the 1 ng/ml level if appropriate functional-group chromatograms are recorded.

The essence of the above overview is that a GC-based separation procedure should be preferred whenever all analytes of interest in a sample are amenable to GC without prior derivatization or conversion. Admittedly, this is a somewhat rash statement since no comparison with other separation techniques, notably LC, has yet been made. Still, a

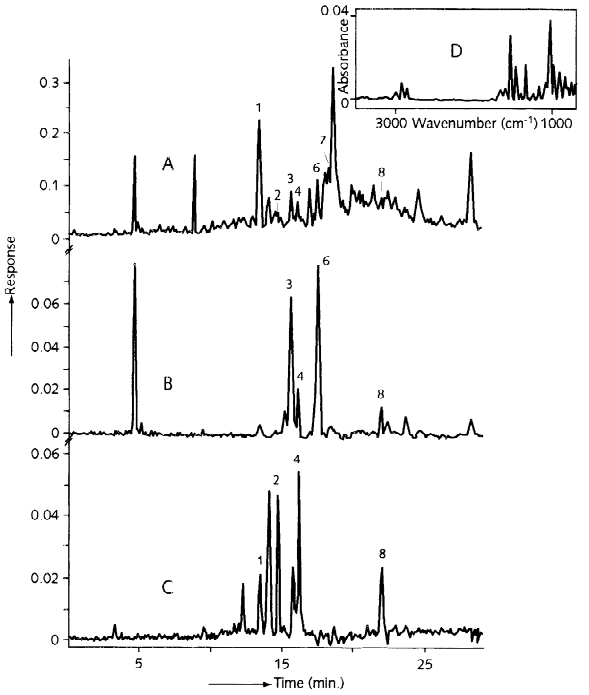


Fig. 4. On-line SPE–GC–IR analysis of 20 ml tap water spiked at the 1.0 μg/l level with several microcontaminants. The Gramm–Schmidt chromatogram is shown. Peak assignments: 1, diethyl phthalate; 2, sulfotep; 3, atrazine; 4, diazinon; 5, caffeine; 6, simetryn; 7, metolachlor; 8, triazophos [21].

large majority of all experienced analytical chemists will agree. There is, however, an increasing interest in non-GC-amenable compounds. To give a few examples, these include 15–20% of the present-day pesticides, e.g., phenylureas and carbamates, and the phenoxyalkanoic acids, and a large majority of all pesticide TPs. In addition, many substituted anilines and phenolic compounds easily create problems during GC analysis, and many detergents, most dyes and essentially all breakdown products of chemical warfare agents and a majority of all toxins require a non-GC approach. Finally, whenever aqueous samples or (largely) aqueous sample extracts have to be analysed, subjecting them to an SPE–LC rather than to an SPE–GC procedure (irrespective of the detector to be used) will be the general preference because of the easier interfacing, specifically because most LC are reversed-phase LC (RPLC) procedures.

In summary, in view of the rapidly growing interest in polar and ionic chemical compounds and in, almost invariably rather polar, breakdown products of pesticides and, also, many other classes of compounds, there is an urgent need to develop and evaluate LC-based separation-plus-detection procedures which can compete with, and be complementary to, their GC-based counterparts.

3. Pesticide analysis: LC-based procedures

In GC analysis, integration was introduced (before the word had been coined for the purpose) at the back-end of the system, with GC–MS becoming the best-known example. In LC, however, the set-up of integrated systems was a typically front-end affair. Early attempts at utilizing LC–LC column-switching procedures for heartcut operations showed these to be, in principle, possible. However, they also clearly demonstrated that the LC eluent switch almost invariably required to effect a change of separation mechanism often caused serious interfacing problems, such as the removal of buffers, the evaporation of fairly large amounts of water, or organic solvent–water incompatibility problems. Next, it was recognized by a few authors—but not immediately understood by all of them, which is somewhat difficult to believe today!—that introduction of an aqueous sample solution on an alkyl-bonded silica column—then, as now, usually a rather hydrophobic C_{18}- or C_8-bonded silica column—caused a dramatic trace enrichment of most analytes in that sample at the very top of such a column. Once the beneficial effects of this phenomenon in terms of improved analyte detectability if expressed in concentration units, had been realized, its potential was rapidly put to good use with the design of so-called precolumns.

Such precolumns, more often called SPE cartridges today, typically had—and still have—dimensions of 1–2 cm length and 1–4 mm I.D. and contain some 50–60 mg of a sorbent. As a rule of thumb one may add that, for almost all conventional sorbents, such a mass can retain at least 50–60 pg of analytes. Since detection problems (and avoiding these is what SPE primarily is about) usually are at, or well below, the 50–60 ng level, it will be clear that insufficient capacity is not a real issue during most SPE–LC procedures. What, on the other hand, is a real issue is the breakthrough volume, V_B, of the individual

analytes under realistic conditions, i.e., at trace-level concentration in real samples. Initially, the required time-consuming experiments were faithfully performed for each individual analyte, and it took some time before it was realized that there should be a direct correlation between the V_B values and the capacity factors of the analytes, if extrapolated in the proper way to pure water, κ'_{water}, and/or with the logarithms of the octanol–water partition coefficient, log K_{ow}. An excellent review on this subject has been written by Hennion [22].

Extensive experimental studies in this area have meanwhile made it abundantly clear that moderately polar analytes with log K_{ow} values of 2–4 or higher, can be preconcentrated from, at least, 30–100 ml aqueous solutions onto a conventional-size C_{18}-bonded silica cartridge without breakthrough [23,24]. Changing over to the much more hydrophobic styrene–divinylbenzene copolymers such as PLRP-S and PRP-1 typically increases the V_B values found for C_{18}-type sorbents 20–50 fold [25] (cf. Fig. 5). This implies that adequate retention now can also be achieved for analytes as polar as phenol, aniline and oxamyl, with their log K_{ow} values of less than 1.0–1.5. An additional sorbent of interest is graphitized carbon black (GCB) which has been studied extensively by Hennion and co-workers [26,27]. The sorption mechanism of GCB is notably different from that of the conventional hydrophobic materials discussed above. This causes surprisingly high retention of polar compounds like breakdown products of atrazine, desisopropyl- and desethylatrazine. A distinct drawback of GCB is that efficient desorption of the trapped analytes requires relatively large volumes of, e.g., pure acetonitrile, methanol or dichloromethane [28,29]. Initially, this made on-line combination with LC seem essentially impossible. However, more recently it has been convincingly demonstrated that diluting such an organic effluent with an excess of water (which can be done on-line) allows efficient retrapping on a conventional C_{18}-cartridge, and eliminates the main deficiency of GCB as a sorbent in on-line SPE–LC [30]. With all of these procedures, one should be aware that the presence of large amounts of humic acids in, e.g., surface and pond water, may seriously decrease breakthrough volumes (see, e.g., Refs. [31,32]). An excellent review on multiresidue methods using SPE for monitoring priority pesticides in ground and surface water was published by Sabik et al. [33].

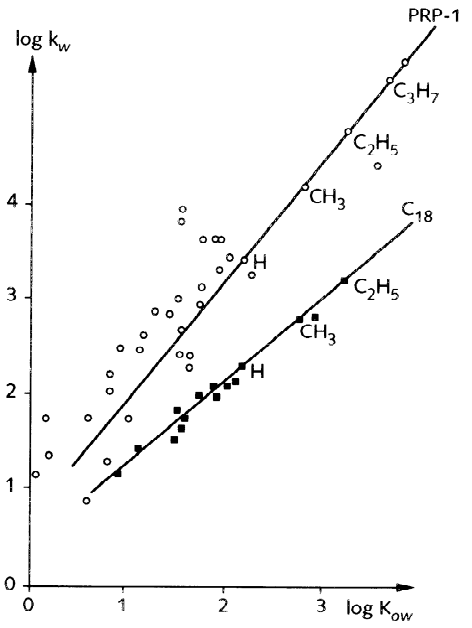


Fig. 5. Relationship between octanol–water partition coefficient (log K_{ow}) and retention factor estimated or measured in water (log k_w) on C_{18} silicas and PRP-1 copolymer for various organic compounds [34].

Most more selective types of sorbent, such as ion exchangers, metal-loaded sorbents, PAH-selective sorbents and restricted-access media, do not play a major role in SPE–LC-based environmental analysis, because the emphasis is much more frequently on trapping whatever microcontaminants may be present in a sample than on selecting one or a few of them only. They will, therefore, not be considered here. The only exception are the immunoaffinity-type sorbents (IASPEs) [35–43]. As selective sorbents par excellence, they are, in principle, in the same class as the other selective sorbents and should therefore also be disregarded. However, they continually attract attention because of the many successful applications in the biomedical and veterinary field. A recent application [44] of IASPE-LC–MS–MS for the determination—and simultaneous confirmation, one may add—of carbendazim in water and soil samples illustrates the potential and weakness of the approach. Not unexpectedly, excellent LODs of below 30 ng/g (soil) and 10 ng/l (water) were obtained by

the authors, which contrasted sharply with some 100-fold higher LODs for UV detection. However, the need to use such a sophisticated approach for a single analyte will be quite limited, and the added value of either the IASPE step or, alternatively, the tandem MS mode, was not really made clear in the paper. In this context, the approach of Hennion and co-workers [35–40] is more attractive. These authors successfully tried to prepare and/or mix IASPE sorbents which show more or less the same reactivity towards all members of a class of analytes, such as the phenylureas or triazines—which is a much more relevant option in environmental analysis. A typical result is shown in Table 2, which summarizes results obtained for phenylureas when bonding polyclonal anti-isoproturon or anti-chlortoluron antibodies, and for triazines when bonding anti-atrazine or anti-simazine antibodies. In another study, Lawrence et al. [35] used immunoaffinity cartridges in the clean-up of urea herbicides in plant samples. The extracts obtained were clean enough to permit quantitative LC with UV detection at low ng/l levels in plant extracts. For an interesting multi-residue IASPE-GC study, applied to river and waste water and soft drinks, the reader should consult Ref. [42].

