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Review

Recent and future developments of liquid chromatography in pesticide trace analysis

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Abstract

Until recently, the application of liquid chromatography (LC) in pesticide analysis was usually focused on groups of compounds or single compounds for which no suitable conditions were available for analysis with gas chromatography (GC). However, recent developments in both detection and column material technology show that LC significantly enlarged its scope in this field of analysis. Obviously, the most striking example is the rather abrupt transition of LC coupled to mass spectrometric detection (MS) from an experimental and scientifically fashionable technique to a robust, sensitive and selective detection mode rendering LC–MS being increasingly used in pesticide trace analysis. Other recent major developments originate from the innovation of new LC column packing materials, viz. immuno-affinity sorbents, restricted access medium materials and molecular imprinted polymers improving considerably the screening of polar pesticides by means of reversed-phase LC with UV detection. In this review the merits and perspectives of these important LC developments and their impact to current and future applications in pesticide trace analysis are presented and discussed.

Keywords: Stationary phases, LC; Molecular imprinting; Restricted-access media; Liquid chromatography–mass spectrometry; Environmental analysis; Water analysis; Soil; Extraction methods; Reviews; Pesticides

Contents

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1. Introduction

The survey of pesticide residues in both the areas of the environment and the public health is faced with the identification and quantification of hundreds of pesticides with widely different physico–chemical properties in very different types of matrices. Hence, a major task of the analytical discipline is to provide reliable and cost-effective methods.

Since the introduction in the late 1960s of gas chromatography (GC) and the inherent remarkable feature to perform on a packed column multi-residue analysis, the technique became rapidly adopted. Further important developments such as capillary columns providing high separation capacity and sensitive and selective detectors significantly enlarged the number of pesticides efficiently analyzed in one run. These attractive features and the favorable development of both the performance and the costs of the instruments make capillary GC the most widely applied and productive technique in pesticides residue analysis [1].

For example, in the Netherlands a multi-residue method (MRM) employing capillary GC with mass spectrometric (MS) detection for regulatory purposes [2] comprises of the determination of nearly 300 pesticides in foodstuffs which covers about 60% of the pesticides mentioned in the Dutch Regulation on Pesticides in Foodstuffs [3].

As a result of changing and extending use patterns of pesticides and an on-going product development, several trends can be observed in pesticide science. For example, there has been a clear shift from the use of 'long-life' persistent insecticides such as organochlorine compounds to more polar and readily degradable 'short-life' pesticides such as *N*-methylcarbamate pesticides. Other major trends are the extensive use nowadays of 'traditional' herbicides, e.g. triazines, chlorophenoxy acids and phenylureas and so-called 'modern' herbicides, e.g. sulfonylureas and imidazolinones, with favorable properties such as a low dose rate of application and a high degree of (bio)degradation. Most of these pesticides are (very) polar, low volatile and/or thermo-labile compounds not directly amenable for GC. Because of its powerful features, GC–MS methods involving a derivatization step remain attractive for thermo-stable polar compounds, e.g. chlorophenoxy acids [1,2].

However, most of the (very) polar pesticides can be efficiently separated with reversed-phase liquid chromatography (RPLC) without a preceding laborious derivatization step. Hence, since the introduction of RPLC equipped with a suitable/robust UV or fluorescence detector in the field of pesticide residue analysis — which occurred around 1980 — LC became rapidly adopted as a viable technique complementary to GC for the determination of various classes of polar pesticides.

The wide application range, long-term stability, easy use, low cost and improved selectivity (diode array) makes the UV detection mode most widely used in residue analysis. However, confirmation becomes difficult for pesticides of the same class due to the high degree of similarity between UV spectra. This makes that RPLC–UV based methods are most effective for a fast screening of samples, but usually require additional confirmatory analysis in case of positive samples.

Fluorescence detection (FLD) is distinctly more selective and sensitive for analytes that normally undergo fluorescence, however, its applicability is limited because only few pesticides have a favorable fluorophore. Pre- or post-column derivatization techniques enlarged the scope of FLD to multi-residue analysis.

For example, LC-based robust and reliable multi-residue methods are applied by the Dutch Food Inspection Service for the control of residues of phenylureas (12 compounds) or *N*-methylcarbamates (22 compounds) in foodstuffs [2]. These methods are based on the work of De Kok et al. using post-column hydrolysis of *N*-methylcarbamate pesticides with a solid-phase reactor followed by *o*-phthalaldehyde (OPA) reaction with FLD [4]. The same approach including automated sample preparation and applied for the on-line trace analysis of 22 *N*-methylcarbamates and 12 metabolites in environmental waters [5], demonstrates clearly the importance of LC in this field of analysis.

During the last few years some remarkable developments have taken place (potentially) extending the applicability of RPLC in pesticide residue analysis. The recent introduction into the market of robust and easy-operating LC–MS instruments provides a new way for analyzing polar pesticides more efficiently.

Concerning column technology, the input of sev-

eral new materials undoubtedly will improve cost-effective pesticide residue analysis. The selectivity enhancement of these materials widens the scope of 'simple' and low-cost UV detection in trace analysis.

Starting with column materials the use and the potential of these new developments including our recent experience and results with bench-top LC–MS will be overviewed and discussed.

2. Column material technologies

2.1. Immunosorbent materials

Immunoassay technology is based on the specific reactions between antigens and antibodies and has been used for a long time for analysis and sample pretreatment in the field of biomedical analysis. Since the availability of immunosorbents focussed at the bonding of pesticides and conveniently packed in solid-phase cartridges and/or precolumns, the technique became more accessible to be included in LC procedures for pesticides [6].

Different from immunoassays, immunosorbent based procedures involve, after a desorption step, in a second step analytical separation and detection of the individual analytes [6].

In the early 1990s the first immunosorbent applications involved the selective trace analysis of single pesticides such as carbofuran in soils [7], atrazine in water [8] and chlortoluron in water [9]. The merit of this approach towards more productive and cost-effective multi-residue analysis was achieved by Hennion and co-workers [6,10–17]. They beneficially exploited the unavoidable cross reactivity of antibodies for structurally related compounds and developed immunosorbents performing in one step both extraction and cleanup for a class of compounds. The combination of the selective immunosorbent technique with RPLC–UV is an attractive approach for the cost-effective determination of polar pesticides in a variety of matrices.

The first applications in multi-residue analysis involved off-line solid-phase extraction (SPE) with immunosorbents for the selective trace enrichment of phenylurea and triazine herbicides in environmental waters [10,11]. SPE immuno-technology involved the production and purification of polyclonal antibodies against isoproturon and atrazine and the selection of silica-based sorbents instead off rigid hydrophilic polymers for the covalent bonding of the antibodies. The SPE immunosorbent enabled the trapping of most of the tested phenylureas (9 out of 13) and triazines (6 out of 9) emphasizing its potential to multi-residue analysis. The high selectivity was clearly demonstrated by the fact that no interferences were found in RPLC–UV (244 nm) of phenylureas in extracts of Seine river samples and that the baselines corresponded to that of drinking water samples extracts. Limits of detection (LODs) were obtained in the range of 0.1 μg/l after 200 ml of preconcentration on the immunosorbent cartridge [10,11].

This approach was successfully applied to the determination of herbicides in foodstuffs [12,13]. The use of the immunosorbent SPE cartridge considerably simplified the analysis of phenylureas and triazines in the tested food samples carrots, celery, corn, grapes, onions, potatoes and strawberries. Methanolic SPE extracts of the matrices were simply concentrated, diluted with water, and processed with RPLC–UV. Because of high selectivity, this approach is rapid and LODs were obtained of about 25 μg/kg for most of the matrix/compound combinations. An additional cleanup step using a strong anion-exchange (SAX) SPE cartridge was required for some food commodities. With the combined SPE–SAX and immunosorbent cleanup, the detection limits in the foodstuffs were about 2–5 μg/kg.

The good performance of this approach is illustrated in Fig. 1, showing the RPLC–UV analysis of extracts of a potato and carrot sample spiked with phenylurea herbicides at a level of 25 μg/kg.

A powerful development in this field is the use of immunosorbents in precolumns for on-line SPE–LC, which in turn provides fully automated procedures for the assay of polar herbicides in environmental water samples [14–17]. The silica-based immunosorbent as applied in the off-line SPE cartridge [10,11] was applied in column switching and after 50 runs the loss in capacity was less than 10% allowing the unattended SPE–RPLC–UV trace analysis of phenylureas [14] and triazines [15] in environmental waters to the sub ppb level.

