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Determination of pesticides in environmental waters by automated on-line trace-enrichment and liquid chromatography

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Abstract

Automated on-line trace-enrichment, liquid chromatographic analysis and UV diode-array detection (DAD) were investigated for the determination of pesticides at the 0.1 μg/l level in drinking and surface waters. The selection of the preconcentration parameters (sorbent, sample volume, reproducibility) is discussed. Conditions are specified for quantification methods such as using calibration graphs constructed with spiked samples, standard addition methods and a calculation method included in the DAD software depending on the sample nature. Detection limits of 0.1 μg/l were obtained using 150 ml of river waters without any clean-up.

1. Introduction

Liquid chromatography (LC) has been shown to be an effective technique for the determination of pesticides and organic pollutants in aqueous media [1–3]. Its increasing availability is easily explained by its suitability for analysing simultaneously thermolabile and non-volatile organic compounds over a wide range of polarity without any derivatization such as is required in gas chromatographic (GC) analysis. Other reasons are its easy on-line coupling with the enrichment step using solid-phase extraction on precolumns [2,4–8] and the development of sensitive UV diode-array detection (DAD). Trace enrichment and LC analysis with DAD can be automated and this methodology has been applied for the automated monitoring of a broad range of pesticides and pollutants in drinking and surface waters [9]. On-line methodology coupling solid-phase extraction to LC separation is easily performed in any laboratory. In its simplest form, a precolumn is placed in the sample-loop position of a six-port switching valve. After conditioning, sample application and cleaning via a low-cost pump, the precolumn is coupled to an analytical column by switching the valve into the inject position. The extracted compounds are then eluted directly from the precolumn to the analytical column by a suitable mobile phase which permits the separation of the trapped compounds. The sequence described above can be totally automated using commercially available programmable systems, *e.g.*, the Prospekt module. As there is no sample manipulation between preconcentration and analysis, no loss or contamination risk can occur and one can expect more accurate quantitative results. Another advantage is that the whole species are analysed, allowing the handling of a smaller volume in comparison with off-line procedures where only an aliquot is usually analysed. One constraint of on-line techniques is the need to avoid a decrease in the analytical column ef-

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ficiency when coupling the precolumn. This is obtained by selecting a precolumn of small dimensions, typically 1 cm × 0.2 cm I.D., packed with LC-grade stationary phases. Therefore, when low detection levels are required, one has to select a stationary phase giving a high retention of analytes in water in order to handle a sufficiently large volume without breakthrough. A comparison of reversed-phase extraction sorbents has shown that the retention volumes of many organic compounds were about 25 times higher with apolar copolymers (PRP-1 or PLRP-S) than with octadecylsilicas [10].

Most of the studies using on-line trace enrichment, LC separation and DAD have discussed the identification of co-eluting analytes with similar UV spectra or with weak UV absorption. Limits of determination in LC-grade waters were shown to be lower than 0.1 μg/l, but determinations at this level in drinking waters were difficult [9]. In river water, the limits of detection are generally above 1 μg/l, although a multi-residue analysis of pesticides in river water at the 0.3 μg/l level using an on-line solid-phase disk extraction has been reported [11].

The aim of this study was not to determine in one run as many pesticides as possible, but to investigate the quantitative aspects encountered in trace-level determinations carried out using automated on-line trace enrichment and LC analysis with DAD. The effects of the sample matrix and optimization of the preconcentration parameters are discussed. Detection limits that can be obtained for drinking and river waters in multi-residue analysis and for triazine and phenylurea groups are presented.

2. Experimental

2.1. Apparatus

LC analyses were performed with a Varian LC System Workstation including a Varian Star 9010 solvent-delivery system and a Model 9065 Polychrom diode-array detector. The analytical column was connected to a Valco valve (VICI, Houston, TX, USA). Trace enrichment was performed on disposable cartridges using the Prospekt (Spark Holland, Emmen, Netherlands), which is an automated programmable sample preparation unit allowing direct elution to the LC column. Conditioning of the cartridges and sampling were performed via a solvent-delivery unit (SDU) (Spark Holland).