3.1. Dual-precolumn set-up

In an early paper on the analysis of industrial wastewater [45], the advantages of using a serial precolumn set-up consisting of C_{18}-bonded silica/PRP-1 copolymer/ion exchanger were vividly demonstrated (Fig. 6) (note that all but two test compounds show up on a single precolumn only). An early diode array UV detector was used for confirmation of identity. Since then, three or more precolumns in series have appeared in very few papers. One notable exception is the use of four precolumns in a study on the determination of sugars in fermentation broths and spent sulfite liquor [46]. Combination of the SPE^4–LC set-up with a post-column enzyme reactor and UV and electrochemical detection yielded a noise-free baseline for the recognition of as little as 10^{-4}–10^{-5} *M* of the sugars.

Table 2
Potential of class-selective immunosorbents (ISs) as measured by analyte recoveries from samples spiked with a mixture of phenylureas or triazines

Analyte	Anti-isoproturon IS	Anti-chlortoluron IS
Phenylureas		
Metoxuron	21	80
Monuron	98	78
Chlortoluron	95	95
Isoproturon	99	90
Difenoxuron	37	17
Buturon	64	62
Linuron	61	85
Chlorbromuron	95	102
Diflubenzuron	101	76
	Anti-atrazine IS	Anti-simazine IS
Triazines		
Desisopropylatrazine	<5	56
Hydroxyatrazine	60	<5
Desethylatrazine	98	30
Simazine	99	93
Cyanazine	91	74
Simetryn	63	17
Atrazine	99	88
Prometron	65	<5
Sebutylazine	88	91
Propazine	101	57
Terbutylazine	98	85

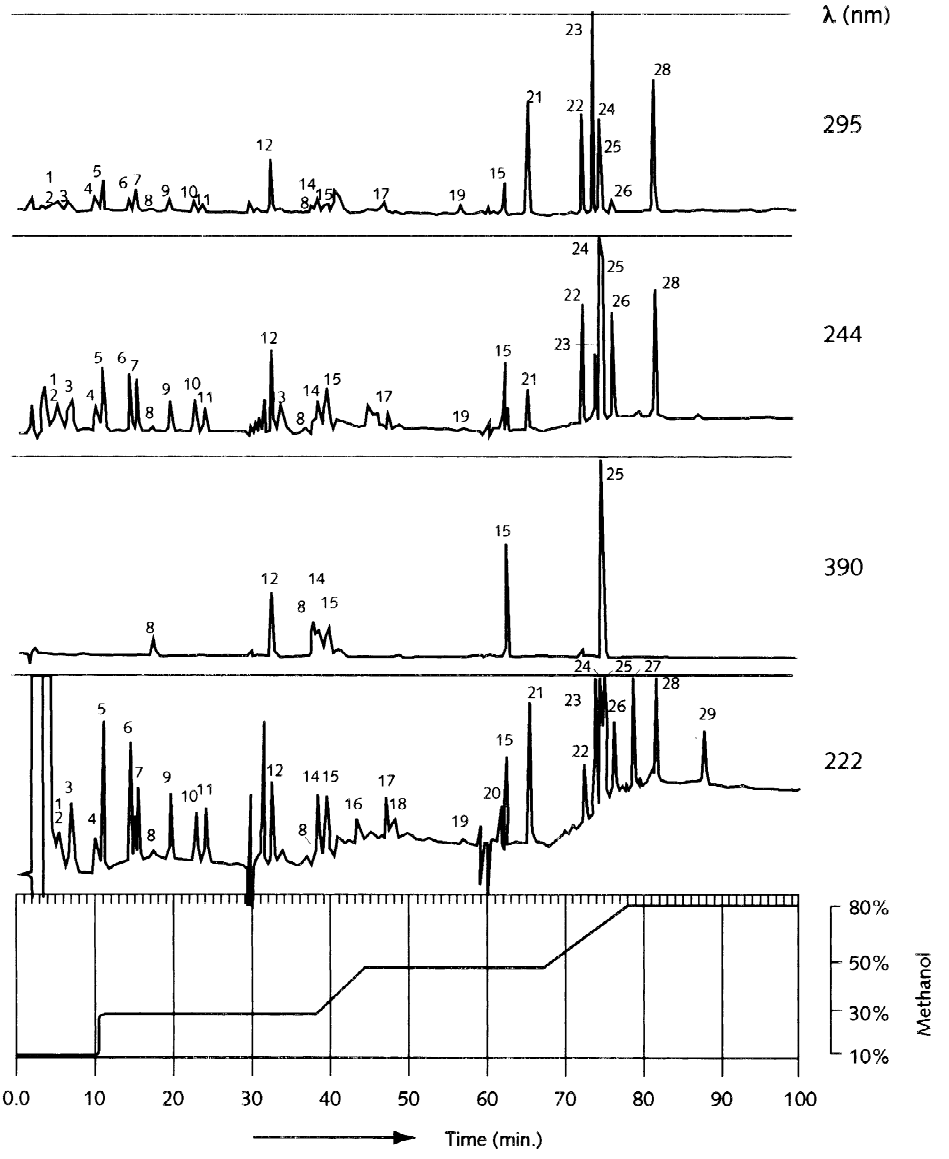


Fig. 6. Multiwavelength plot of a 5-ml standard solution containing 200 μg/l of selected pollutants using C_{18} silica/PRP-1 copolymer/ion-exchange SPE and on-line LC–UV analysis [45].

Today, it is more common to find dual-precolumn set-ups—both in the environmental and other application areas—in which the first precolumn serves to get rid of suspected ''background contamination'' while the second precolumn takes care of the actual trace enrichment. An elegant example was presented by Brouwer and Brinkman [47] who used a PLRP-S/ENVI-Chrom P combination to determine phenol which, with UV detection, is often obscured by humic/fulvic acids. Out of a 50-ml sample, the first 45 ml was passed through the first precolumn only. The humic/fulvic acids as well as all substituted phenols less polar than the parent phenol are trapped/enriched, but phenol itself breaks through rapidly and is largely lost. The final 5 ml of sample is sent through both precolumns in series: the humic/

fulvic acids again are trapped on the first column, and the second one receives an essentially interference-free phenol solution. The considerable improvement so obtained is illustrated by the LC–UV traces shown in Fig. 7. Detection limits for 50-ml samples were as low as 0.5 μg/l. In this context, one should also mention the successful C_{18}/cation-exchange tandem SPE studies on atrazine ozonization products, which bracketed the polarity range from the parent compound through to cyanuric acid [48].

Very recently it was shown that the above principle cannot be used with real confidence for analytes which are distinctly more polar than phenol, such as, e.g., oxamyl and desethylhydroxyatrazine [49]. Too much then depends on the composition of each type of sample offered for analysis, and a solution has to be found via increased selectivity provided by electrospray ionization (ESI)-MS–MS detection. With 50-ml surface water samples, LODs now are as low as 1–50 ng/l. Should one argue that, in that situation, the humic/fulvic acids removal is superfluous, a strong argument in favour of maintaining the dual-precolumn set-up is that the frequently observed signal suppression often caused by such compounds, now will be largely eliminated (see also Section 4). Rather similar results were reported for six very polar organo-P pesticides such as, e.g., acephate and omethoate. Here, 1-ml LVI–LC–atmospheric pressure chemical ionization (APCI)-MS–MS was used for surface, ground and tap water analysis, with 10–30 ng/l LODs for the analytes of interest [50].

4. Pesticide analysis: SPE–LC-based systems

Most of the early applications of SPE–LC, which were largely of the SPE–LC–UV type, were in the biomedical field. One should mention the work of Zech and co-workers [51,52] who used the on-line parallel precolumn design and analysed over 15 000 urine and serum samples containing urapidil and its hydroxylated and demethylated metabolites over a 2-year period, and amply demonstrated the ruggedness of the analytical procedure (UV and electrochemical detection).

In the field of environmental analysis most of the initial work was devoted to limited numbers of analytes (with Ref. [45] quoted above, as a notable

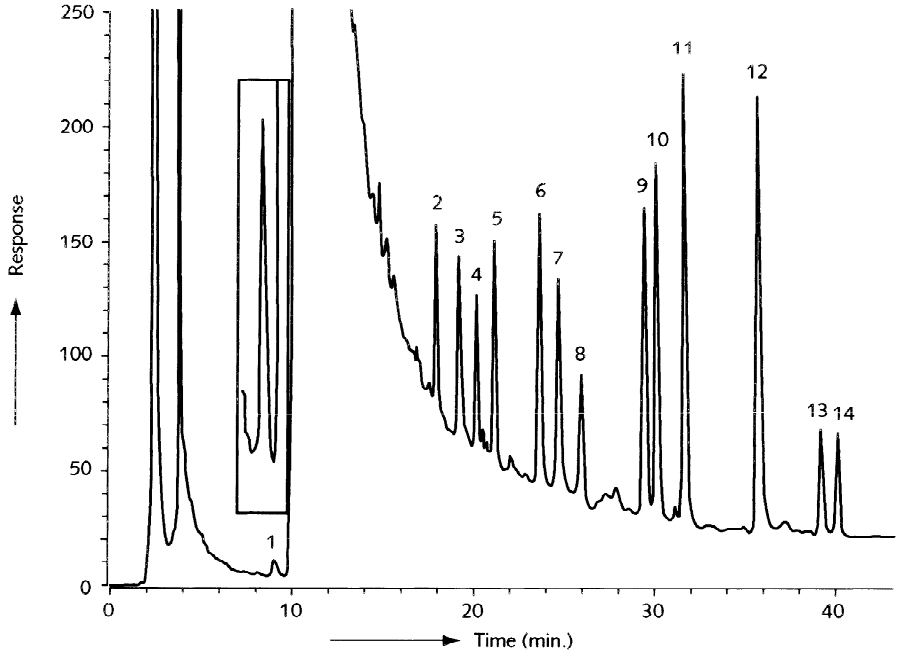


Fig. 7. On-line SPE–LC–DAD UV chromatogram (λ=210 nm) of 50 ml surface water, spiked with 14 phenols (5 μg/l each), using two precolumns (PLRP-S/ENVI-Chrom P) in series. Insert shows blow up of peak No. 1 (phenol) [47].