The use of mixed immunosorbent containing antiisoproturon and antichlortoluron antibodies immobilized on aldehyde-activated silica was successfully applied for the on-line analysis of five phenyl-

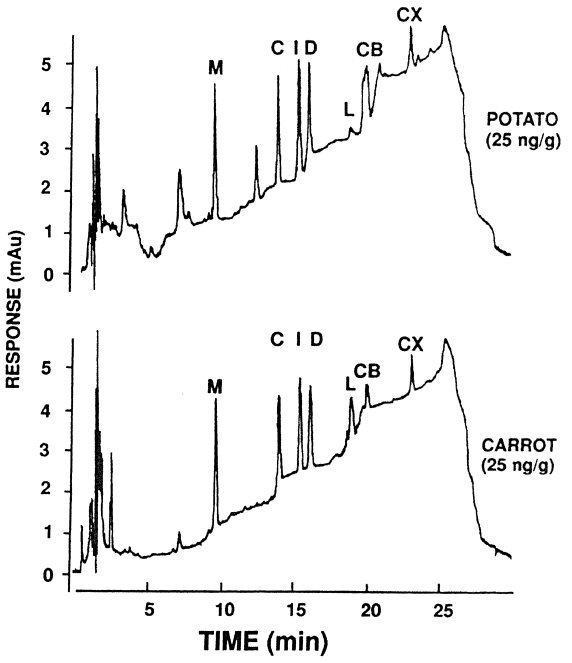

Fig. 1. RPLC–UV (244 nm) of extracts obtained after SPE on immunosorbent of a potato sample (upper chromatogram) and a carrot sample (lower chromatogram) and spiked with phenylurea herbicides at the level of 25 μg/kg. Linear gradient elution (40–80% methanol in water over 30 min). Peaks: M, monuron; C, chlortoluron; I, isoproturon; D, diuron; L, linuron; CB, chlorbromuron; CX, chloroxuron. (Reprinted with permission from Ref. [12]).

urea compounds in ground and river waters [16]; by percolating only 10 ml of water sample the on-line system provided detection limits in the range of 0.01–0.03 μg/l.

An inter-laboratory study using certified freezed dried water samples was performed to validate the use of the anti-isoproturon immunosorbent for the determination of phenylureas in environmental waters [17]. The method involved automated SPE of 50 ml of water sample followed by the analysis of the obtained extracts with LC with UV diode array detection (DAD) and LC with atmospheric pressure chemical ionisation mass spectrometric (APCI–MS) detection. Concerning the certified material, acceptable results were obtained, however, the low breakthrough on the tested sorbent of many phenylureas hampered the overall sensitivity [17].

2.2. Molecular imprinted polymer materials

The molecular imprinting technique has a high potential to pesticide residue analyses. The technique is based on the preparation of polymeric receptors, which bind small molecules with affinities and selectivities to a degree comparable to that of an immuno-based interaction. The materials are obtained by creating three-dimensional polymer networks that have a memory of the shape and functional group positions of the template molecule (target analyte). The resulting molecular imprinted polymers (MIPs) can selectively recognize and, consequently, retain the analyte used in the imprinting process.

Molecular imprinting has become increasingly popular and excellent reviews on their favorable features, preparation and (potential) application range have been published [18–21]. Potential advantages of the so-called ‘plastic’ antibodies over biological ones are stability, capacity, cost and ease of preparation.

The first application in pesticide residue analysis involved the use of MIPs against atrazine for the cleanup of organic extracts of beef liver [22]. In comparison to the crude extract the accuracy and precision were improved, and the detection limit of the analytical HPLC method was lowered by using SPE with a MIP.

As regards the use of MIPs in HPLC most work has concentrated on the resolution of chiral compounds providing highly selective chiral stationary phases [19,20,23].

Depending on the procedure of preparation the bonding of the target analyte to the MIP can be covalent or non-covalent [18–20]. Obviously, the slow kinetics of the strong covalent bonding will lead to excessive band broadening in LC. Hence, if applied as a stationary phase the non-covalent interactions are preferably used in which the optimum bonding will be a good compromise between a strong interaction (selectivity) and a fast desorbtion of the analyte (sensitivity).

The particle size distribution of the applied MIP materials usually varies from 10 to 25 μm [20] making them not very efficient in comparison to the 3–5 μm conventional LC packing. In addition, in case of hydrophobic-based interactions optimal sepa-

rations on MIPs are obtained when using aqueous poor mobile phases. This means that when coupling a MIP-packed column with an analytical RPLC column one deals with problems like a phase-switch and band broadening of analytes.

Recently, these problems have been nicely solved by Bjarnason et al. [24] for the on-line SPE of triazine herbicides. In this study a coupled-column system was used consisting of a combination of a column (150×4.6 mm I.D.) packed with laboratory-made 10–25 μm MIP material and an analytical 5 μm C_{18} column (250×4.6 mm I.D.). In the instrumental analysis, a C_{18} precolumn (55×2 mm I.D.) was placed before the MIP column and used for the SPE of the water sample. After sampling, all extracted species were transferred with 100% acetonitrile to the MIP column for retaining the analytes and allowing the contaminants to pass. With a small volume (200 μl) of pure water the analytes were captured in a 100 μl loop of a switching valve and then injected into the analytical C_{18} column using a mobile phase of acetonitrile–sodium acetate buffer pH, 7.0 (50:50, v/v) at 1 ml/min. The obtained selectivity of the column switching procedure was tested for several complex aqueous samples, viz. humic acid rich water, apple extract and urine.

The impressive selectivity of this approach for the determination of the triazines in humic acid rich water (20 mg/l) is very well illustrated in Fig. 2. Enrichment was observed in all cases, and triazine-enrichment factors of up to 100 could be recorded with an extraction efficiency of about 75% for each analyte.

2.3. Restricted access medium columns

Originally, restricted access medium (RAM) columns have been successfully developed in the field of biomedical analyses for the determination of low-molecular-mass target analytes in body fluids containing high-molecular-mass compounds, e.g. proteins [25–27]. The basic concept of the retention of analyte's and exclusion of large size molecules of the two adopted and commercially available materials, viz. internal surface reversed-phase (ISRP) [25] and semi permeable surface (SPS) [26], is shown in Fig. 3.

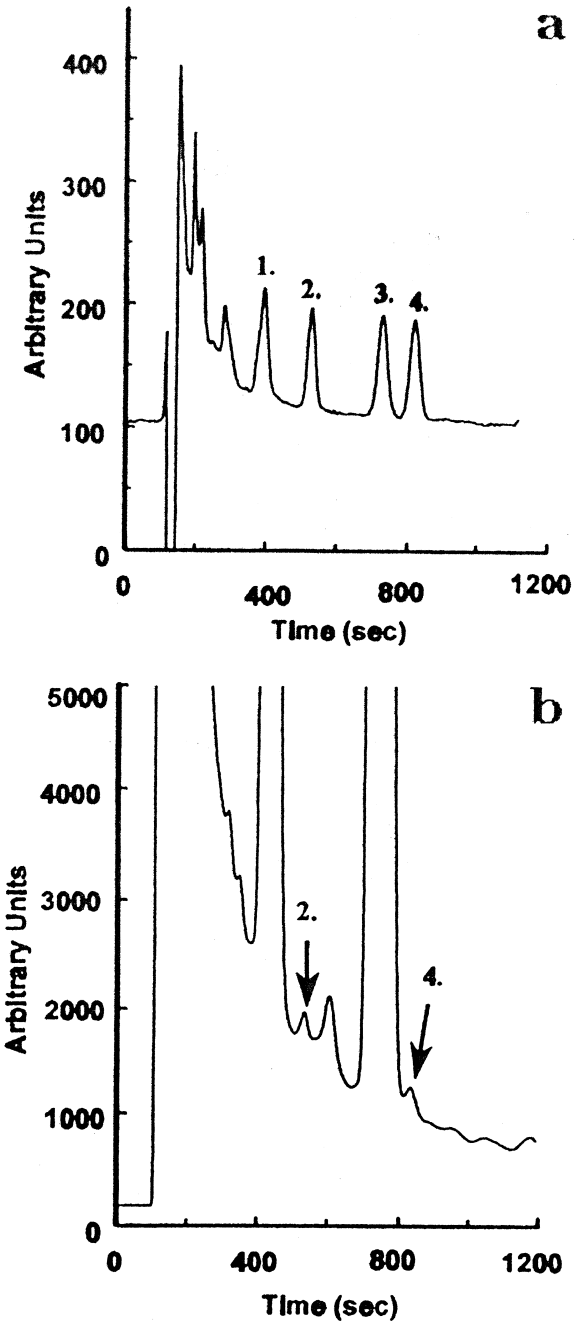

Fig. 2. On-line SPE–RPLC–UV with (a) and without (b) the use of a MIP column in the LC system of 200 ml solution containing 20 mg/l humic acid and spiked with 0.5 μg/l of each triazine. Peaks: 1, simazine; 2, atrazine; 3, propazine; 4, terbutylazin. (Reprinted with permission from Ref. [24]).