2.2. Stationary phases and columns

The analytical column was 25 cm × 4.6 mm I.D. prepacked with 5-μm Varian ODS-TSK 80TM octadecylsilica (Toyo Soda Manufacturing). Samples were preconcentrated on 10 mm × 2 mm I.D. cartridges prepacked with styrene–divinylbenzene copolymer (15–25 μm PLRP-S; Polymer Laboratories, Church Stretton, UK) and the octadecylsilica (Baker, Deventer, Netherlands).

2.3. Chemicals

HPLC-grade acetonitrile was kindly given by J.T. Baker. Methanol was purchased from Prolabo (Paris, France). LC-quality water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were obtained from Prolabo, Merck or Fluka.

The various pesticides were supplied by Riedel-de Haën (Seelze, Germany) or Promochem (Wesel, Germany). Stock standard solutions of selected solutes were prepared by weighing and dissolving them in methanol.

Three standard solutions were prepared, one containing ten representatives of the main classes of pesticides (simazine, atrazine, methabenzthiazuron, isoproturon, linuron, carbaryl, propanil, fenamiphos, fenitrothion and parathion), another containing most of the triazines used in France and some degradation products (deisopropylatrazine, deethylatrazine, hydroxyatrazine, hexazinone, simazine, cyanazine, simetryne, atrazine, prometon, sebutylazine, propazine and terbutylazine) and the third containing phenylurea herbicides (fenuron, methoxuron, monuron, methabenzthiazuron, chlortoluron, fluometuron, monolinuron, isoproturon,

diuron, difenoxuron, buturon, linuron, chloroxuron, chlorbromuron, diflubenzuron and neburon). These standard solutions were stored at 4°C and were used for the preparation of dilute working standard solutions and for spiking water samples. No change in the chromatogram of the standard solutions was observed during the 3 months of the study. The final spiked samples did not contain more than 0.5% of methanol.

2.4. Procedure

Automation of on-line trace enrichment was performed using the Propekt system equipped with a disposable cartridge unit. This system contains an SDU for conditioning and washing the trace-enrichment cartridges and percolating samples.

The procedure was as follows: (1) washing the cartridges with 10 ml of acetonitrile; (2) conditioning them with 10 ml of methanol and then 10 ml of LC-grade water; (3) percolation of samples; and (4) desorption from the cartridge to the analytical column by an acetonitrile gradient with phosphate buffer (pH 7). Three different gradients were used for the separation of triazines, phenylureas and multi-residue pesticides and are described in the figure captions.

3. Results and discussion

3.1. Analytical LC separation

The pesticides used are some of the most commonly applied in the European Community. Three standard solutions were prepared, one containing pesticides from different classes such as triazines, phenyureas, carbamates, organophosphorus and propionanilides and the other two containing twelve triazines and sixteen phenylureas, respectively. As each solution contained pesticides with a wide range of polarity, the analytical separation was carried out by reversed-phase chromatography using a C_{18} analytical column and an acetonitrile gradient with phosphate buffer (pH 7). The chromatographic conditions are reported in Fig. 2 for the multiresidue separation and in Fig. 6 for the separation of phenylurea and triazine herbicides.

3.2. Preconcentration parameters

Choice of sorbent

Once the analytical separation is obtained by direct injection of the standard solution onto the analytical column, it is possible to determine the detection limit for each compound and then to calculate the sample volume that has to be percolated without breakthrough in order to detect the analyte of interest at the required concentration. As an example, quantitative analysis at the 0.1 μg/l level necessitates at least a minimum limit of detection of 0.05 μg/l and, if the detection limit measured by direct injection is 5 ng, a sample volume of 100 ml has to be handled. Therefore, the main parameter of the preconcentration procedure is the choice of a sorbent that gives for this analyte a convenient breakthrough volume (V_b). The choice of the sorbent and the knowledge of the V_b values of the analytes are more important when using on-line preconcentration, in contrast to off-line preconcentration where it is possible to increase the breakthrough volume by increasing the amount of sorbent in the cartridge.