exception). From among these, a study on the carbamate pesticide carbaryl merits attention [53]. It combined C_{18}-SPE–LC with on-line post-column hydrolysis on an ion exchanger at 100–120 °C to yield methylamine which was, next, reacted with *o*-phthaldialdehyde, the reaction product being monitored fluorimetrically. The procedure was adopted by de Kok and co-workers [54–56] and widely applied for the trace-level determination of *N*-methylcarbamates and their TPs in water as well as fruit and vegetables (Fig. 8). The real breakthrough came when Huen et al. [57] and Brinkman and his group [58–62] started to use SPE–LC–diode array detection (DAD) UV for large numbers of pesticides and related compounds, and introduced semi- and full automation. The design of the latter workers was extensively tested in the framework of the Rhine Basin Program, and became known as the SAMOS (System for the Automated Measurement of Organics in Surface water) system. Typically, 100 ml of surface water is pumped through a PLRP-S SPE cartridge and, after brief flushing of the cartridge, separated by means of an acetonitrile–water gradient and detected at appropriate UV wavelengths. A typical real-life result, the detection and DAD UV-based confirmation of the ubiquitous diuron, is shown in Fig. 9. Today, the system is used—either in its original or in an adapted form—for early-warning and/or monitoring purposes in many European countries, and especially in the UK [63,64]. The SAMOS approach has been reported in detail in many papers and need not detain us here. However, a few remarks should be made which are relevant in view of our subsequent discussions. Firstly, a SAMOS LC run typically takes about 50 mm. This is not bad considering that: (i) two samples can be handled simultaneously [LC of *n*th sample plus SPE of (*n*+1)th sample], and (ii) sample taking on big rivers can be limited to 3–4 times per 24 h. However, in view of the typical real-life situation of only a few, and

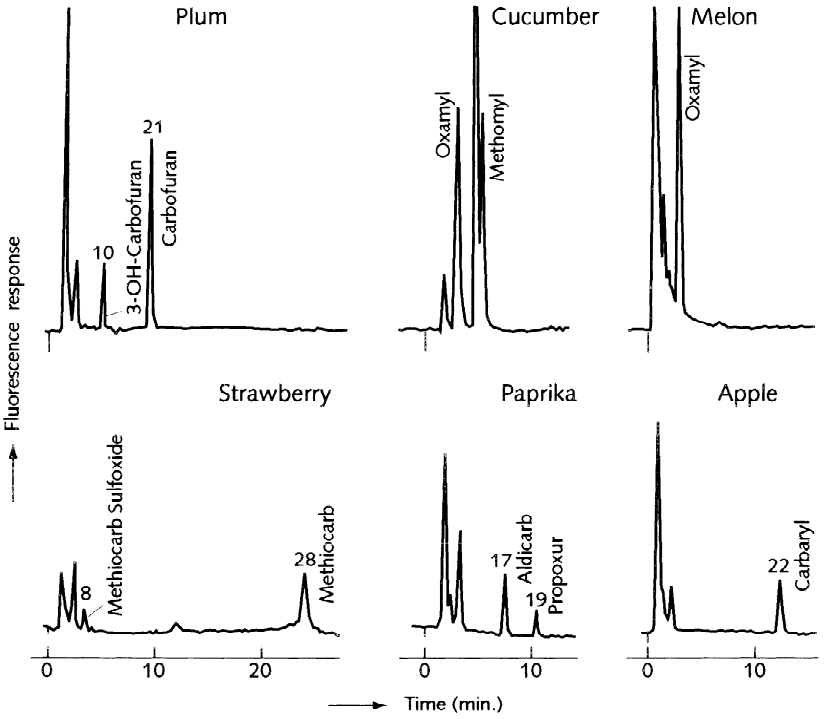


Fig. 8. RPLC with post-column fluorescence reaction detection of crop sample extracts containing real residues of various *N*-methylcarbamates. Residue concentrations: (melon) 0.04 μg/g oxamyl; cucumber 0.28 μg/g oxamyl and 0.07 μg/g methomyl; (plum) 0.02 μg/g carbofuran and 0.01 μg/g 3-hydroxycarbofuran; (apple) 0.05 μg/g carbaryl; (paprika) 0.008 μg/g aldicarb and 0.003 μg/g propoxur; (strawberry) 0.02 μg/g methiocarb and 0.005 μg/g methiocarb sulfoxide [54].

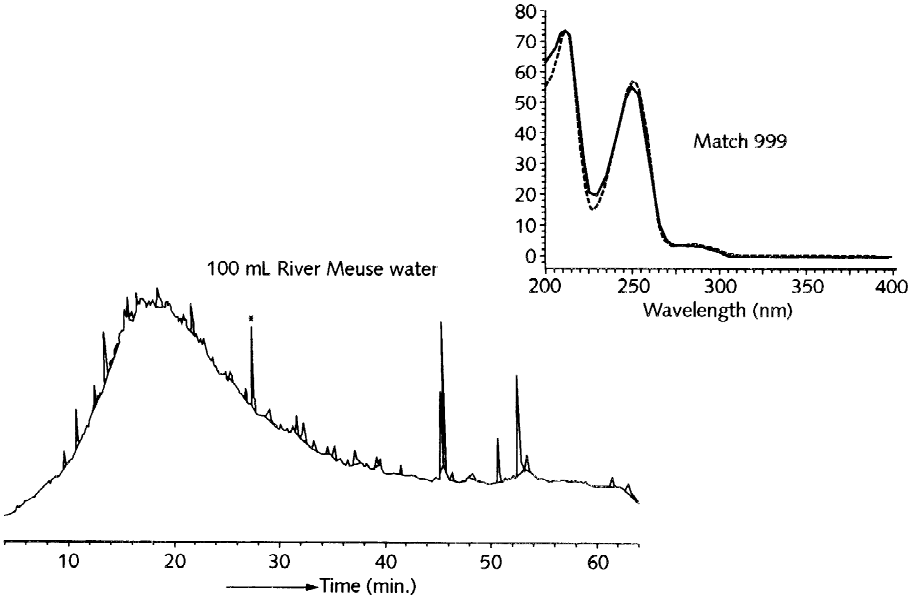


Fig. 9. On-line SPE–LC–DAD UV chromatogram (λ=210 nm) of 100 ml surface water (river Meuse). Insert shows library DAD spectrum and spectrum recorded for peak at 27.72 min.

often no suspected peaks per run (cf. Fig. 9), one may well be tempted to set up a faster system for rapid screening purposes. Secondly, every DAD UV-based suspected positive result of higher than ca. 0.3–1 μg/l has to be confirmed by an MS-based procedure and, again, now because of cost-effectiveness, this confirmatory run should be as short as possible. Finally, although the SAMOS system meets the usual alert level of 1 μg/l for most analytes of interest, improving its performance without increasing the sample volume, is desirable.

There is no single solution to all of the above problems, because too much depends on the specific demands made with each individual problem. In the next sections, we shall discuss alternatives such as SPE–LC–MS or –MS–MS for confirmation and improved selectivity, the use of a single short column or even no column at all, i.e., flow-injection analysis (FIA), to speed up analysis and, possibly, have better detectability, and the adaptation of the complete set-up for the target determination of a single analyte known to be the only violative compound (possibly) present.

With few exceptions, conventional detection methods other than (DAD) UV have not been used to any extent for wide-ranging screening purposes. However, it is interesting to add that, recently, SPE–LC–UV was combined with at-line ToxPrint detection for (geno)toxcicity screening of subsequent 1-min fractions [65]. Exceptions are the use of fluorescence detection, whether in combination with post-column reaction detection (cf. Refs. [53–56] above and Refs. [66–68]), or for direct detection, e.g., for naphthalene sulfonates by means of micro-LC [69] or capillary electrophoresis [70] and of electrochemical detection for, e.g., a variety of chloroanilines [71] and chloro- and nitrophenols [72]. The only detector in frequent, and increasing, use today next to the UV detector, is the quadrupole or ion-trap mass spectrometer already briefly referred to above, which will be discussed in some detail below. The emphasis will be on applications; for detailed information on interface design, operation and optimization, the reader is referred to [73,74]. An interesting overview of the achievements [75] and obstacles [76] of LC–atmospheric pressure ionization (API)-MS in water analysis was published a few months ago.

4.1. MS-based detection

In the present section, only the more recent

literature will be discussed. This implies that three types of interfaces will be mentioned, the TSP, particle beam (PB) and, specifically, API interfaces, with the ESI and APCI interfaces as the representatives of the API type.