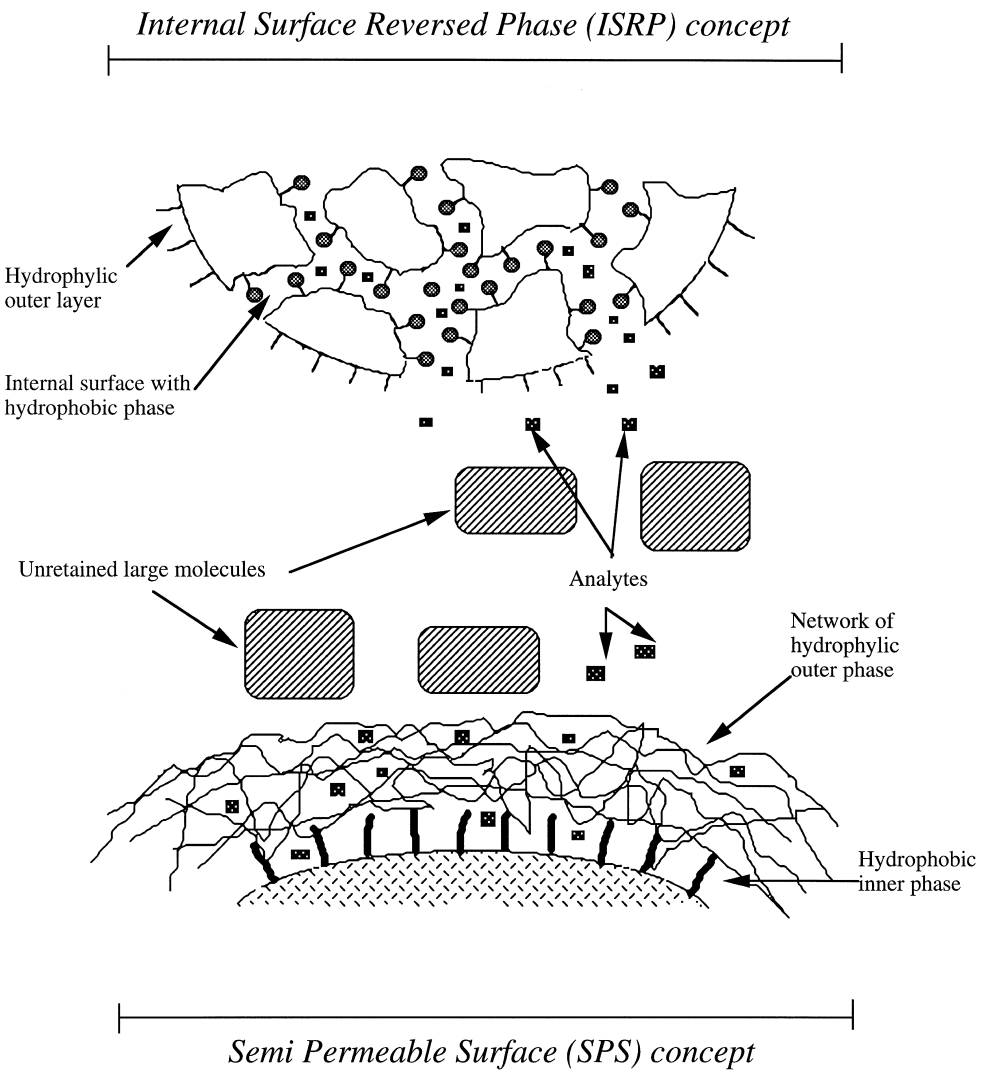

Fig. 3. Schematic presentation of the retention mechanisms of restricted access medium (RAM) materials.

The first RAM material developed by Hagestam and Pinkerton [25] is of the ISRP concept using chemically and enzymatically modified silica particles. It consists of a hydrophobic bonded phase (glycine–L-phenylalanine–L-phenylalanine, GFF) inside the small pores (80 Å) and outside a hydrophilic glycine.

The second concept, SPS, makes use of silica particles on which first a covalently bonded polyoxyethylene polymer network is formed followed by bonding a conventional phase, such as C_8 or C_{18}, underneath the polymer.

The revolutionary feature of analytical columns packed with 5 μm silica-based RAM particles is that they combine on the same column a powerful cleanup, viz. size exclusion of large molecular compounds, and an efficient reversed-phase separation of analytes, allowing the direct processing of serum and plasma samples. Overviews have been made about the available RAM materials and their

applications in biomedical analysis including their use as analytical columns or precolumns [28,29].

The use of an analytical RAM column in the coupled-column mode (LC–LC) has been applied for the high-speed analysis of β-agonists in serum in combination with tandem mass spectrometric (MS–MS) detection [30]. Beside the important advantage to prevent elution of proteins into the thermospray ionization (TSP)–MS interface, this approach provides small peak volumes (sensitivity) and, with a time of analysis of less than 10 min, a very high sample throughput.

Coupled-column RPLC has proven to be an attractive approach in pesticides residue analysis [31–34]. The approach of simply dividing the separation power over two analytical columns of similar selectivity by means of column switching usually improves significantly the selectivity in comparison to a one-column separation [31–34].

As clearly demonstrated in many applications the most powerful feature of the LC–LC approach is to elucidate peaks in the first part of the chromatogram, which will be obscured by the excess of early eluting interferences when applying a one column separation.

This favorable effect is caused by the separation power of the first column (C-1) preventing the co-elution of a major part of early eluting interference with the analyte(s) to the second column (C-2). Obviously, attainable selectivity depends on the size of the transfer volumes, being maximal in case of single residue methods. However, it must be emphasized that just the removal of a part of the early eluting interferences (cleanup) usually significantly improves chromatographic analysis making LC–LC also attractive in case of multi-residue analysis.

Unfortunately, the LC–LC approach is less successful in the screening of acidic pesticides in environmental samples when using UV detection at low wavelength. In this type of applications RPLC–UV is usually severely hampered by co-extracted humic substances, e.g. humic and fulvic acids. These interferences show up in the chromatogram as a broad hump causing a severe baseline deviation and obstructing, in many cases, the determination of the analytes at low levels. In LC–LC performed with C_8 and/or C_{18} columns, the co-eluted (small) fraction of humic/fulvic acids interferences persists in bad chromatographic behavior on the second column, making the improvement less pronounced in comparison to the analysis of neutral and/or basic compounds.

Because of the large difference in molecular size between target analytes (small molecules) and humic substances (large molecules) the use of the RAM columns seems to be attractive. In the early 1990s precolumns packed with 5 μm ISRP of Pinkerton substantially improved the RPLC–UV analysis of chlorophenoxy acid herbicides in environmental waters [35]. Unfortunately, the precolumns showed poor reproducibility limiting its further application in this type of analysis.

As encountered before in the processing of serum [30], the use of an analytical ISRP column (50×4.6 mm I.D.) instead of a precolumn appeared to be more a viable approach in the RPLC–UV analysis of the chlorophenoxy acid herbicide, mecoprop, in soils. The efficient removal of humic substances on the ISRP column allowed the LC–LC–UV (228 nm) processing of uncleaned soil extracts and the screening of mecoprop to a level of 20 μg/kg (ppb) [36].

The use of an analytical 5 μm SPS-C_{18} column (150×4.6 mm I.D.) as a second column in coupled column RPLC–UV was favorable for the trace analysis of acidic pesticides in ditch water samples originating from Dutch agricultural locations [37]. In combination with an efficient 3 μm C_{18} column (50×4.6 mm I.D.), the SPS column provides a favorable elution of the co-extracted humic/fulvic acid interferences allowing the determination of bentazone and bromoxynil down to a level of 0.05 μg/l in the ditch water samples investigated. The large improvement in selectivity obtained with the LC–LC (C_{18}/SPS) configuration in comparison to a one-column separation is clearly displayed in Fig. 4. It shows the RPLC–UV (217 nm) analysis of an uncleaned extract of a ditch-surface water sample spiked at a level of 0.5 μg/l; sample pretreatment consisted of SPE on a 500 mg C_{18} cartridge of 200 ml sample (brought at pH 2.2).

The instrumental time of analysis of less than 15 min in combination with a rapid SPE procedure efficiently allowed the assay of hundreds of samples. The validation data obtained during a five-month period and GC–MS confirmation clearly indicated the good performance and robustness of the coupled-

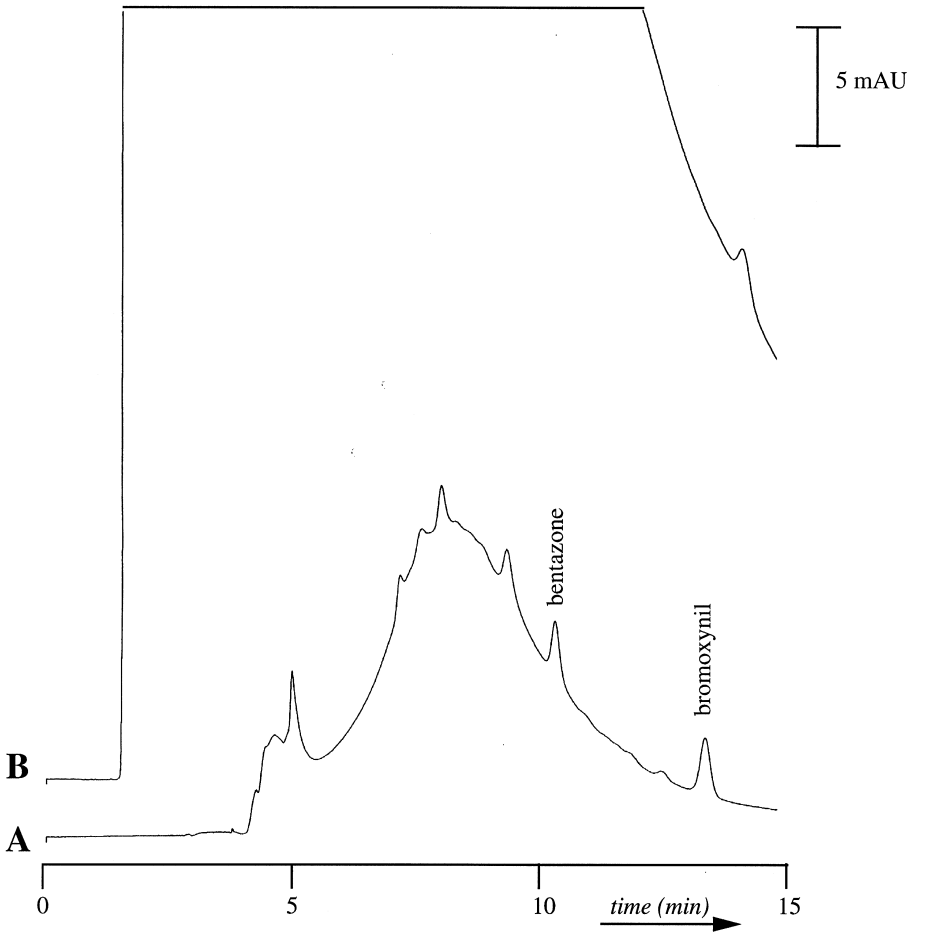

Fig. 4. RPLC–UV (217 nm) analysis of 300 μl of extract of a ditch surface water sample (corresponds to about 60 ml of sample) and spiked with bromoxynil and bentazone at a level of 0.5 μg/l. (A) LC–LC with 3 μm C_{18} column (50×4.6 mm I.D.) as first column (C-1) and 5 μm SPS C_{18} column (150×4.6 mm I.D.) as second column (C-2); first and second mobile phase, acetonitrile–0.03 *M* phosphate buffer, pH 2.4 (35:65, v/v) at 1 ml/min; cleanup volume, 2.9 ml; transfer volume, 0.9 ml. (B) LC on 5 μm SPS C_{18} column (150×4.6 mm I.D.) with mobile phase as in A.

column RPLC–UV (217 nm) screening method [37].