Breakthrough volumes

The breakthrough volume can be measured on a "breakthrough curve" obtained by percolating a spiked solution through the precolumn and recording the UV signal of the effluent [2,12]. This method is very time consuming and the direct UV recording requires a solution spiked at the 0.1 mg/l level. An experimental method for determining both breakthrough volumes and recoveries has been described and is easily performed with the on-line set-up [12,13]. A small volume spiked with a trace concentration (μg/l level) of all the analytes is percolated through the precolumn and the chromatogram corresponding to the on-line elution is recorded and peak areas are measured. This first volume is chosen so that breakthrough does not occur for any solute that can be verified approximately by

direct loop injection of the same amount. The sample volume is then increased and the concentration decreased in order to have a constant amount of analytes in the percolated samples. Provided that breakthrough does not occur for any analyte, the amounts concentrated remain constant and peaks areas measured on chromatograms obtained with on-line elution are constant. The breakthrough volume of an analyte is calculated when the peak area begins to decrease and the corresponding recovery can be also calculated by dividing the peak area obtained for the sample volume by the constant peak area obtained for sample volumes before breakthrough. One advantage of this method is that these values can be obtained from three or four on-line preconcentrations for all the analytes and under experimental conditions that correspond to those used for real analysis (trace level and several analytes together).

This method was applied and breakthrough volumes were compared using cartridges packed with C_{18} silica and PLRP-S. The results are reported in Fig. 1a and b. On C_{18} precolumns, breakthrough occurs rapidly for a volume between 40 and 60 ml for the four more polar compounds of the multi-residue standard solution whereas on PLRP-S precolumns V_b is between 300 and 400 ml for simazine and methabenzthiazuron and above 500 ml for atrazine. The differences in V_b values between C_{18} silica and the apolar copolymer are consistent with retention measurements [10]. Nevertheless, the V_b values measured here are higher than some published values obtained using the same cartridges [7,9]. For example, values of 50 and 90 ml were obtained for simazine and atrazine on PLRP-S cartridges. Nevertheless, the same workers have also measured V_b values of 180 ml for simazine and >400 ml for atrazine with a 10 mm × 2 mm I.D. precolumn laboratory packed with PLRP-S [8]. We have also obtained similar results for these two compounds on a similar precolumn packed with the PRP-1 copolymer [12]. As the V_b values in ref. 9 were measured by recording the breakthrough curves, the lower values obtained are likely to be due to the high concentrations of the spiked solutions and overloading of the precolumn.

It has also been reported that the breakthrough volume was the same when measured in LC-grade water, drinking water and surface waters [8]. The more polar the analytes, the lower are the V_b values on both C_{18} silica and apolar copolymers. Many modern pesticides and degradation products are more polar than simazine; Liska *et al.* [7] reported a method allowing the screening of about 50 "polar" pesticides, which are nowadays applied and which were classified in order of increasing retention times. In a first approximation, this order reflects the polarity order and simazine is

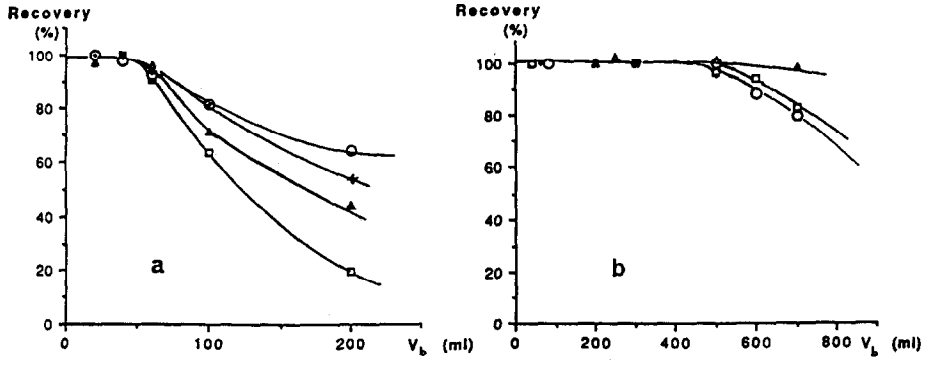

Fig. 1. Experimental variations of recovery with percolated Milli-Q-purified water samples having a constant amount (about 100 ng) of pesticides. Precolumn: (a) C_{18}; (b) PLRP-S. Analytical column, Varian ODS (25 × 0.46 cm I.D.); flow-rate, 1 ml/min; mobile phase, acetonitrile gradient with 0.05 *M* phosphate buffer at pH 7, gradient 30% acetonitrile from 0 to 38 min, 30 to 45% from 38 to 44 min, 45 to 47% from 44 to 52.5 min, 47 to 100% from 52.5 to 70 min; detection at 220 nm.