LC–TSP-MS was much in vogue 5–10 years ago, and a number of selected applications are summarized in Table 3 [77–86]. As is evident from the entries, substantial analyte enrichment by means of LLE (off-line) or SPE (off-line or on-line) is necessary to reach the 0.1–1 μg/l detection limits that are typically required today. Fig. 10 provides an interesting example: with on-line SPE–LC–TSP-MS, and the use of time-scheduled (TS) SIM, 50 ml of surface water suffice to confirm the presence of the three analytes of interest, simazine, atrazine and diuron at the 0.5–1 μg/l level. The detection limits of about 5–15 ng/l that can be calculated for these analytes, are up to modern standards, and similar results can be achieved for a large number of

Table 3
Selected applications of LC–TSP–MS(MS)

Analytes	Sample volume and matrix	Extraction technique	LC eluent	MS detection mode	Limit of detection (ng/l)	Ref.
• 39 polar pesticides+ carbamates+phenylureas+ organophosphorus pesticides	50–100 ml drinking and surface water	On-line SPE C_{18}	0.1 *M* ammonium acetate–methanol	TS-SIM	2–90	[77]
• 15 phenylureas	50 ml surface water	On-line SPE C_{18} membrane or PLRP-S	0.1 *M* ammonium acetate–methanol	TS-SIM	5–20	[78]
• Phenoxy acids+bentazone	150 ml surface water	On-line SPE C_{18}	0.05 *M* ammonium formate–acetonitrile	TS-SIM	5–100	[79]
• 128 polar pesticides	1 l water	Off-line SPE C_{18}	Methanol–water; 175 m*M* ammonium acetate postcolumn	LC–MS–MS daughter-ion mode TS-SIM	10–100	[80]
• 39 polar pesticides	100 ml surface water	On-line SPE PLRP-S	0.1 *M* ammonium acetate–acetonitrile	LC–MS–MS RF[a]-only FS	50–1000	[91]
• Nitroaromatic explosives+TPs	water	SPE PLRP-S	Methanol–water	MS–MS SRM	10–100	[81]
• Carbamates	100–500 ml water	Off-line SPE Empore C_{18} on-line SPE C_{18}	0.05 *M* ammonium acetate–methanol or acetonitrile	SIM	1–10	[82]
• Azo dyes	1000 ml waste water	Off-line LLE dichloromethane	0.1 *M* ammonium acetate–methanol	MS–MS	180–2000	[83]
• Triazines	100 ml water	off-line SPE C_{18}	0.05 *M* ammonium acetate–methanol	MS–MS SRM	10–100	[85]
• Uron and carbamate pesticides	500 ml drinking water	Off-line SPE C_{18}	0.1 *M* ammonium acetate–methanol	SIM	40	[86]
• 52 pesticides	Water	Off-line/on-line SPE C_{18} LLE	0.1 *M* ammonium acetate–acetonitrile	FS	1000–25 000	[87]
• Triazines	48 ml surface water	On-line C_{18} SSC	0.1 *M* ammonium acetate–methanol	SSC–LC–MS–MS product ion mode	1000–10 000	[92]
• 10 organo-P pesticides and TPs	100 ml river water	On-line SPE C_{18}	0.05 *M* ammonium formate–acetonitrile	TS-SIM	10–100	[88]
• Triazines+TPs	150 ml surface water	On-line SPE LiChrolut EN	0.01 *M* ammonium acetate–acetonitrile	TS-SIM	100–10 000	[89]
• Triazines+TPs	10–50 ml ground water	SCX	Methanol–water (ammonia)	LC–MS–MS SIM	100	[90]
• Pesticides+TPs	150 ml surface water	On-line SPE PLRP-S	0.05 *M* ammonium formate–acetonitrile	SIM	10–3000	[84]

[a]RF; radio frequency.

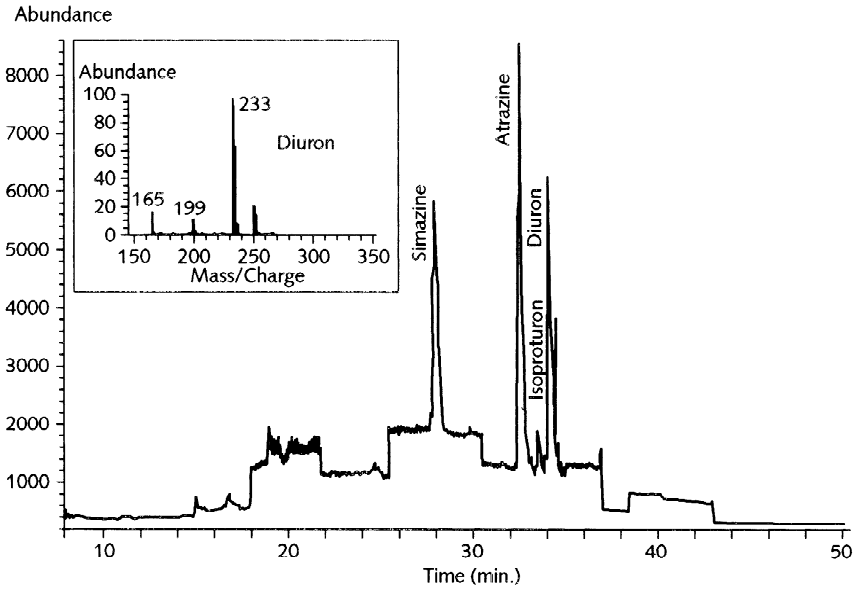


Fig. 10. On-line SPE–LC–TSP-MS of 50 ml of river Meuse water (ions at m/z 202, 207, 216 and 233 monitored) [77].

pesticides, notably phenylureas, triazines, organo-P pesticides and carbamates. Despite such rewarding results TSP is, today, not on the scene anymore. However, one should not forget Arpino's statement that the introduction of the thermospray interface undoubtedly was a milestone in the establishment of LC–MS as a routine analytical tool in the laboratory [93].

The role of LC–PB-MS for trace-level analysis has always been hotly debated. Distinct disadvantages are the non-linear response (which has, however, been eliminated in more modern studies) and, more importantly, the rather poor sensitivity. The latter moreover differs from compound (class) to compound (class) in an often unpredictable way. This phenomenon argues against low transfer efficiency as the only or main cause of the low sensitivity. On the other hand, the unique advantage of the PB over other interfaces for LC–MS is that electron ionization (EI) type spectra are generated which provide an abundance of structural information and allow searches to be made via conventional ''GC–MS'' libraries. This characteristic helps to explain why quite a number of papers on LC–PB-MS and SPE–LC–PB-MS report the detection and identification of many tens of organic microcontaminants in environmental samples. It also explains the continued usage of the technique, with the recent development of a microflow PB interface [94] and the re-interpretation of an initial erroneous identification of bentazone in river water as dichlorobenzoic acid [95], as elegant examples.

The potential and limitations of PB-MS can be illustrated by quoting the several studies on the Multianalysis system [96–99]. This offered the use of a single SPE module to serve both an LC and a GC system, with a DAD UV detector at the LC outlet, and a single mass spectrometer to provide GC–MS as well as LC–PB-MS detection. System operation was satisfactory, and the practicality of the approach was demonstrated during a 2-year monitoring programme of the Nitra river [99]. Each of the three detection techniques contributed to the final goal, the identification of microcontaminants. However, GC–MS was much more successful than the two LC-based procedures and, more importantly, analyte detectability was considerably better for GC–MS, with LODs of 1–10 ng/l (10 ml samples) as against 1–5 μg/l (200 ml samples) for LC–PB-MS as the most notable difference.

In the early 1990s, API ousted TSP as competitive interface for LC–MS. Most API-MS instruments are of the bench-top type and have userfriendly software (Windows 95/NT). Additional structural information

can often be obtained by means of collision-induced dissociation (CID) in the preanalyser region. Not surprisingly LC–API-MS instruments are used routinely in the fields of biology, biochemistry, and environmental, food and pharmaceutical chemistry.

In API, three steps can be distinguished [73,74,100,101] in the ionization process (see Fig. 11): (1) nebulization of the sample solution, (2) liberation of ions from droplets, and (3) transportation of ions from the API region into the vacuum and mass analyser of the mass spectrometer. In the APCI interface the column effluent is pneumatically nebulized into a heated tube, where the solvent evaporation is completed. In the same region, the electrons from a corona discharge needle initiate the ionization and the generated ions are sampled into the high vacuum of the MS system. In the ESI interface, pneumatic nebulization of the column effluent is assisted by application of a 3 kV potential between the capillary and the counter electrode.

Generally speaking, acidic and basic—i.e., more polar—compounds can best be analysed by ESI-MS, while less polar compounds are more amenable to APCI-MS [102,103]. Both APCI and ESI effect soft ionization with a high ionization efficiency; that is, they provide molecular mass information and excellent sensitivity—the drawback is that, with little fragmentation, proper identification is not always straightforward. In the case of ESI, there is no need to use high temperatures which seriously reduces the risk of decomposition of thermolabile compounds.