In a comprehensive study [38] various types of RAM columns were investigated on their RPLC–UV performance in the trace analysis of acidic herbicides of different chemical families in environmental water samples. Reference water samples with 3, 6 or 12 mg/l DOC (dissolved organic carbon) content were studied and before instrumental analysis processed with SPE on 500 mg C_{18} cartridges. Information on the tested columns and there mode of use is given in Table 1. LC configurations involved the single RAM column mode (LC) and the column switching mode (precolumn–LC or LC–LC) employing one RAM column in combination with an analytical C_{18} column or two RAM columns.

This study [38] also included the testing of the single residue method (SRM) approach with model compound mecoprop, and the multi residue method (MRM) approach with metsulfuron-methyl, bentazone, bromoxynil, 4-chloro-2-methylphenoxyacetic acid (MCPA) and mecoprop as model compounds.

The testing of the SRM approach revealed that the LC–LC approach involving the use of at least one analytical RAM column easily allows the determi-

Table 1
Information on analytical RAM columns used in LC–LC for the trace analysis of acidic pesticides in environmental samples

Material[a]	Dimensions $L \times$I. D (mm)	N[b] (plates/m)	Used as[c]	SRM/MRM	Ref.
RAM columns:					
5 μm Hisep (Supelco)	50×4.6	38 000	C-1, C-2	SRM	[38]
5 μm Pinkerton ISRP GFF-II (Regis)	50×4.6	20 000	C-1	SRM/MRM	[36,39]
5 μm SPS-5PM-S5-100-C_{18} (Regis)	50×4.6	70 000	C-1, C-2	SRM/MRM	[38]
5 μm SPS-5PM-S5-100-C_{18} (Regis)	150×4.6	76 000	C-2	SRM/MRM	[37,38]
C_{18} columns:					
3 μm Microsphere C_{18} (Chrompack)	50×4.6	118 000	C-1, C-2	SRMMRM	[36–38]
3 μm Microsphere C_{18} (Chrompack)	100×4.6	138 000	C-2	SRM/MRM	[38]

[a] Supplier between brackets.
[b] Number of column plates per meter, $N = (t_r/\sigma)^2$.
[c] C-1, first column; C-2, second column; SRM, single residue method; MRM; multi-residue method.

nation of mecoprop in water with high DOC content (12 mg/l). This is nicely illustrated in Fig. 5 showing the LC–LC–UV analysis of uncleaned SPE extract of a water sample containing 12 mg/l DOC and spiked with analyte at a level of 1 μg/l.

The alkyl-diol-silica (ADS) precolumn (25×4 mm I.D.) hardly improved the selectivity making the precolumn in comparison to the analytical columns less suitable in this field of analysis.

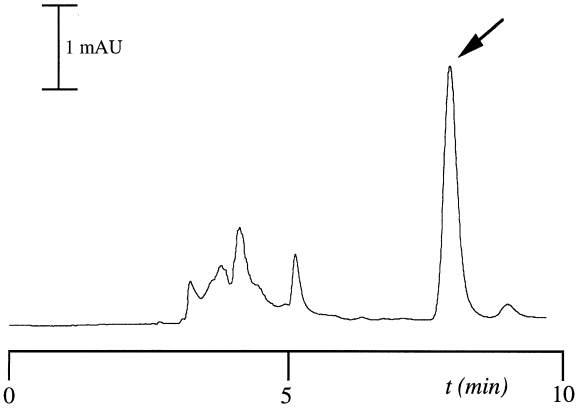

Fig. 5. LC–LC–UV (220 nm) using 5 μm Hisep/3 μm C_{18} columns (both 50×4.6 mm I.D., see also Table 1) of an uncleaned SPE extract of a water sample containing 12 mg/l DOC and spiked with mecoprop at a level of 1 μg/l. Injection volume 300 μl (corresponds to about 20 ml of sample). First and second mobile phase, methanol–0.05% trifluoracetic acid in water (40:60, v/v) at 1 ml/min; cleanup volume, 2.8 ml; transfer volume, 0.80 ml.

In the MRM approach ISRP/C_{18} or ISRP/SPS column combinations were most favorable for the simultaneous analysis of the heterogeneous group of pesticides in water with a high DOC level. For samples with a medium DOC content (6 mg/l) C_{18}/SPS using short columns (50×4.6 mm I.D.) and isocratic elution is an efficient alternative.

3. Liquid chromatography with mass spectrometric detection

3.1. General aspects

The powerful features of LC–MS such as efficient separation, identification, and quantification of polar analytes makes this technique very attractive to the field of pesticide residue analysis. In addition, an important aspect of MS is to perform confirmation and quantification of compounds of a same class (e.g. phenylureas) and compounds lacking a chromophore (e.g. phosphonic acids). This allows the determination of a broad spectrum of pesticides and, hence, productivity in pesticide residue analysis.

Especially, the availability nowadays of the robust atmospheric pressure interface technique that combines high sensitivity/selectivity with reliable quantification largely contributes to the breakthrough of LC–MS.

The types and principles of the various LC–MS

interfaces have been described in detail [39] as well as the state-of-the-art and the impressive progress of LC–MS in the past decade [40]. Probably the most striking achievement of LC–MS is the sudden recent change of going from a technique requiring a specialist for operation into a more routinely applicable technique.

Until the mid 1990s most applications in pesticide residue analysis involved thermospray (TSP) or particle-beam (PB) interfaces [41]. Despite successful applications these interfaces were not very well adopted in regulatory practice for reasons such as

(i) high costs.
(ii) significant variation in sensitivity between the different classes of compounds or even for compounds from the same class (both PB and TSP).
(iii) poor compatibility of PB with RPLC.
(iv) in case of TSP, highly variable compound-dependent responses and the need for a critical control of relevant temperatures during LC–TSP–MS analysis.

Nevertheless, an attractive feature of LC–PB–MS is that it provides electron impact (EI) spectra data comparable to GC–MS data bases; evidently, this advantage is limited to GC-amenable pesticides such as triazines and organophosphorous compounds [42].

Since the development and availability (about mid-1990s) of atmospheric pressure ionization (API) interfaces most of the problems mentioned above were reduced or eliminated [39,43]. Generally, in API a distinction is made between electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The ionization mechanisms of both interfaces have been described [39] and detailed information is beyond the scope of this review.

A major difference between ESI and APCI is the recommended flow of the column entering the interface of the MS. The working range of ESI is about 10–100 μl/min while for APCI optimal flows are at about 1 ml/min, making the latter a flexible technique regarding conventional LC flow-rates. It must be stressed that besides the development of API interfaces a parallel progress can be observed in the MS instruments. The fully automated computer-controlled optimization procedures impressively improved the performance of LC–MS, and moreover, the ease in operating the instruments.

Until now, there is little substantial information about which type of interface is most suitable in pesticide residue analyses. Based on our recent experience and information we have the impression, that the selection of an interface is partly based the experience of the laboratory and the type of instrument. As will be shown later, the performance of both interfaces regarding sensitivity/selectivity is very well comparable for many pesticides.

Despite the lacking of scientific support it is generally considered that concerning detection/quantification APCI is less prone to matrix interferences than ESI, which can be an important issue in trace analysis.

API applied in the LC–MS methods discussed below is a soft ionization technique that predominantly produces the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecular ions in positive (PI) or negative (NI) ionization mode, respectively. Increase in compound identification can be obtained by MS–MS or MS^n based on detection of product ion(s) (daughter ions) formed by collision-induced detection (CID) of the initially formed (de)protonated molecular ion (parent ion). In pesticide residue analysis triple quadruple (MS–MS) and/or ion trap (MS^n) instruments will offer highest selectivity and in most cases highest sensitivity due to improvement of the signal/noise ratio.

Unfortunately, in comparison to current LC detectors, LC–MS(MS) instruments are (still) much more expensive. Hence, adoption in pesticide residue analysis will depend on the economic pay off by factors such as, (lower) instrument-price development, sample processing time and method development time.

The development of LC–MS instruments is an on-going fast process. Therefore, the reviewing of LC methods was arbitrarily restricted on work published during about the last three years in order to provide as much as possible up-to-date information. Based on the type of LC–MS applications discrimination has been made between the analysis of environmental water samples and solid matrices.

3.2. Methods for water samples

The literature search over the period 1997–1999 revealed that most LC–MS applications involved the trace analysis of pesticides in environmental water

samples. The good compatibility of aqueous samples with RPLC, contributes to the fact that, especially in case of fully automated procedures, LC–MS is already frequently applied this field of analysis.