the 23rd compound. Therefore, it is advisable to use PLRP-S cartridges for trace-level determinations of pesticides in water. On a C_{18} precolumn, the sample volume that can be handled without any breakthrough for many moderately polar pesticides is lower than 50–100 ml.

Reproducibility

One advantage of automation in on-line preconcentration is that more reproducible results are expected, provided that the precolumns are packed with the same amount of sorbent and with the same efficiency. The repeatability of peak areas and heights obtained by direct loop injections into the analytical column was first studied. Table 1 (top) reports results from five 10-μl injections of a 2.5 mg/l solution containing four pesticides. The relative standard deviation (R.S.D.) is between 3–7% and 3–5% when measuring peak areas and peak heights, respectively. The reproducibility between cartridges was measured by preconcentrating 50 ml of LC-grade water spiked with 0.5 μg/l of the same pesticides. Five experiments were carried out using a new precolumn in each run. As can be seen in Table 1 (bottom), the R.S.D. is around 10% for measurements of both peak areas and peak heights. These results indicate that the precolumns were packed under reproducible conditions.

When percolating 50 ml of the spiked solution, breakthrough does not occur for each compound and therefore recoveries of 100% are expected. As the spiked solutions contained 25 ng of each compound, the peak areas obtained by preconcentration should be equal to those obtained by direct injection, as the amount injected directly is also 25 ng of each analyte. The average ratio between peak areas obtained by preconcentration and direct injection is 76%; it is 78% when using peak heights. If a decrease in efficiency was to be observed owing to the coupling of the precolumn, the ratio calculated from peak heights should be different from that calculated from peak areas. The difference observed is due to the volume of the injection loop, which is specified to an average accuracy of 20%. Calibration of a 20-μl loop is a delicate operation and is not necessary. When using on-line techniques, quantitative analyses should not be carried out by comparison with direct injections. Once the sample volume has been selected, calibration graphs should be constructed with spiked solutions under the same experimental conditions as selected for the analyses of unknown samples.

Flow-rate

Flow-rates of 2 and 5 ml/min for the preconcentration step were studied. The peak areas

Table 1
Test of reproducibility

Method	Compound	Peak area		Peak height	
		Mean ± S.D.	R.S.D. (%)	Mean ± S.D.	R.S.D. (%)
Direct injection[a]	Simazine	31 473 ± 866	2.7	1740 ± 45	2.6
	Cyanazine	19 558 ± 686	3.5	1182 ± 59	5.0
	Atrazine	22 460 ± 1570	7.0	1964 ± 99	5.0
	Londax	4059 ± 210	5.2	448 ± 18	4.0
Preconcentration[b]	Simazine	23 414 ± 2071	8.8	1256 ± 121	9.6
	Cyanazine	14 983 ± 1580	10.5	984 ± 111	11.3
	Atrazine	17 000 ± 1929	11.3	1520 ± 152	10.0
	Londax	3057 ± 245	8.0	349 ± 30	8.6

[a] Data obtained from five direct injections of 10 μl of a 2.5 mg/l solution of each analyte (amount injected 25 ng) in methanol.
[b] Data obtained from five preconcentrations of 50 ml of Milli-Q-purified water spiked with 0.5 μg/l of each compound (amount injected 25 ng) using different PLRP-S precolumns.

and heights of the different pesticides were measured under the same experimental conditions as those used for the results in Table 1 (bottom). Reproducibility was observed within a similar R.S.D. of 10%. For experiments allowing the measurement of breakthrough volume or calibration with LC-grade waters, the same precolumn was often reused after washing with pure acetonitrile and a flow-rate of 2 ml/min was applied. For natural water such as river water containing many interferences that cannot be eliminated easily by a washing step, a new precolumn was used in each run and the percolation was carried out at a flow-rate of 5 ml/min.