TS-SIM and positive-negative (PI–NI) polarity switching are options that are widely used today, to enable the determination of most compounds at the required low detection limits and under suitable operating conditions. With SPE–LC of 100 ml samples, Slobodnik et al. [99] compared ESI-MS and APCI-MS detection. LODs for a test mixture of 17 pesticides with APCI and ESI were similar, viz., 0.007–3 μg/l in full-scan and 0.1–200 ng/l in TS-SIM. The authors also demonstrated the successful use of a laboratory-made library with 60 compounds for searching product-ion spectra at low analyte levels (10 ng/l) in tap water. Aguilar et al. [104] used automated on-line C_{18}-SPE to concentrate 200-ml samples. Pesticides were determined by APCI-MS in the TS-SIM mode with LODs of 0.8–4 ng/l (PI mode) and 4–20 ng/l (NI mode). To monitor

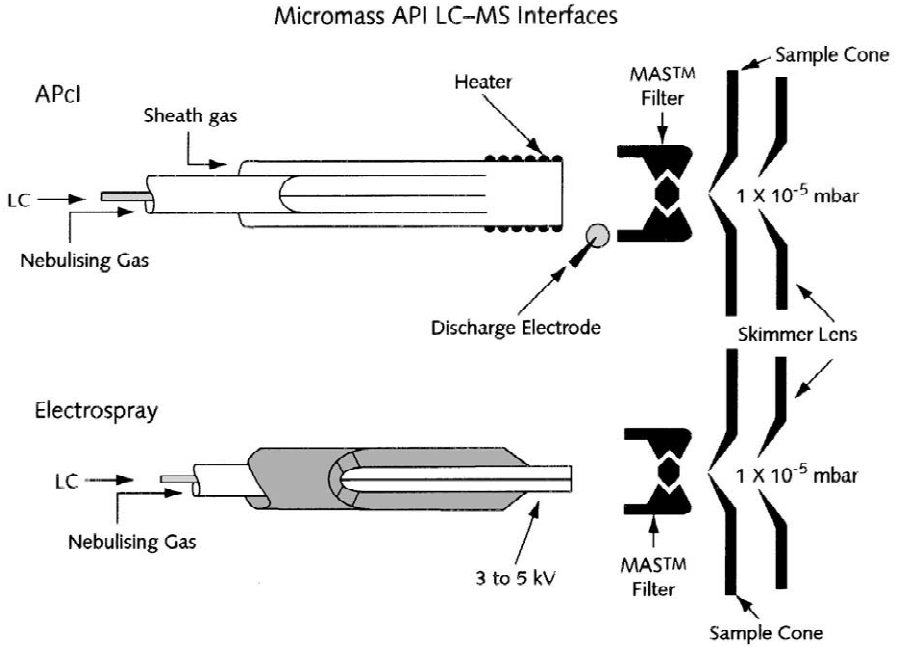


Fig. 11. Schematic presentation of API ionization process. (i) Nebulization of sample solution, (ii) liberation of ions and (iii) transportation of ions into the vacuum.

analytes in water in the ng/l range, large sample volumes were extracted by D'Ascenzo et al. [105]. One liter of ground, or 2 l of tap water were enriched on GCB to determine base/neutral pesticides by LC–ESI-MS in the PI mode, and acidic pesticides in the NI mode, with LODs of 3–10 ng/l (tap) and 6–20 ng/l (ground water) using TS-SIM. Out of a 200-μl final extract, 50 μl was injected. Di Corcia et al. [106] determined phenylureas and some of their degradation products in natural water with LC–ESI-MS. Samples of 4 l were enriched on GCB and LODs were 3–11 ng/l (tap water), 6–21 ng/l (ground water) and 36–75 ng/l (surface water). These studies demonstrate that ng/l detection limits can be achieved by modern LC–API-MS-based procedures, provided there is sufficient analyte enrichment during sample preparation. Selection of the interface is largely determined by the application as was demonstrated for 10 polar pesticides (mainly carbamates) in strawberries and plums which could not be included in current GC–MS multi-residue techniques. Generally, similar results were obtained with the APCI and ESI interfaces. However, APCI-MS was found to be the more sensitive technique for, e.g., carbofuran and aldicarb and its TPs and showed, in the authors' hands, greater flexibility regarding LC flow-rates. It was, therefore, the interface of choice [107].

Other classes of compounds that have been determined in aqueous samples by means of LC–API-MS include the arylphenoxypropionic herbicides [108] and antifouling agents [109] and, in industrial effluents, polar benzene and naphthalene sulfonates [110,111]. Some further examples will be quoted in a subsequent section when discussing the single-short-column approach.

One topic that has not yet been discussed is the comparison of RPLC-based separation procedures combined off-line and on-line with SPE. Scrutiny of the literature reveals that much the same results—i.e., LODs in terms of concentration units—are obtained with sample volumes of 1–2 l for the off-line, and 50–100 ml for the on-line situation. This reflects the well-known advantage of a ''minor aliquot'' versus ''total sample'' analysis. With the added merits of less manipulation, no use of organic solvents and easier automation, it is obvious that for all situations in which large numbers of samples have to be analysed, the on-line approach is to be preferred. A number of illustrative examples are presented in Tables 4 and 5 and a typical result obtained when comparing LC–ESI-MS and LC–ESI-

Table 4
Selected examples of LC–API-MS(–MS) combined off-line with sample treatment for the determination of pesticides and TPs in water

Analyte class	Volume (ml)	Water sample	Sample prep.	Ionization/ scan mode	LODs (ng/l)	Ref.
Phenoxy acids+sulfonylureas	2000	Tap	SPE	ESI-NI, FS/SIM	1–10	[112]
Triazmes+phenylureas	1000	River	SPE	ESI-PI, FS	1–20	[29]
Organo-Ps+carbamates+ acetanilides	4000	Tap	SPE	ESI-PI, FS	0.2–0.6	[113]
Triazines+phenylureas+ acetanilides	1000	Ground	SPE	ESI-PI, SIM	5–10	[105]
Phenoxy acids+dinitrophenol+ benzothiazole	1000	Ground	SPE	ESI-NI, SIM	5–10	[114]
Triazmes+phenylureas	1000	Tap	LLE	APCI-PI, SIM	1–6	[115]
Triazines+TPs+phenylureas	1000	Tap+ river	LLE/SPE	APCI-PI, FS	10–50	[116]
Phenoxy acids+sulfonylureas	1000	River	SPE	ESI-NI, FS-MS–MS	2–20	[117]
Triazines+phenylureas+ acetanilides+TPs	1000	River	SPE	ESI-PI, SRM	0.2–8	[7]
Triazines+TPs+phenylureas+ acetanilides	1000	River	LLE/SPE	APCI-PI ion trap	2–6	[118]
Base-neutral and acidic pesticides	500	River	SPE	ESI, PI+NI	5–15	[119]

Table 5
Selective examples of LC–API-MS(–MS) combined on-line with sample treatment for the determination of pesticides and TPs in water

Analyte class	Volume (ml)	Water sample	Sample prep.	Ionization/ scan mode	LODs (ng/l)	Ref.
Triazines+phenylureas+ organo-Ps	200	Tap	SPE	APCI-PI, SIM	1–4	[120]
Phenoxy acids+bentazone+TPs	50	River	SPE	ESI-NI, SIM	10–30	[121]
Phenoxy acids+TPs+ bentazone+TPs	50	Estuarine	SPE	APCI-NI, SIM	20–100	[122]
Organo-Ps+TPs	200	Ground	SPE	ESI-PI, SIM	10–200	[118]
Triazmes+phenylureas	20	Ground	SPE	APCI-PI, SIM	1–5	[123]
Triazines	50	River	SPE	ESI-PI, SRM	1–5	[48]
Phenoxy acids	50	River	SPE	ESI-NI, SRM	2–50	[48]
Triazines+TPs+phenylureas+ acetanilides	100	River	SPE	APCI-PI, FS-MS–MS	10–200	[124]
Phenoxy acids	100	River	SPE	APCI-NI, FS-MS–MS	20–150	[124]
Triazines+ phenylureas	4	River	SSC	APCI-PI, SRM	10–50	[126]
Quatenary N^+	30	Tap	SPE	ESI, FS-MS–MS	10–70	[125]

MS–MS in the selected reaction monitoring (SRM) mode is depicted in Fig. 12. The general move to lower sample volumes is also evident from a study on about 30 pesticides, where 1.3 ml water samples sufficed to obtain fully satisfactory results down to at least 0.1–0.5 pg/l when using on-line SPE–LC–ESI-MS–MS [127]. Additional literature on this topic is quoted in a recent review [128]. One further remark that may be added here is that the information on quantification reported in the studies quoted in Tables 4 and 5 and other such papers is not always as complete as one would like it to be. On the other

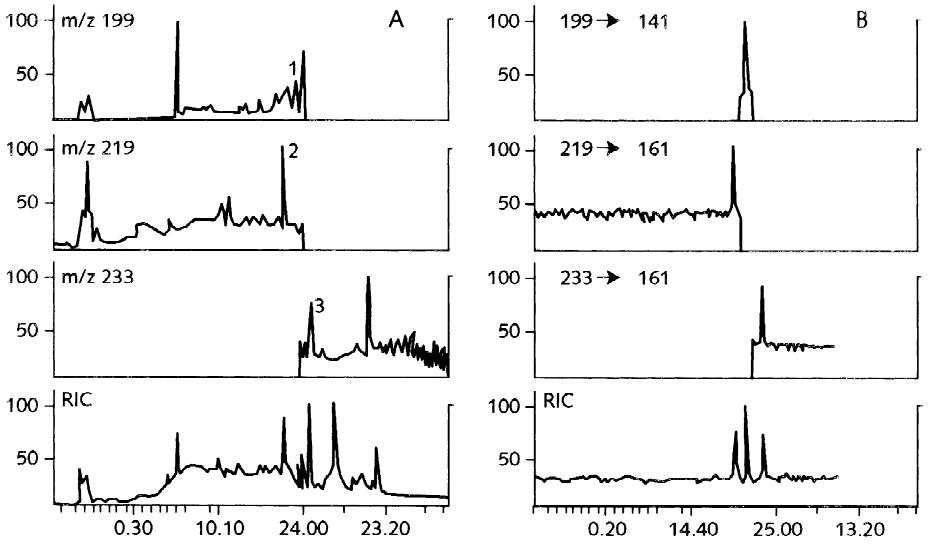


Fig. 12. Typical chromatograms from an LC–ESI-MS analysis of an authentic ground water sample following pre-concentration by SPE. The sample was analysed using single MS in SIM mode (A) and, subsequently, by MS–MS in SRM mode (B) for verification. Compound identification: MCPA (1), 2,4-D (2) and dichlorprop (3) [114].

hand, whenever such data are presented, for either MS- or MS–MS-based procedures, good linearity over 2–3 orders of magnitude (typically in the 10 ng/l to 10 pg/l range) is almost invariably reported.