As demonstrated by recent developed single residue methods, LC–MS makes it possible to determine in an efficient and selective way, so-called 'difficult' pesticides such as the quaternary ammonium compounds diquat and paraquat [44,45] and the phosphonic acid glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) [46].

Our first LC–MS applications carried out recently involved the trace analysis of several acidic herbicides of various classes in water samples originating from field studies investigating the fate of pesticides in agricultural use areas. As illustrated in Fig. 6, our usual approach consists of the screening of samples with a RPLC–UV method followed by a confirmation of analytes in (a selection) of the samples by means of GC–MS involving a preceding derivatization of the analytes [34,37]. As outlined in Section 2.3, in the case of acidic compounds we, nowadays, employ coupled-column LC involving at least one RAM analytical separation column [37,38]. As Fig. 6 clearly shows, LC–MS reduces considerably the total time of analysis in comparison to the existing procedure. Another important feature to mention is that for the study involving the determination of bentazone and mecoprop in surface water samples at a required level of 10 ng/l the LC–MS method was developed within two days. The convenient operation of the Series 1100 benchtop LC–MS system (Hewlett-Packard) in finding suitable APCI–NI–MS conditions largely contributed to the relatively short time spent at method development. SPE was performed by sampling 250 ml of water sample (brought to pH 2.4 with a 10% HCl solution in water) on a preconditioned (3 ml methanol, 3 ml acetone, 3 ml methanol, 6 ml 0.1% HCl) 500 mg C_{18} cartridge. After loading, the cartridge was dried for 30 min by passing air and the analytes were desorbed with 2 ml acetone. One ml of acetone was evaporated and the residue redissolved in 250 μl methanol–0.4% formic acid in water (60:40, v/v). Analysis was performed by injecting 100 μl of extract onto the LC–MS system. The compounds were separated on a 150×4.6 mm I.D. column packed with 5 μm SPS-5-PM-5S-100 ODS (Regis, USA) with a mobile phase consisting of methanol–0.4% formic acid in water (60:40, v/v) and detected with APCI–MS in the NI mode and selected ion monitoring (SIM). Recoveries of drinking water samples spiked with bentazone and mecoprop at levels between 10 and 500 ng/l (n=8) were between 82 and 96% (RSDs 2–10%); The LC–APCI–NI–MS analysis of an extract of a surface water sample containing bentazone (10 ng/l) and mecoprop (5 ng/l) is shown in Fig. 7. It nicely shows the good performance of this approach, especially if one compares the LC–LC–UV analysis of a surface water sample containing bentazone displayed in Fig. 4.

It must be noted that the LC–MS analysis is performed on a one-column system without any cleanup. Despite the presence of co-eluting compounds visualized by the baseline pattern (see Fig. 7) the LC–MS results were in good agreement with data obtained by the GC–MS confirmation analysis [37]. For example, for the sample displayed in Fig. 7 and analyzed with GC–MS, concentrations of 10 ng/l and <10 ng/l were found for bentazone and mecoprop, respectively.

From the point of view of productive analysis, MRMs are most attractive. Therefore, an overview is made in Table 2 of MRMs involving LC–MS for the assay of polar pesticides in water samples. As can be seen, these methods include various classes of pesticides employing ESI [47–58] or APCI [59–63] for interfacing.

Table 2 shows that sample pretreatment is carried out off-line with a SPE procedure [47–57], on-line by an integration in the analytical procedure [58–60] or not applied in so-called single short column approach [61–63].

Most of the off-line SPE methods [47–55] involve the use cartridges packed with a graphitized carbon black (GCB) sorbent (Carbograph 1 or 4). This approach, originating from Di Corcia and co-workers [47–50] enables the fast sampling of a large volume of water, the preconcentration of pesticides with a large range in polarity, and moreover, enhancement of selectivity by means of class fractionation during the desorbtion step. All methods employ ESI for interfacing and allow the multi-residue analysis of many pesticides of various classes, most of them at the 5–20 ppt level with good reproducibilities

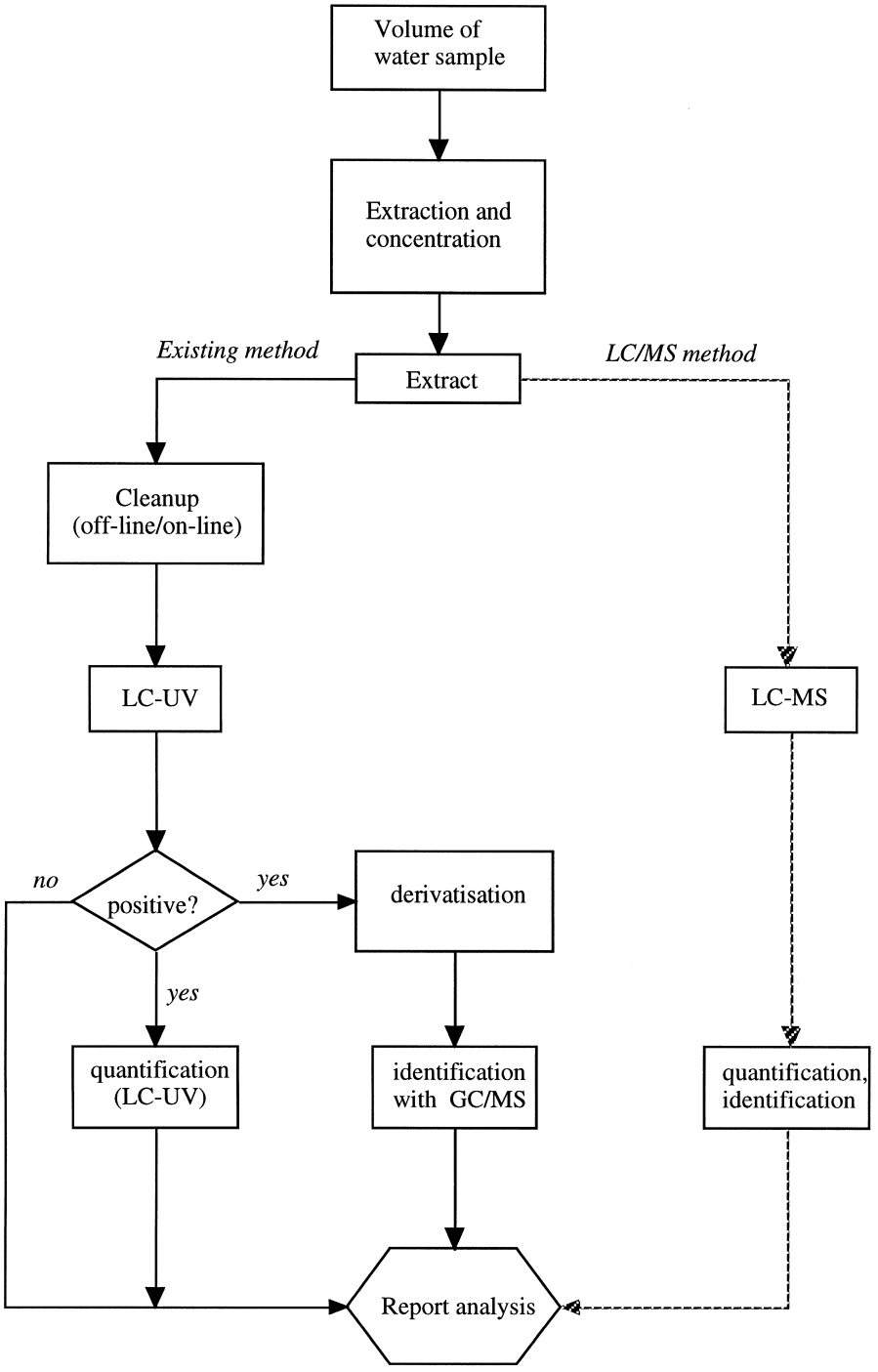

Fig. 6. Comparison between the existing method and the LC–MS method for the assay of polar pesticides in water samples.

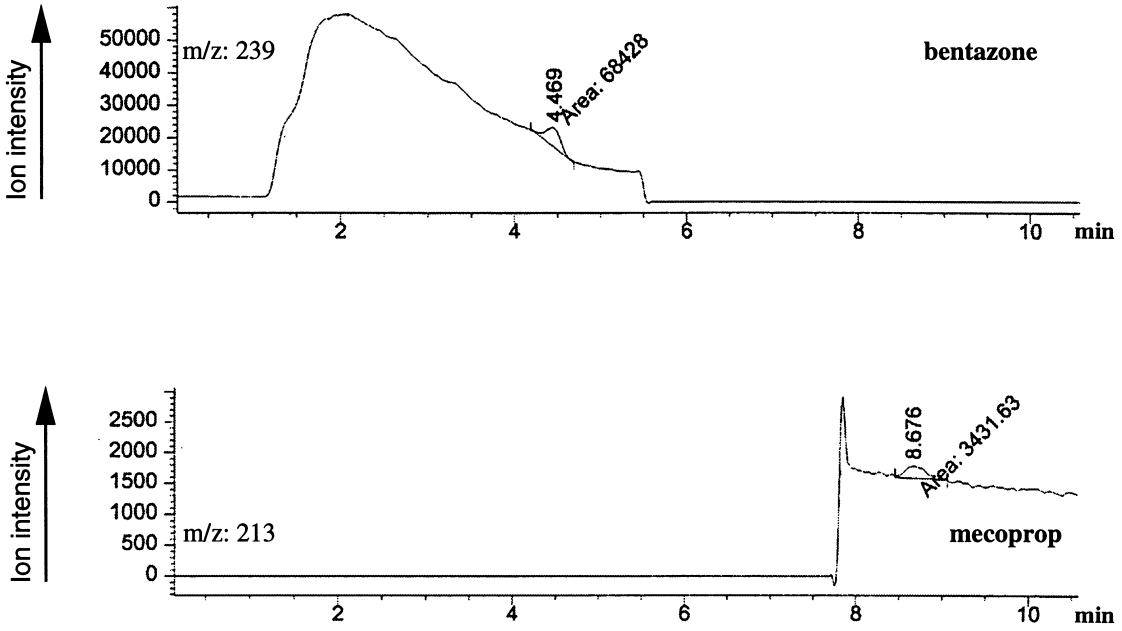

Fig. 7. LC–APCI–NI–MS ion chromatograms of an extract of a surface water sample containing bentazone and mecoprop concentrations of 10 ng/l and 5 ng/l, respectively (ion intensity in arbitrarily units). *x*-axis in minutes.