3.3. Determination in different matrices

It was recommended that calibration graphs should be obtained in the same conditions as for real analyses of unknown samples. Nevertheless, it is interesting to assess whether a calibration graph that was obtained with spiked LC-grade waters could be used for drinking or river waters. Some quantification methods are also included in the software of diode-array detectors and calculations are often made from data from spiked LC-grade solutions.

Matrix interference peaks

PLRP-S is a non-selective sorbent and many other compounds from the matrix of natural samples are preconcentrated and can be eluted together with the analytes of interest. Interferences depend on the nature of the water. They have an effect on both detection limits and quantification [9]. No clean-up can be applied such as by washing the precolumn after the percolation, because the more polar analytes would be eluted.

Different waters, LC-grade water, drinking water and Seine river water, were spiked with the standard multi-residue solution at a concentration of 0.3 μg/l and 150 ml of each sample were analysed on-line. The chromatograms obtained are represented in Fig. 2. The blank (Fig. 2a) indicates the baseline obtained at 220 nm with the gradient applied. LC-grade water (Fig. 2b) contains some impurities at the end of the chromatogram. In drinking water (Fig. 2c), interferences are visible at the beginning of the chromatogram by a broad peak spread over 8 min and several other peaks that do not correspond to the standard solution. In Seine water (Fig. 2d), the interfering peak at the beginning

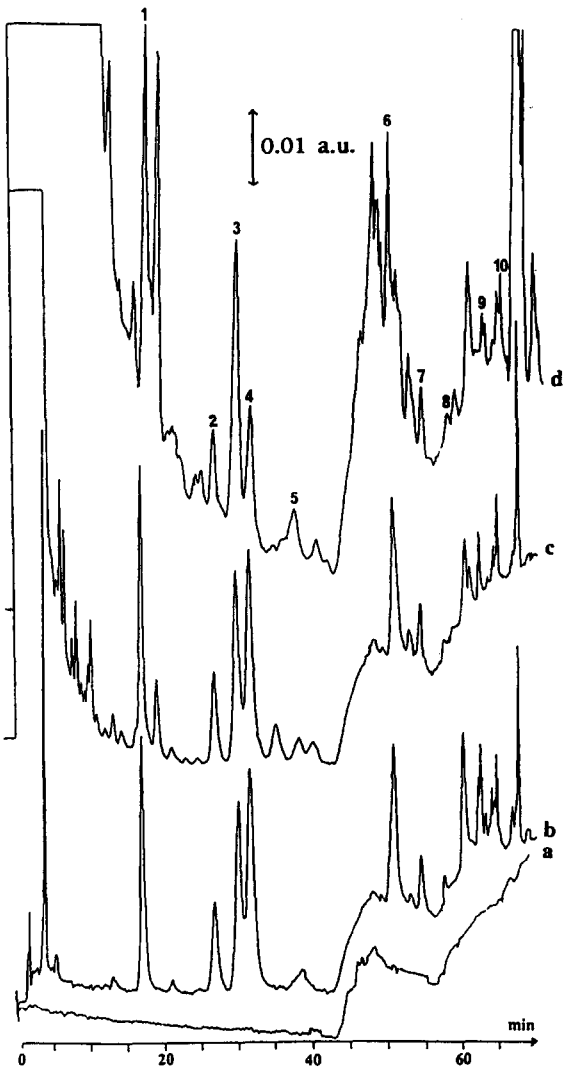

Fig. 2. On-line analysis of 150 ml of different water samples spiked with 0.3 μg/l of (1) simazine, (2) methabenzthiazuron, (3) atrazine, (4) carbaryl, (5) isoproturon, (6) propanil, (7) linuron, (8) fenamiphos, (9) fenitrothion and (10) parathion. Precolumn, PLRP-S; other experimental conditions as in Fig. 1. (a) Blank gradient; (b) Milli-Q-purified water; (c) drinking water; (d) surface water from the Seine (June 28th, 1993).

of the chromatogram is much broader than in drinking water and is spread over 15 min, and there are also other interfering peaks from around 40 min until the end of the gradient. The non-spiked sample of surface water is represented in Fig. 5.