4.2. Ion suppression

The selection of the organic modifier to be used in the RPLC separation and, consequently, for introduction of the analytes into the MS system can markedly affect the analyte responses [124,129,130]. To quote an example, in an LC–TSP-MS study, the intensity of the carbofuran signal decreased 94% upon changing the post-column solvent from water–acetonitrile (60:40) to pure acetonitrile [131], and reducing the percentage of methanol from 100 to 50% resulted in a twofold decrease of the intensity of the sodium adduct ions for all carbamates tested. Similar results were reported for 12 carbamates in fruits and vegetables: the signal intensities in LC–ESI-MS decreased significantly when acetonitrile was used as organic modifier instead of methanol [129]. Steen et al. [7] who used gradient LC–ESI-MS, found that methanol gave much better results than acetonitrile and that phenylureas were more susceptible to solvent changes than triazines. In another study, both the nature of the modifier and the buffer (acetate or formate) were found to have a considerable effect on the signal intensity of several pesticides in LC–ESI-MS, especially in the PI mode. Typical examples are quoted in Table 6. In recent FIA–MS studies [124,132], even traces of acetonitrile were found to pronouncedly influence the signal intensities of desethyl- and desisopropylatrazine but, somewhat surprisingly, not that of the parent compound atrazine [132]. The, sometimes considerable, influence of the eluent flow-rate on analyte response in LC–API-MS–MS has been demonstrated for, especially, hydrophobic pesticides [133] and the advantages of ultralow flow for ESI-MS have been highlighted [134].

One should add that, next to the organic modifier and/or the buffer present in the LC eluent, sample constituents are another serious cause of signal reduction. During ionization, these compounds—which sometimes do not even show up in the MS spectrum themselves—compete with the analytes of interest and cause ion suppression. It is increasingly being recognized that this phenomenon can easily create problems in quantitative analysis [124,135,136]. One good solution is to use matrix-matched standards. In actual practice, both effects may, of course, play a role. For example, Steen et al. [7] found that co-extracted humic material caused a strong signal suppression in ESI-MS–MS. The degree of suppression was determined by both sample pH and the nature of the sample, surface or estuarine water.

One worthwhile approach to remove humic material is to use a dual-precolumn setup (cf. Section 3.1). Rather similarly, Geerdink et al. [137] used tandem SPE. On the first precolumn, the humic acids were retained at pH 1–2. The triazine test compounds were quantitatively recovered in the eluate

Table 6
Relative response of test analytes for different eluent compositions in LC–ESI-MS–MS (PI mode)

LC eluent*		Atraton	Atrazine	Diuron	Linuron	Metolachlor	Diazinon
A	B						
Pepperpot							
AA	AcN	45	50	15	0	70	65
AA	MeOH	65	80	50	60	100	100
AF	AcN	65	55	30	5	45	90
AF	MeOH	100	100	100	100	90	90
Crossflow							
AA	AcN	30	40	10	0	45	20
AA	MeOH	45	50	45	40	60	25
AF	AcN	58	55	55	65	95	55
AF	MeOH	100	100	100	100	100	100

*AA ammoniumacetate; AF, ammoniumformate; AcN, acetonitrile; MeOH, methanol.

and, after a pH switch to pH 7, isolated by C_{18}-SPE. The mass spectra recorded with this procedure were much more reliable than the mass spectra obtained after a single SPE step. The method was a marked improvement over an earlier tandem-SPE procedure which involved the aminopropyl/LiChrolut EN combination [7]. A somewhat similar observation was recently made with regard to polycyclic aromatic hydrocarbons. Here, the negative effects of humic acid during trace enrichment on C_{18}-SPE could be obviated by using dynamic ion-exchange SPE instead; the analyte recoveries increased 10–30% [138].

Finally, one should note that, whereas in LC–MS the changing nature of the LC eluent—which is the dominant chemical ionization phase during the ionization process—is a distinct cause of problems, almost any (constant) carrier stream composition can be used in FIA. In addition, in FIA the flow-rate can be freely adjusted to optimize the performance of the MS system. In other words, there is much versatility with regard to optimization for specific compound classes in that case, while instrumental conditions can be set for maximum responses of individual analytes [139,140].

4.3. Tandem MS operation

Many methods quoted above use single-stage MS, preferably in the TS-SIM mode, in order to detect very small amounts of target analytes. However, if confirmation of the identity of analytes or provisional identification are main issues, more structural information is required that can be obtained by means of pre-analyser-CID or MS–MS. To quote some examples, Slobodnik et al. [98] used TS-SIM to detect analytes at the 10 ng/l level; in a second run, time-scheduled product-ion scan MS–MS was used to confirm the results. Køppen and Spliid [114] determined 18 acidic herbicides (phenoxy acids, sulfonylureas, phenols) in groundwater using LC–ESI-MS–MS to arrive at their proper identification. In this instance, the loss of detectability caused by using MS–MS detection was 3–4-fold.

Hogenboom et al. [126] analysed 4 ml river water for the target analysis of herbicides. Trace enrichment and subsequent elution and separation were performed by means of a single short column of only 10 mm×2 mm I.D. (also see below) and LODs obtained with APCI-MS–MS were 0.01–0.1 μg/l. At least 40 analyses could be performed without loss of column stability or chromatographic efficiency. Baglio et al. [141] determined polar pesticides with LC–API-MS^n. Carbamates, triazines and phenylureas were best ionized as positive ions, while phenoxy acids, nitrophenols and bentazone yielded stronger signals with negative ionization. In another study [118], LC–DAD, LC–MS and LC–MS–MS were used for 48 pesticides and some of their TPs. Different extraction techniques were used for surface water samples from France and Canada and LODs of 20–100 ng/l (LC–MS full scan) and 2–6 ng/l (LC–MS–MS SIM) were obtained. For a series of 60 samples, the correlation between results found by LC–DAD UV and LC–MS was good ($r=0.93$).

Tandem MS techniques are especially relevant when analytes have to be determined at the ultra-trace level and when screening for unknown TPs of pesticides is required. In such cases, the complementary use of precursor-ion, product-ion and/or constant-neutral-loss scanning is highly relevant. Recent examples of such studies include the screening for (bio)degradation products of the pesticides fenchlorazole-ethyl and furthiocarb [142], and a detailed study of the degradation of alachlor [143]. In the latter instance, GC–MS and LC–time-of-flight (TOF)-MS (for accurate mass determination) were also included. This combined effort gave rewarding results; however, because of the closely related structures of most of the (over 10–15) TPs, complete unravelling of the problem at hand was not possible. In another recent study, the potential of LC–TOF-MS and MS–MS for screening and identification of unknown microcontaminants in surface water has been convincingly demonstrated [144]. The increasing interest in the use of LC–TOF-MS is also apparent from a recent study on the potential of the technique (ESI mode) for the confirmation of the identity of non-volatile and/or thermolabile pesticides (mean errors, 0–5 ppm) [145]. An interesting study on ultra-trace analysis of polar pesticides and TPs in estuarine water demonstrated that LC–ESI-MS–MS (off-line combined with SPE) can be used for the detection of these compounds down to low ng/l concentrations [146]. Finally, as was shown in [147] FIA–MS–MS procedures can also be very

useful: a 10-ml extract suffices to screen for the presence of specific classes of compounds and, next, to perform target screening and/or SRM-based confirmation.

An interesting MS–MS procedure, the radio frequency mode (RFD) scan mode, was used by Kienhuis and co-workers [99,148–150]. With this procedure the offset voltage varies from scan to scan which results in several spectra being recorded which provide different information, e.g., molecular mass as against fragment-ion data. The spectral information and analyte detectability were found to be similar to that of full-scan MS–MS (Fig. 13). The method was validated for 40 compounds and LODs were 0.05–1 μg/l in the full-scan mode. In surface water the presence of unknown compounds like tris(2-butoxy-ethyl)phosphate, carbetamide, carbamazepine and *N*-butylbenzene sulfonamide could be established.

4.4. Shorter and short LC columns

Already many years ago, the idea was launched to use much shorter columns than the conventional 20–25 cm ones, specifically for the target analysis of limited numbers of very polar analytes that cannot easily be included in standard LC–UV gradient runs. The problem was studied in much detail by Hogendoorn and co-workers [151–156] who came up with the set-up schematically shown in Fig. 14A. From the two high-pressure packed, 5-cm long columns used, the first one serves to remove a large proportion of the interfering polar sample constituents and, after analyte peak transfer, a slightly less polar eluent is used for the actual separation. Successful applications were reported for analytes which display, next to their highly polar character, unfavourable characteristics such as high water solubility or low UV absorbance. Relevant data for some such compounds, chloroallylalcohol (TP of the soil fumigant 1,3-dichloropropene), ethylenethiourea (a key TP of the ethylenebisthiocarbamate fungicides) and methylisothiocyanate (a soil fumigant), are compiled in Table 7 [157]. As a typical result, the ultra-trace-level determination of bentazone in rain water is shown in Fig. 14B. It is good to add that, for larger numbers of analytes, one will probably prefer an SPE–LC approach which involves a selective washing step

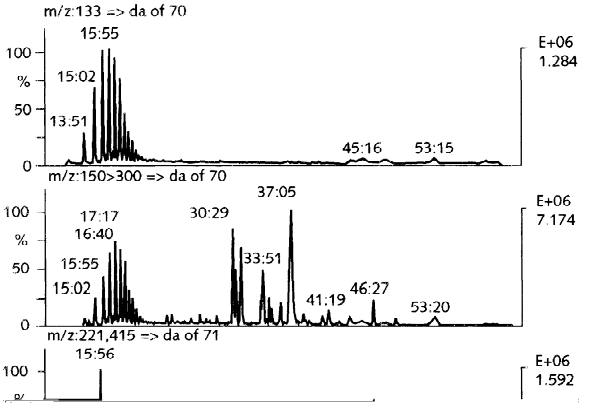
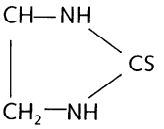
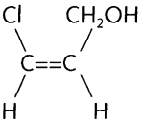


Fig. 13. SPE–LC–APCI-RFD-MS of a 100-ml river Meuse sample in PI mode. From bottom to top: RIC; user trace representing the UV chromatogram at 220 nm; the combined filtered mass traces (collision offset −7 V) of m/z 221 (internal standard, atrazine-dS) and m/z 415 [one of the protonated molecular ions of polyethylene glycol (PEG)]; a filtered mass trace (collision offset −23 V) showing the combined intensities of all masses between m/z 150 and 300; the filtered mass trace of m/z 133 (−23 V) showing a common daughter ion of PEG components [149].