(<10%) and recoveries above 80%. For the assay of ground water samples, the combination of LC–ESI–MS with off-line SPE with cartridges packed with polymeric [54] or C_{18} material [55] was used for the determination of several groups of acidic herbicides and sulfonylurea herbicides, respectively, at levels ranging between 5 and 10 ng/l.

Off-line SPE on polystyrene–divinylbenzene resin cartridges (RP-102) for concentration and SAX cartridges for cleanup allowed confirmation and quantification of sulfonylurea, imidazolinone and sulfonamide herbicides at the low ng/l level in surface water using LC–ESI–PI–MS [57].

The methods mentioned above combine off-line SPE with LC–ESI–MS and show that in many cases PI or NI ionization can been used for a same class of pesticide without a significant difference in method performance.

Our own experience showed that phenylurea herbicides could be efficiently analyzed in various types of water to a level of at least 10 ng/l by combining a rapid SPE step and LC–APCI–PI–MS. The SPE step consisted of the sampling of 100 ml of water on a preconditioned (3 ml methanol, 3 ml acetone, 3 ml methanol, 6 ml water) 500 mg C_{18} cartridge. After loading, the cartridge was dried for 30 min by passing air over it and the analytes were desorbed with 2 ml acetone. After solvent evaporation the residue was redissolved in 200 μl methanol–water (50:50, v/v). Analysis was performed by injecting 100 μl of extract onto the Series1100 bench-top LC–MS system of Hewlett-Packard. The compounds were separated on a 150×4.6 mm I.D. column packed with 5 μm SPS-5-PM-5S-100 ODS (Regis) with a mobile phase consisting of methanol–water (50:50, v/v) and detected with APCI–MS in the PI mode and SIM. The analyses of drinking water samples spiked with five phenylurea herbicides at levels between 10 and 500 ng/l (n=8) provided average recoveries ranging between 67 and 91% (RSDs 4–6%); various types of environmental water samples spiked with the herbicides at levels of 10 and 50 ng/l (n=6) provided recoveries between 70 and 100% (RSD range 8–20%). The performance of this approach is illustrated in Fig. 8 showing the LC–MS–APCI–PI–MS analysis of an extracted of a surface water sample spiked with the analytes at a level of 10 ng/l.

Table 2
LC/MS multi-residue methods for pesticides in environmental waters

Pesticide class	Water[a]	Sample pretreatment[b]	MS-mode[c]	LODs (ng/l)	Ref.
Acetanilide	GW	Off-line,1 l, SPE, Carbograph 4	ES–PI–MS	5–10	[55]
Aminopropionate	GW	Off-line, 1 l, SPE, Porapak Rdx	ESI–NI–MS	5–10	[54]
APPA[d]	GW	Off-line, 2 l, SPE, Carbograph-4	ESI–PI–MS	about 5	[51]
APPA	GW	Off-line, 1 l, SPE, Porapak Rdx	ESI–NI–MS	5–10	[54]
APPA	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	5–10	[55]
Benzonitrile	GW	Off-line, 1 l, SPE, Porapak Rdx	ESI–NI–MS	5–10	[54]
Benzonitrile	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	10–20	[55]
Benzothiazole	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	5–10	[54]
Benzothiazole	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	10–20	[55]
Dinitrophenol	GW	Off-line, 1 l, SPE, Porapak Rdx	ESI–NI–MS	5–10	[54]
Imidazolinone	SW	Off-line, 0.2 l, SPE, RP-102; cleanup on SAX cartridge	ESI–PI–MS	20–30	[57]
Imidazolinone	GW	Off-line, 2 l, SPE, Carbograph-4	ESI–PI–MS	about 5	[51]
Imidazolinone	DW	Off-line, 2 l, SPE, Carbograph-4	ESI–PI–MS	about 1	[52]
Imidazolinone	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	5–10	[55]
Imidazolinone	SW	Off-line, 1 l, SPE, Carbograph 1	ESI–PI–MS	30–70	[56]
Phenoxy acid	GW	Off-line, 1 l, SPE, Porapak Rdx	ESI–NI–MS	5–10	[54]
Phenoxy acid	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	10–20	[55]
Phenylurea	SW	Off-line, 1 l, SPE, Carbograph-4	ESI–PI–MS	6–75	[47]
Phenylurea	SW	Off-line, 1 l, SPE, Carbograph-4	ESI–PI–MS	1–20	[48]
Phenylurea	GW	Off-line,1 l, SPE, Carbograph 4	ESI–PI–MS	5–10	[55]
Phenylurea	SW	Off-line, 0.1 l, SPE C_{18}	APCI–PI–MS	5–10	t.w.[e]
Phenylurea	SW	On-line, 10 ml on PC (PDMS)	ESI–PI–MS	about 10	[58]
Phenylurea	SW	On-line, 10 ml on AC (3 μm C_{18})	APCI–PI–MS	10–200	[60]
Phenylurea	GW	Automated SPE-immuno, 20 ml	APCI–PI–MS	1–5	[59]
Phenylurea	SW	None, 4 ml on SSC	APCI–PI–MS–MS	50–500	[61]
Phenylurea	SW	None, 4 ml on SSC	APCI–PI–MS–MS	10–100	[62]
Phenylurea	SW	None, 4 ml on SSC	APCI–PI–MS–MS	10–100	[63]
Phosphate	SW	None, 4–15 ml on SSC	APCI–PI–MS–MS	10–100	[63]
Sulfonamide	SW	Off-line, 0.2 l, SPE, RP-102; cleanup on SAX cartridge	ESI–PI–MS	20–30	[57]
Sulfonylurea	SW	Off-line, 0.2 l, SPE, Carbograph-4	ESI–PI–MS	13–40	[50]
Sulfonylurea	GW	Off-line, 2 l, SPE, Carbograph-4	ESI–PI–MS	about 5	[51]
Sulfonylurea	GW	Off-line, 2 l, SPE, C_{18} + cleanup on silica cartridge	ESI–NI–MS	5–10	[53]
Sulfonylurea	GW	Off-line, 1 l, SPE, Porapak Rdx	ESI–NI–MS	5–10	[54]
Sulfonylurea	SW	Off-line, 0.2 l, SPE, RP-102; cleanup on SAX cartridge	ESI–PI–MS	20–30	[57]
Sulfonylurea	GW	Off-line,1 l, SPE, Carbograph 4	ESI–NI–MS	10–20	[55]
Triazine	GW	Automated SPE-immuno, 20 ml	APCI–PI–MS	1–5	[59]
Triazine	SW	Off-line, 1 l, SPE, Carbograph-4	ESI–PI–MS	1–20	[48]
Triazine	SW	Off-line, 1 l, SPE, Carbograph-4	ESI–PI–MS	40–300	[49]
Triazine	GW	Off-line,1 l, SPE, Carbograph 4	ESI–PI–MS	5–10	[55]
Triazine	SW	None, 4 ml on SSC	APCI–PI–MS–MS	10–50	[61]
Triazine	SW	None, 4 ml on SSC	APCI–PI–MS–MS	30–100	[62]

[a] DW, drinking water; GW, ground water; SW, surface water.
[b] SPE, Solid Phase Extraction; PC, precolumn; AC, analytical column; SSC, single short column; PDMS, polydimethylsiloxane; SAX, strong anion exchanger.
[c] ESI, electrospray; APCI, atmospheric pressure chemical ionization; PI and NI, positive and negative ionization mode.
[e] t.w., method presented in this work.
[d] APPA, aryloxyphenoxypropionic acid.