First, with this gradient applied, a peak will not be detected in river water if its retention time is lower than 15 min. Peaks 1–4,6 and 7 are easily visible at this wavelength even in river water, in spite of the low concentration. It is important to note that within an R.S.D. of 10%, the peak heights are close in LC-grade water, drinking water and river water. Calibration graphs for these compounds should be similar in these three different matrices. Peak 3 is higher in river water owing to the presence of atrazine in the raw sample.

Effect of the gradient applied

Determination of a compound in natural samples is more difficult if a matrix interference peak is co-eluted. The shape of the matrix peaks depends on the nature of the sample and also of the gradient which is applied. It can appear as a broad peak at the beginning of the sample, and this corresponds in general to a gradient with an initial content of acetonitrile higher than 20–30%, as shown in Fig. 3a. With the same water and preconcentration parameters, but with a gradient containing only 5% of acetonitrile at the beginning, the matrix interfering peak has a different shape and appears in the middle of the chromatogram, as shown in Fig. 3b. This was also observed by Slobodnik *et al.* [9], with a gradient from 10% to 100% acetonitrile in 55 min. Low-level detection means that the interfering peak in river water is not too large and is well situated. This is often incompatible with the simultaneous determination of a large number of compounds spread over a large polarity range, which requires a gradient from pure water to pure acetonitrile or methanol. As an example, the retention time of simazine is 17.7 min with the gradient in Fig. 3a and 22.2 min with the gradient in Fig. 3b. If a limit of detection in river

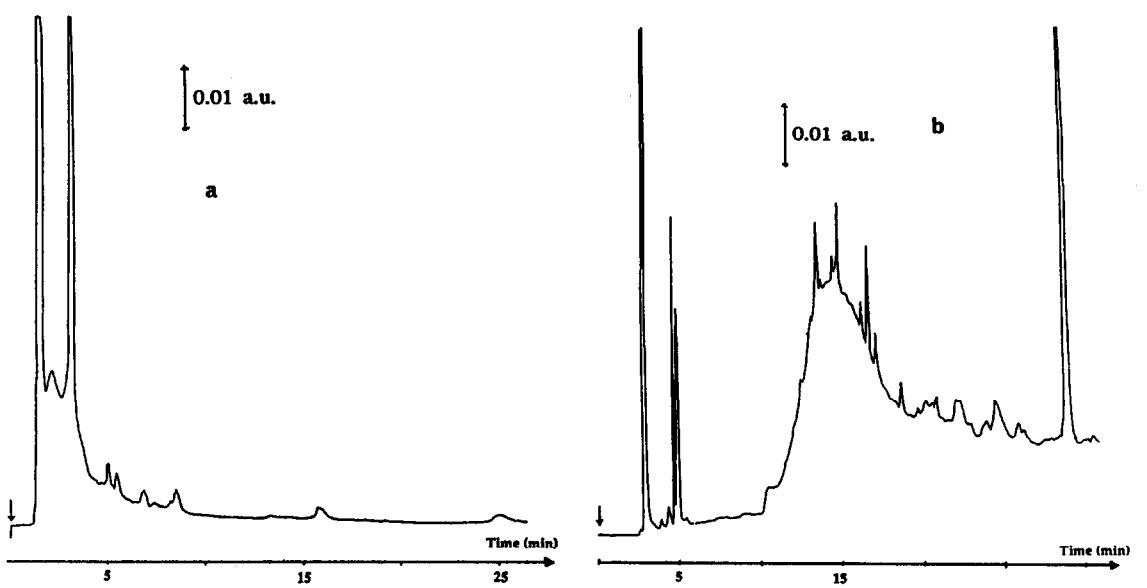

Fig. 3. On-line analysis of 150 ml of drinking water with two different acetonitrile gradients for the elution of the precolumn. (a) Acetonitrile gradient with a 0.05 *M* sodium phosphate solution (pH 7) at a flow-rate of 1 ml/min: 30% acetonitrile from 0 to 38 min, 30 to 45% from 38 to 44 min, 45 to 47% from 44 to 52.5 min, 47 to 100% from 52.5 to 70 min. (b) Acetonitrile gradient with a 0.001 *M* solution of perchloric acid at a flow-rate of 1 ml/min: 5 to 10% acetonitrile from 0 to 5 min, 10 to 35% from 5 to 8.5 min, 35% from 8.5 to 18 min, 35 to 75% from 18 to 30 min. Precolumn, PLRP-S; analytical column, Varian ODS (25 × 0.46 cm I.D.); detection at 220 nm.

waters below 0.5 μg/l is required, a compromise has to be found between the gradient shape and the polarity range of compounds detected.