[31,32]. As was demonstrated for a series of phenoxy acids, with a procedure involving suitable pH variation, the bulk of the sample matrix can then be transferred to waste and only a heart-cut containing the analytes of interest is subsequently desorbed and led to the analytical column.

From the many reported applications, one may conclude that the above approach is robust if target compounds in known matrices are the main aim of an analysis. However, today one would like to see such studies being extended by the inclusion of MS

Table 7
Examples of polar compounds analysed by LVI–column-switching RPLC–UV [157]

Parameter	Ethylenethiourea	Chloroallyl alcohol	Methylisothiocyanate
Structure			H_3–N==C=S
Water solb. (g/l)	20	Infinite	8
κ'*	1.6	7.0	20
λ (nm)	233	205	237
ε (l/mol cm)	18 000	10 000	3000
Sample volume (μl)	200	200	770
LOD (μg/l)**	1	1	1
Time of analysis (min)	5	7	7

*On 5 μm Hypersil ODS; eluent, pure water.
**Detection limit ($S/N=3$) of analyte in ground, surface and rain water.

pounds not amenable to GC analysis (without prior derivatization). One serious drawback is that mass spectral recognition in LC–MS still is much less sophisticated than in GC–MS, with insufficient fragmentation, the influence of (changes in) LC eluent composition and differences in design of the MS instruments all playing a role. This is, therefore, the proper moment to briefly return to the use of GC as an analytical technique, specifically for organic microcontaminants: are there any recent developments which still give GC a lead over LC (although for a more limited set of compounds) in terms of analytical performance? To our opinion, the answer certainly is affirmative, as the subsequent brief discussion will show.

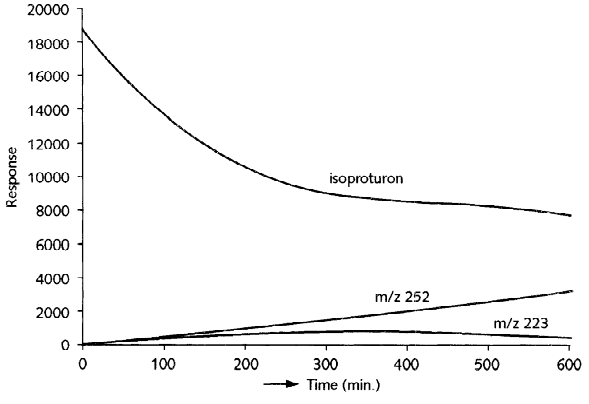


Fig. 15. Response versus time plots of photodegradation of isoproturon in demineralized water. The responses obtained in the precursor-ion m/z 72 scan mode were used to construct the curves [92].

In the past few years, so-called comprehensive GC, or GC×GC, has emerged as a powerful separation technique, and is now rapidly gaining in popularity [163–165]. Briefly, two independent GC separations are applied to an entire sample. The sample is first separated on a conventional high-resolution GC column which usually contains a non-polar stationary phase, and temperature programming is used. A valve-less modulator—either a thermal desorption modulator or, more often today, a cryomodulator—between the two columns accumulates and focuses each subsequent small portion of eluate from the first GC column and, next, causes its injection on the short, narrow and more polar second-dimension column to allow a very rapid and essentially different type of separation in the brief time span of the few seconds available before the next eluate fraction arrives. The reconstructed final chromatogram is usually presented as a two-dimensional contour plot. The distinct advantage of GC× GC over earlier GC-based procedures includes the use of two independent (boiling point and selective interaction) separations, better analyte detectability (due to peak refocusing in the modulator) and the

absence of extra time consumption (analysis is complete in first-run time span).

Until now, most applications are for petrochemical mixtures, but essential oils, fatty acid methyl esters, flavours and mixtures of organohalogens such as PCBs have also been analysed. More important for our topic, very recently, attention has been given to the separation of pesticides and other organic micro-contaminants. One typical example is shown in Fig. 16 which clearly illustrates the beneficial influence of the dramatically increased peak capacity [166], especially when it is compared with the SPE–GC–MS separation of Fig. 2 above, where essentially the same mixture of microcontaminants was separated. What still is missing in this instance, and in most published applications, is the possibility of peak identification by means of MS detection. This requires the use of TOF-MS which is much faster than a quadrupole or ion-trap MS—and is, therefore, compatible with the extremely rapid second-dimension separation of a GC×GC run—and also has excellent deconvolution capabilities for partly co-eluting peaks [167,168]. Fig. 17 shows the most crowded part of a GC×GC–TOF-MS chromatogram of a celeriac extract spiked with 58 pesticides. The extract contains high concentrations of several matrix components (peaks A–E) and three compounds

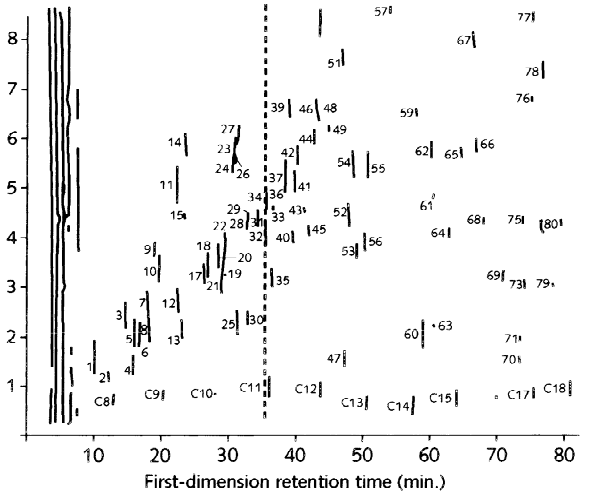


Fig. 16. Contour plot of a GC×GC–FID analysis of a mixture of 80 surface water contaminants, using cryogenic modulation (part above 80 min not shown) [166].

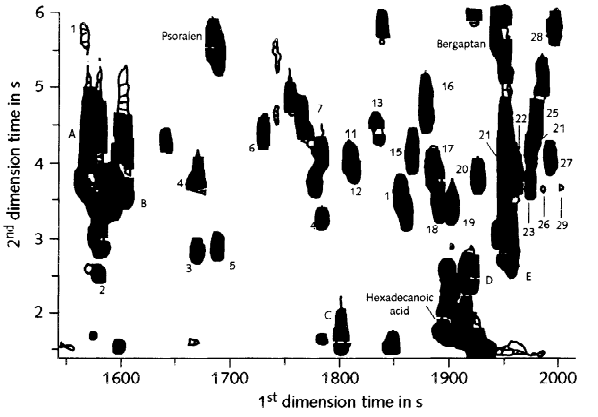


Fig. 17. Detail of GC×GC–TOF-MS chromatogram of extract of celeriac, spiked with pesticides at a level of 0.16 ng/μl. Peak assignment, 1, dimethoate; 19 triadimefon; A–E, non-identified matrix compounds; for other pesticides, see Ref. [175].

identified as bergaptam, psoralen and hexadecanoic acid). All pesticides could be unambiguously identified while this was, for obvious reasons, completely impossible for several analytes—such as dimethoate (No. 1) and triadimefon (No. 19)—in one-dimensional GC–TOF-MS. The various strategies that are available in TOF-MS for the identification/confirmation of target analytes as well as unknowns are explained and illustrated in an extensive recent study [169].

The brief discussion presented above should convince the readers of the potential of the new technique for real-life studies and encourage them to further explore this highly promising approach.

6. Conclusions and perspectives

In a recent review on the trace-level determination of pesticides in aqueous samples, much attention was devoted to summarizing the state-of-the-art and indicating future trends [128]. We agree with the main conclusions of that review and, not too surprisingly, find that what were the main issues then, still are important aspects less than a year later. Nevertheless, several valuable additional comments can be made, and some very recent trends should be highlighted. A mixture of these and the key conclusions of the earlier study is presented below.

Firstly, analytes have to be detected, identified and quantified. Today, for a very large majority of all pesticides (and related industrial chemicals) and also most of their TPs that have been studied in some detail, sample volumes of 30–50 ml suffice to reach LODs of 10–30 ng/l, which complies with most (inter)national directives. Three remarks should be added. (i) Even with target procedures, a limited number of analytes of interest and a sophisticated MS–MS or TS-SIM procedure, sample volumes still have to be 5–10 ml in essentially all cases. In other words, even though exceptions start to show up in the recent literature (see, e.g., Refs. [49,170,171]), trace enrichment is almost invariably required and on-line operation is, consequently, to be recommended. As a rule, enrichment via non-selective SPE will be the preferred approach—with IASPE-type selectivity being used only in well-defined target-analysis procedures. (ii) GC-based procedures still offer better separation efficiency and MS-oriented detectability than do LC-based separations. Consequently, whenever all target compounds are GC-amenable (without prior derivatization, which should be avoided at all cost), GC is the separation technique of choice. This will become even more true with the advent of intriguing GC×GC, which considerably adds to the overall separation efficiency and will therefore be primarily used for sample types more complicated than ''water'', such as food, soil and biological material. (iii) Whilst analyte detection is not a serious problem anymore in many real-life situations, both identification and quantification frequently cause concern. This is briefly discussed in the next paragraphs.