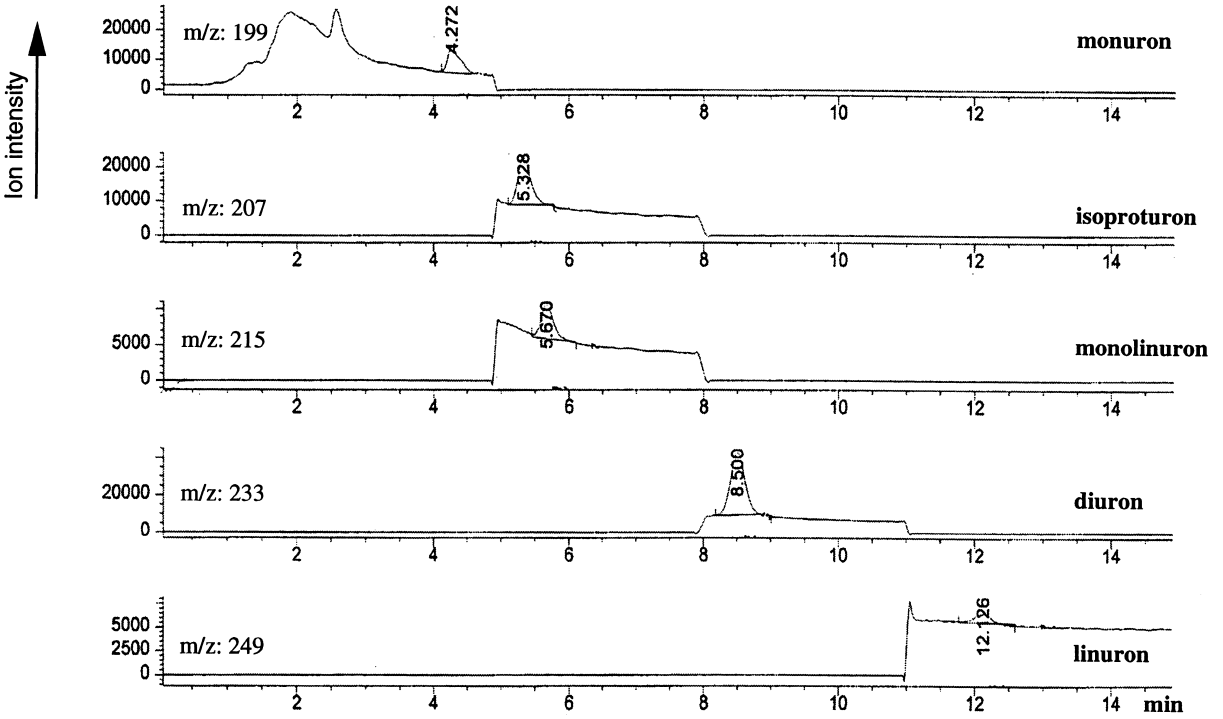

Fig. 8. LC–APCI–PI–MS ion chromatograms of an extract of a surface water sample spiked with phenylurea herbicides at a level of 10 ng/l (ion intensity in arbitrarily units). *x*-axis in minutes.

An automated immunosorbent phase extraction system coupled on-line with LC–APCI–PI–MS (PI mode) appeared to be an original and powerful approach for the sensitive/selective analysis of triazine and phenylurea herbicides in environmental samples [59]; sample volumes of 20 ml of ground water provided LODs in the range 1–5 ng/l.

On-line sample processing by means of column switching using a 10 ml of sample volume allowed the determination of phenylurea herbicides in environmental waters to a level of 10 ng/l [58,60]. Both the precolumn (PC–LC) [58] and the coupled-column (LC–LC) procedure [60] involved an injection of 10 ml of sample and provided an LOD of 10 ng/l for most of the analytes. Remarkably, in case of linuron, APCI–PI–MS [60] appeared to be about 10 times less sensitive in comparison to ESI–PI–MS detection [58].

The single short column (SSC) approach involves the direct injection of a volume of water sample on a short column hyphenated to a tandem mass spectrometry instrument [61–63]. By combining sample enrichment and separation of analytes in one-step the SSC approach aims at the high-speed analysis of target analytes and, therefore, reduction of expensive LC–MS measurement time. For a given LC–MS–MS application the compromise between the minimal time of analysis and the minimal required chromatographic separation will be largely determined by the efficiency of the column (total number of plates, *N*) which in turn will depend on the dimension of the column and efficiency of the packing material. In most cases, suitable conditions for sorption and desorbtion will always include a washing step (removal of inorganic salts) prior to elution and a minimal required separation between the first analyte(s) and the solvent front in order to avoid the unstable ionization conditions caused by the rapidly changing elution solvent composition. For the trace analysis of triazines, phenylurea and phosphate pes-

ticides in water optimal results were obtained by using a 10×2 mm I.D column high-pressure packed with 8 μm C_{18} bonded silica. In combination with a 4 ml sample injection volume APCI–MS resulted in detection limits of 50–500 ng/l (see Table 2).

3.3. Methods for solid samples

Information on selected LC–MS methods for the residue analysis of pesticides in solid matrices published over the period 1997–1999 is given in Table 3. In comparison to water samples (see Table 2) LC–MS seems to be less adopted. Table 3 includes both SRMs [64–67] and MRMs [68–70] and shows that LC–MS has been applied for various types of pesticide/matrix combinations.

Table 3 clearly reveals that the usually laborious sample pretreatment, in all LC–MS methods is simple [66–69] or superfluous [64,65,70]. Beside the advantage of high-speed analysis, the LC–MS procedures provide enhancement of sensitivity [64,65] and/or selectivity [66,67] in comparison to existing analytical methodologies [64–67].

For example, in the trace analysis of imazethapyr in soils with LC–ESI–MS–MS [67] the instrumental analysis time was reduced to that approximating sample preparation time and the drawbacks of in situ methylation required for the GC method were eliminated.

Crescenzi et al. [69] performed multi-residue analysis of 16 herbicides, including acidic and non-acidic analytes, in soils. The combination of sub-critical water extraction with on-line sorbent trap (Carbograph 4) and LC–ESI–MS appeared to be an efficient approach for the simultaneous determination of various classes of pesticides in various types of soils.

Barnes et al. developed a LC–APCI–MS multi-residue method for the determination of ten various pesticides in different types of fruit [70]. After

Table 3
Single- and multi-residue LC–MS methods (SRMs and MRMs) for pesticide residue analysis in solid matrices

Pesticides	Matrix	Sample pretreatment[a]	MS-mode[b]	LODs (μg/kg)	Ref.
SRMs:					
Fenbutatin oxide	fruits	Ethyl acetate extraction, concentration, solvent switch to acetonitrile	APCI–PI–MS	10–20	[64]
Chlormequat	pear	Methanol extraction	ESI–PI–MS–MS	40	[65]
Daminozide	apples, leaves	Methanol extraction; cleanup on Envicarb SPE cartridge (GCB)	APCI–PI–MS–MS	8–20	[66]
Imazethapyr	soil	MASE with buffer (pH 10) as solvent; cleanup on C_{18} SPE cartridge	ESI–PI–MS–MS	1	[67]
MRMs:					
Arylozyphenoxy-propionic acids	soil	SCE with methanol-buffer, pH 10; cleanup on Carbograph-1 SPE cartridge	ESI–NI–MS	5	[68]
Triazines, phenylureas, phenoxy acids, benzonitriles	soil	Heated water extraction with home-made device including trap on SPE Carbograph 4	ESI–NI–MS or ESI–PI–MS	2–10	[69]
Carbamates, diflubenzuron, clofetezine, carbendazim, thiabendazole	fruits	Ethyl acetate extraction, concentration, solvent switch to acetonitrile	APCI–NI–MS or APCI–PI–MS	2–35	[70]

[a] SCE, solid column extraction; MASE, microwave assisted solvent extraction; GCB, graphitized carbon black.
[b] ESI, electrospray; APCI, atmospheric pressure chemical ionization; PI and NI, positive and negative ionization mode.

extraction with ethyl acetate, solvent evaporation, and a solvent switch to acetonitrile, part of the diluted extract was directly processed with LC–APCI–MS using a switching positive/negative ionization mode during each acquisition. The LODs obtained clearly met the required MRLs for these compounds in foodstuffs. Despite the favorable features it was observed that sensitivity is both compound- and matrix-dependent, making next to solvent-based standards (check on column/instrument performance) additional calibration with matrix-matched standards necessary.

4. Conclusions and trends

The development of more efficient (and/or selective) column materials in combination with robust, selective and sensitive mass spectrometric detection leads reversed-phase liquid chromatography (RPLC) to have an excellent future in productive pesticide residue analysis.

The reviewed new LC packing materials, viz. immunosorbents, molecular imprinted polymers (MIPs) and restricted access materials (RAMs), enhance selectivity in such a way that in the determination of polar pesticides in various types of matrices the uncleaned extracts can be processed with column-switching RPLC–UV. As a powerful result, the labor of sample pretreatment can be considerably reduced by including such a material in the analytical procedure.

Methods employing immunosorbent technology offer very high selectivity, allowing the sensitive/selective assay of a large number of triazine and phenylurea herbicides in both solid and aqueous samples. However, antibodies are still developed and produced by a few research groups and do not behave uniformly [71], hence, their acceptance in daily practice will depend on better reproducibility and commercial availability.

Because of advantages such as ease of preparation, stability and low cost of MIPs, these materials are very attractive in cost-effective analysis and one might expect that further developments will include pesticides of various classes, which evidently will lead to increased adoption of MIPs in pesticide residue analysis.

Analytical RAM columns applied in coupled-column RPLC–UV is an attractive approach for analysis of acidic pesticides in environmental samples. The approach substantially reduces the so-called humic-hump and makes it possible to process uncleaned extracts.

LC–MS is rapidly becoming a routine technique for the efficient trace analysis of polar pesticides in various types of samples. In comparison to existing methodologies LC–MS considerably simplifies cleanup procedures, reducing both time of analysis and method development time.

The fact that large size molecules such as humic substances are outside the range of the MS detector, certainly contributes to the successful use of LC–MS in the trace analysis of pesticides in environmental samples

The two powerful features of RPLC–MS, viz. its ability to perform efficient separation of very polar to apolar pesticides and the universal/selective character of the detection mode, undoubtedly makes LC–MS compatible with GC–MS in the near future.