Calibration graphs

When carrying out multi-residue analyses, the chromatograms are often presented with the detector set at 220 or 230 nm, because most compounds absorb at these wavelengths [3]. Nevertheless, when very low levels are required, it is advisable to draw the calibration graphs at wavelengths adapted to the compounds and to the matrix interferences. This is illustrated in Fig. 4 by chromatograms obtained at three wavelengths from the on-line analysis of 150 ml of drinking water spiked with 0.3 μg/l of the multi-residue standard solution. Whereas peaks 1–4 are well detected at 220 nm, peaks 5–10 are not. Peak 5 (isoproturon) is better detected at 239 nm (not represented), peaks 6–8 at 249 nm and peaks 9 and 10 (fenitrothion and parathion) at 268 nm. Although peak 10 is higher at 220 nm, its determination at 268 nm is certainly easier and more accurate because it can also be seen that the background of interfering materials detected after 40 min is higher at 220 nm than at 249 or 268 nm.

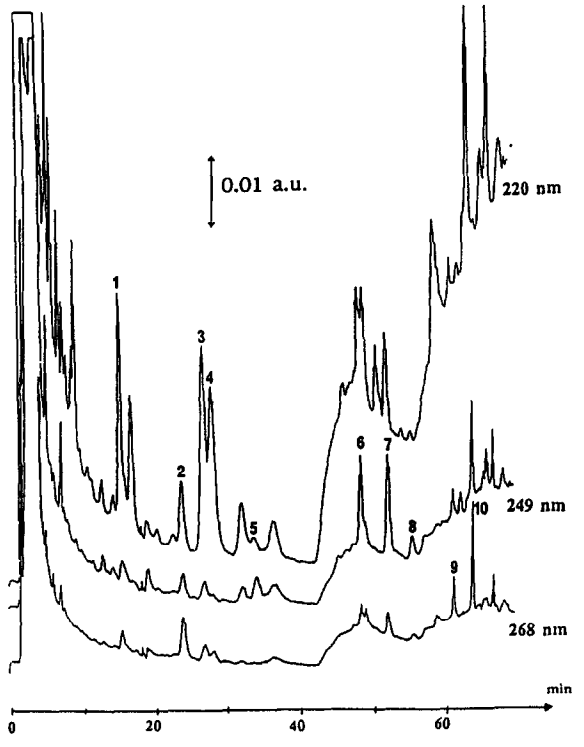

Fig. 4. Chromatograms corresponding to the on-line elution of 150 ml of drinking water at different wavelengths; drinking water spiked with 0.3 μg/l of ten pesticides as in Fig. 2. Precolumn, PLRP-S; analytical column, Varian ODS (25 × 0.46 cm I.D.); flow-rate, 1 ml/min; acetonitrile gradient as described in Fig. 1.

Calibration plots for the ten solutes were drawn for spiked LC-grade and drinking water in the trace-level range of 0.1–1.5 μg/l and using the same experimental conditions with a sample volume of 150 ml. Non-spiked drinking water was also analysed in order to be sure that no peak was obtained at the retention times of the analytes. Calibration equations are reported in Table 2. The correlation coefficients are all satisfactory ($R^2 > 0.99$) except for fenamiphos in LC-grade water. The important point is that calibration equations are not very different when constructed from LC-grade water or drinking water. That means that correct quantitative results can be obtained by on-line preconcentration of a non-spiked drinking water sample, using the calibration equation obtained with spiked LC-grade solution. In river waters, matrix interferences are higher and there are often many peaks for the non-spiked samples, so that it is difficult to obtain calibration graphs in the same range from 0.1 to 1.5 μg/l.