As regards identification/confirmation of identity, the situation in GC is markedly different from that in LC analysis. In the former instance, large libraries are available, and searching, compiling of hit lists and meeting properly defined criteria, are well-known and essentially routine operations. In LC–MS, however, ESI-MS and APCI-MS spectra are influenced—and, frequently, strongly influenced—by parameters as divergent as type of MS instrument used, instrument settings, LC conditions (nature and per cent modifier, pH) and sample type. In addition, the structural information that can be derived, in LC, from API-MS spectra is much more limited than the information that can be obtained, in GC, from EI-MS spectra. [This explains why a PB-directed breakthrough in LC–MS still is eagerly sought]. Formulating identification and confirmation criteria for pesticide analysis in LC–MS should, therefore, receive much more attention—and continuation of a recent study on MS criteria for production spectra generated in FIA-based environmental analysis [132] is urgently required. As for quantification, contrary to what was frequently suggested in earlier days, the linearity of calibration plots over several orders of magnitude is not a real problem in LC–MS or –MS–MS procedures. What does cause concern is (i) the frequently considerable, and often unpredictable, influence of LC conditions on analyte response and (ii) the—again, often large—ion suppression caused by sample constituents. While the use of matrix-matched standards provides an at least partial answer to the second problem, much experimental as well as theoretical work will be required to solve the former: grave errors can readily occur if it is not duly considered. In this respect, the FIA approach that has been discussed in several recent papers, offers an interesting alternative because no chromatographic separation is involved. The additional advantage of FIA operations of creating a several minutes wide plateau for optimized analysis should help to stimulate further work in this direction. For the rest, the reader should not fail to be properly impressed by the many recent developments in MS analysis, both for GC and LC. The speed, sensitivity and deconvolution potential (i.e., selectivity) of TOF-MS for GC are one example, and the many scan modes of MS–MS, and mass accuracy of TOF-MS for LC, another. However, one note of warning should be added. Whatever the degree of sophistication and/or userfriendliness of these instruments, in order to use their full potential, the analytical chemist should acquire in-depth knowledge of what scanning strategies, deconvolution and obtaining structural information really are about. And, of course, with the plethora of data available from, even, one instrument, he should carefully consider how to interpret, use and store them.

Three more aspects require attention, the analytes of interest, the measurement strategies and the selection of a proper set of target analytes.

As for the analytes, scrutiny of the literature reveals that adequate analytical procedures are avail-

able in almost all instances for the parent compounds. When limiting ourselves to the pesticides, it is also true that classes of compounds such as triazines (CC and LC) and phenylureas (LC) are distinctly overrepresented. Organo-P pesticides (GC and LC) and carbamates (LC) are also high on the list but coverage is less complete—which seems to indicate that some of these compounds do not really fit in the (conventional) schemes of analysis. Phenoxy acids (LC) are very prominent too but here one often gets the impression that it is a lack of reliability and robustness of published procedures which makes additional effort necessary. Anyway, it is rather obvious that further improvement of techniques for thoroughly studied analytes should get little priority. Rather, one should attempt to get more difficult analytes under control and, if at all possible (i.e., with some compromising being permitted), include them in multiresidue protocols. This is, after all, an excellent method to reduce the work load and increase sample throughput.

In addition, TPs should be higher on the agenda, as we tried to emphasize in the present review by quoting quite a number of relevant examples. On the one hand, with SSC-MS(–MS) an elegant strategy has become available to rapidly monitor (bio)degradation routes and decide on subsequent action. In this case, method development, both on the SPE and the ESI-MS side, will need due attention—with little information being a priori available on analyte response in MS, and ion suppression being a major danger with these early-eluting compounds. On the other hand, information is increasingly becoming available that TPs are present at the trace level in a variety of water, sediment, etc. samples in the environment (cf. Ref. [7]). Especially also because sufficient data on toxicity and bioavailability are not yet available, while the adverse effects of some such TPs, e.g., triazines, carbamates, phenoxy acids, organo-Ps and dithiocarbamates have been amply demonstrated, attention to this under-evaluated group of compounds is highly desirable [172].

Measurement strategies should also be briefly considered. When degradation studies are a main issue, CC is in principle not a relevant approach and a rapid SSC-MS procedure (cf. above) is to be preferred. If rather limited numbers of analytes have to be studied—and, especially, when they belong to a single class of compounds—selective sorption, i.e., IASPEs, can serve a most useful purpose. Furthermore, dual-short-column LC or SSC can help to eliminate superfluous resolution and reduce run times; in GC, the same goal can be reached by using fast or flash GC. In all such instances, maintaining sensitivity while sacrificing part of the selectivity is the proper adage. Or, in other words, much more can be done in LC if MS rather than UV is used for detection. In GC, MS detection is to be preferred anyway, with a proper selective GC detector (or AED as an umbrella-type alternative) for complementary purposes. In addition to all this, water-producing companies and (governmental) bodies charged with the control of water quality via early-warning and monitoring, are faced with the need to perform as wide-ranging analyses as is possible. For such work, automated on-line systems are to be recommended with LVI– or SPE–GC–EI-MS and SPE–LC–DAD UV as complementary approaches. Subsequent confirmation (for target compounds) and provisional identification (for unknowns) will invariably require MS-based strategies—with MS–MS being a most useful tool for both separation techniques.

If there is an urgent need for rapid action, the FIA–MS–MS approach repeatedly quoted above is an attractive alternative procedure: it combines speed of analysis with high selectivity. A completely different but, in principle, highly promising approach is the use of SPE–LC combined (via microtitre plate extraction) with sensitive effect-related (geno)toxicity detection—a powerful tool when monitoring the overall quality of surface water [173]. Also, the potential of another newly emerging technique, data-dependent scanning, for multiresidue screening will have to be assessed in the next few years.

Finally, as regards the selection of a proper set of target analytes for monitoring programmes, it is worthwhile to briefly discuss a strategy introduced by RIZA in 1999, the squeeze-box model (Dutch: harmonica model). In the initial stage, a wide-ranging survey was made at a limited number of sites in the Netherlands and in the various agriculturally relevant periods of the year. Both surface water (374 compounds; 345 pesticides) and suspended matter (244 compounds; 232 pesticides) were analysed. From among the compounds actually detected one or

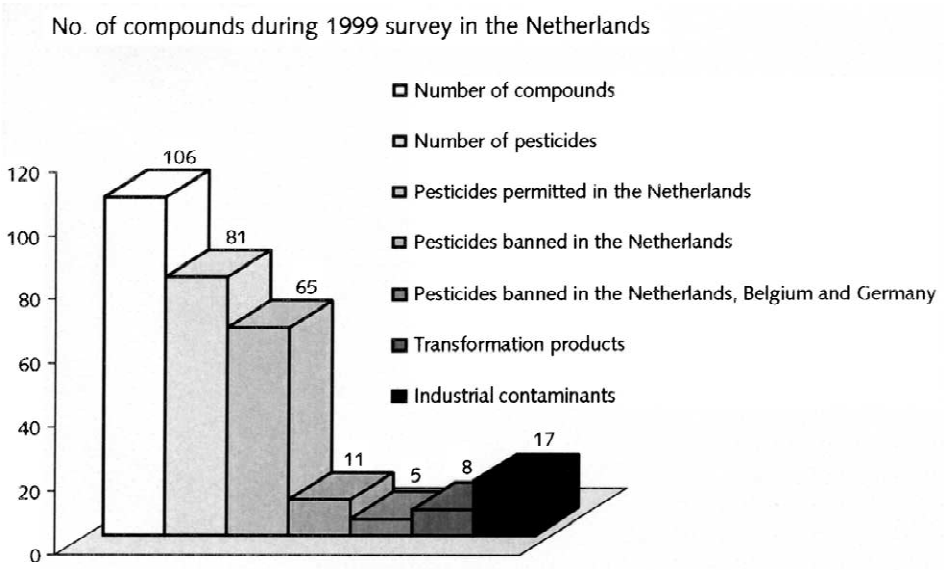


Fig. 18. Number of microcontaminants detected during 1999 survey in the Netherlands and breakdown into permitted and banned compounds, TPs and industrial contaminants [174].

more times (total for 1999: 106; for a breakdown, see Fig. 18 [174], a restricted number were selected for the second, the real monitoring, stage. The criteria were: (i) detection at a concentration over the maximum tolerance risk (MTR) value; (ii) detection at a concentration over the European Union (EU) drinking water legislation value; (iii) detected at least five (out of 21) times.

In 1999, 46 compounds were found to meet one or more of the above criteria. At a first glance, this number does not differ too much from the 59 compounds included in the on-going conventional RIZA monitoring programme. However, a more detailed comparison revealed that 26 out of the 46 inventoried compounds were not on the RIZA list, while 39 compounds appearing on that list, could be deleted because they did not meet a single criterion! In other words, the main advantage of the squeeze-box approach is that it is highly efficient and properly targets at the real-life situation. This has the additional benefit that—since now, for the first time, a large proportion of all pesticides actually present is determined—the experimental results can be examined for compatibility with that often forgotten second criterion of the EU drinking water directive, viz, that the sum total of all pesticides should not exceed 0.5 μg/l.

Improving the quality of the pesticide (plus TP) targeting for monitoring studies, while reducing the cost of operation, certainly is no mean feat. Further testing and validation of the squeeze-box model, and using it also for other applications, is, therefore, urgently recommended.

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