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References

[1] G.R. van der Hoff, P. van Zoonen, J. Chromatogr. A 843 (1999) 301.
[2] P. van Zoonen (Ed.), Analytical Methods for Pesticide Residues in Foodstuffs, 6th ed., General Inspectorate for Health Protection, Ministry of Public Health, Welfare and Sport, The Netherlands, The Hague, 1996.
[3] Regeling Residuen van Bestrijdingsmiddelen, Staatscourant, 5 November 1998 (Stcrt. 212).
[4] A. de Kok, M. Hiemstra, C.P. Vreeker, J. Chromatogr. 507 (1990) 459.

[5] M. Hiemstra, A. de Kok, J. Chromatogr. A 667 (1994) 155.
[6] V. Pichon, M. Bourzige, M.-C. Hennion, Anal. Chim. Acta 376 (1998) 21.
[7] G.S. Rule, A.V. Mordehai, J. Hennion, Anal. Chem. 66 (1994) 230.
[8] D.H. Thomas, M. Beck-Westermeyer, D.S. Hage, Anal. Chem. 66 (1994) 3823.
[9] S.J. Shahtaheri, M.F. Katmeh, P. Kwasowki, D. Stevenson, J. Chromatogr. A 697 (1995) 131.
[10] V. Pichon, L. Chen, M.-C. Hennion, R. Daniel, A. Martel, F. Le Goffic, J. Abain, D. Barceló, Anal. Chem. 67 (1995) 2450.
[11] V. Pichon, H. Rogniaux, N. Fischer-Durand, S. Ben Rejeb, F. Le Goffic, M.-C. Hennion, Chromatographia 45 (1997) 289.
[12] J.F. Lawrence, C. Menard, M.-C. Hennion, V. Pichon, F. LeGoffic, N. Duran, J. Chromatogr. A 732 (1996) 277.
[13] J.F. Lawrence, C. Menard, M.-C. Hennion, V. Pichon, F. LeGoffic, N. Duran, J. Chromatogr. A 752 (1996) 147.
[14] V. Pichon, L. Chen, M.-C. Hennion, Anal. Chim. Acta 311 (1995) 429.
[15] V. Pichon, L. Chen, N. Durand, F. Le Goffic, M.-C. Hennion, J. Chromatogr. A 725 (1996) 107.
[16] A. Martin-Esteban, P. Fernandez, D. Stevenson, C. Camara, Analyst 122 (1997) 1113.
[17] I. Ferrer, V. Pichon, M.-C. Hennion, D. Barceló, J. Chromatogr. A 777 (1997) 91.
[18] A.G. Mayes, K. Mosbach, Trends Anal. Chem. 16 (1997) 321.
[19] B. Sellergren, Trends Anal. Chem. 16 (1997) 310.
[20] D. Kriz, O. Ramstrom, K. Mosbach, Anal. Chem. 69 (1997) 345A.
[21] V.T. Remcho, Z.J. Tan, Anal. Chem. 71 (1999) 248A.
[22] M.T. Muldoon, L.H. Stanker, Anal. Chem. 69 (1997) 803.
[23] M. Kempe, Anal. Chem. 66 (1996) 1948.
[24] B. Bjarnason, L. Chimuka, O. Ramstrom, Anal. Chem. 71 (1999) 2152.
[25] I.H. Hagestam, T.C. Pinkerton, Anal. Chem. (1985) 1757.
[26] C.P. Desilets, M.A. Rounds, F.E. Regnier, J. Chromatogr. 544 (1991) 25.
[27] K.K. Unger, Chromatographia 31 (1991) 507.
[28] K.-S. Boos, A. Rudolphi, LC·GC Int. 11 (1998) 84.
[29] K.-S. Boos, A. Rudolphi, LC·GC Int. 11 (1998) 224.
[30] E.A. Hogendoorn, P. van Zoonen, A. Polettini, G. Marrubini Bouland, M. Montagna, Anal. Chem. 70 (1998) 1362.
[31] E.A. Hogendoorn, U.A.Th. Brinkman, P. van Zoonen, J. Chromatogr. 644 (1993) 307.
[32] E.A. Hogendoorn, R. Hoogerbrugge, E. Dijkman, S.M. Gort, U.A.Th. Brinkman, P. van Zoonen, J. Chromatogr. Sci. 31 (1993) 433.
[33] E.A. Hogendoorn, P. van Zoonen, J. Chromatogr. A 703 (1995) 149.
[34] E.A. Hogendoorn, R. Hoogerbrugge, R.A. Baumann, H.D. Meiring, A.P.J.M. de Jong, P. van Zoonen, J. Chromatogr. A 754 (1996) 49.
[35] J.v. Sancho-Llopis, F. Hernandez-Hernandez, E.A. Hogendoorn, P. Van Zoonen, Anal. Chim. Acta 283 (1993) 287.
[36] P. Parilla, P. kaim, E.A. Hogendoorn, R.A. Baumann, Fresenious J. Anal. Chem. 363 (1999) 77.
[37] E.A. Hogendoorn, K. Westhuis, E. Dijkman, H.A.G. Heusinkveld, A.C. den Boer, E.A.I.M. Evers, R.A. Baumann, J. Chromatogr. A 858 (1999) 45.
[38] E.A. Hogendoorn, E. Dijkman, R.A. Baumann, C. Hidalgo, J.V. Sancho, F. Hernandez, Anal. Chem. 71 (1999) 1111.
[39] W.M.A. Niessen, Liquid Chromtography–Mass Spectrometry, 2nd ed., Chromatographic Science Series, Vol. 79, Marcel Dekker, New York, 1999.
[40] W.M.A. Niessen, J. Chromatogr. A 856 (1999) 179.
[41] J. Slobodnik, B.L.M. van Baar, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 81.
[42] C. Aguilar, F. Borrull, R.M. Marce, J. Chromatogr. A 805 (1998) 127.
[43] W.M.A. Niessen, J. Chromatogr. A 794 (1998) 407.
[44] J.C. Marr, J.B. King, Rapid Commun. Mass Spectrom. 11 (1997) 479.
[45] V.Y. Taguchi, S.W.D. Jenkins, P.W. Crozier, D.T. Wang, J. Am. Soc. Mass Spectrom. 9 (1998) 830.
[46] R.J. Vreeken, P. Speksnijder, I. Bobbeldijk-Pastorova, Th.H.M. Noy, J. Chromatogr. A 794 (1998) 187.
[47] A. Di Corcia, A. Costantio, C. Crescenzi, R. Samperi, J. Chromatogr. A 852 (1999) 465.
[48] C. Crescenzi, A. Di Corcia, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 479.
[49] A. Di Corcia, C. Crescenzi, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 1658.
[50] A. Di Corcia, C. Crescenzi, R. Samperi, L. Scappaticio, Anal. Chem. 69 (1997) 2819.
[51] G. D'Ascenzo, A. Gentili, S. Marchese, A. Marino, D. Perret, Environ. Sci. Technol. 32 (1998) 1340.
[52] G. D'Ascenzo, A. Gentili, S. Marchese, D. Perret, J. Chromatogr. A 800 (1998) 109.
[53] R. Bossi, B. Koppen, N.H. Spliid, J.C. Streibig, J. AOAC Int. 81 (1998) 775.
[54] B. Koppen, N. Spliid, J. Chromatogr. A 803 (1998) 157.
[55] G. D' Ascenzo, A. Gentili, S. Marchese, A. Marion, D. Perret, Chromatographia 48 (1998) 497.
[56] A. Lagana, G. Fago, A. Marino, Anal. Chem. 70 (1998) 121.
[57] M. Rodriguez, D.B. Orescan, Anal. Chem. 70 (1998) 2710.
[58] E. Baltussen, H. Snijder, H.-G. Janssen, P. Sandra, C.A. Cramers, J. Chromatogr. A 802 (1998) 285.
[59] I. Ferrer, M.-C. Hennion, D. Barcelo, Anal. Chem. 69 (1997) 4508.
[60] E. van der Heeft, E. Dijkman, R.A. Baumann, E.A. Hogendoorn, J. Chromatogr. A 879 (2000) 39.
[61] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 794 (1998) 201.
[62] A. Hogenboom, P. Speksnijder, R.J. Vreeken, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 777 (1997) 81.
[63] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, LG·GC Int. 16 (1998) 264.
[64] K.A. Barnes, F.J. Fussell, J.R. Startin, H.J. Mobbs, R. James, S.L. Reynolds, Rapid Comm. Mass Spectrom. 11 (1997) 159.

[65] J.R. Startin, S.J. Hird, M.D. Sykes, J.C. Taylor, A.R.C. Hill, Analyst 124 (1999) 1011.
[66] H.G.J. Mol, R.C.J. van Dam, R.J. Vreeken, O.M. Steijger, J. Chromatogr. A 833 (1999) 53.
[67] S. Stout, A.R. DaCunha, M. Safarpour, J. AOAC Int. 80 (1997) 426.
[68] A. Lagana, G. Fago, A. Marion, M. Mosso, Anal. Chim. Acta 375 (1998) 106.
[69] C. Creszenzi, G. DíAscenzo, A. Di Corcia, M. Nazzari, S. Marchese, R. Samperi, Anal. Chem. 71 (1999) 2157.
[70] K.A. Barnes, R.J. Fussell, J.R. Startin, M.K. Pegg, S.A. Thorpe, S.L. Reynolds, Rapid Commun. Mass Spectrom. 11 (1997) 117.
[71] A. Martin-Esteban, P. Fernadez, C. Camara, Fresenious J. Anal. Chem. 357 (1997) 727.