Determination using standard addition method and DAD software

In river water, the standard addition method is expected to be more accurate because of the high background of interfering compounds on the baseline. A non-spiked 150-ml sample of Seine river water was analysed on-line and the chromatogram obtained at 220 nm is presented in Fig. 5. Peaks corresponding to the retention times of simazine and atrazine were obtained. DAD identified atrazine, but not simazine, as can be seen by the match of the UV spectra. Aliquots of the same water samples (150 ml) were spiked with 15, 45 and 75 ng of each compound of the multi-residue standard solution. The slope calculated as concentration is

Table 2
Calibration data and detection wavelengths for multi-residue analysis (in the range 0.1–1.5 μg/l) in LC-grade water and drinking water

Compound	Detection wavelength (nm)	LC-grade water			Drinking water		
		N^a	Calibration equation[b]	R^2	N^a	Calibration equation[b]	R^2
Simazine	220	6	$y = 2058 + 1.12 \cdot 10^5 x$	0.999	7	$y = -2017 + 1.48 \cdot 10^5 x$	0.994
Methabenzthiazuron	220	6	$y = 790 + 6.26 \cdot 10^4 x$	0.997	7	$y = 164 + 7.65 \cdot 10^4 x$	0.998
Atrazine	220	6	$y = 1485 + 9.63 \cdot 10^4 x$	0.992	7	$y = 4880 + 1.16 \cdot 10^5 x$	0.998
Carbaryl	220	6	$y = 1290 + 1.92 \cdot 10^5 x$	0.998	7	$y = 3768 + 2.09 \cdot 10^5 x$	0.996
Isoproturon	239	6	$y = -5425 + 6.14 \cdot 10^4 x$	0.998	7	$y = -309 + 5.96 \cdot 10^4 x$	0.995
Linuron	249	6	$y = -1596 + 5.63 \cdot 10^4 x$	0.992	7	$y = -1352 + 5.73 \cdot 10^4 x$	0.981
Propanil	249	6	$y = -281 + 5.59 \cdot 10^4 x$	0.993	6	$y = -4382 + 6.19 \cdot 10^4 x$	0.998
Fenamiphos	249	6	$y = -426 + 1.67 \cdot 10^4 x$	0.960	4	$y = -0.14 + 7.47x$	0.994
Fenitrothion	268	6	$y = -1106 + 1.63 \cdot 10^{-4} x$	0.995	6	$y = -751 + 1.80 \cdot 10^4 x$	0.985
Parathion	268	6	$y = 69 + 1.89 \cdot 10^4 x$	0.995	6	$y = -85 + 2.09 \cdot 10^4 x$	0.997

Sample volume: 150 ml. Data from peak areas, except for fenaminphos where peak heights were used for the calibration in drinking water.
[a] Number of experimental points.
[b] y = area; x = concentration (ppb).

close to that obtained for calibration with LC-grade or drinking water. Nevertheless, it must be pointed out that the baseline around the atrazine peak is correct and does not contain too much interfering material. The calibration graph with LC-grade water gives a concentration of 0.30 μg/l in the raw sample. Standard addition calculations give a concentration of 0.28 μg/l.

Another quantification method was used which is included in the Polyview software and named MultiComponent Analysis (MCA). This spectrophotometric-based method allows the identification and determination of several analytes in an unknown sample by comparison with standard solutions. Pesticides are characterized by their retention time, their spectrum and the amount injected, and these data are stored in a library. In our experiment, the data were introduced from a chromatogram corresponding to the on-line analysis of a 150-ml spiked LC-grade water sample, and not by direct injection. The report of the MCA method gives the spectra from the library and the spectrum recorded on the peak of the unknown solution (Fig. 5). The result of quantification via the MCA software is 0.26 μg/l. Taking into account an average R.S.D. which can be estimated to be at least 15%, the values given by the three methods are consistent. The MCA calculation is, of course, the fastest and has also the advantage of indicating rapidly by means of the spectrum if there is a co-eluting analyte in the peak. No conclusion can be drawn in that case. This is clearly shown for the identification of simazine. It is visible in the chromatogram that there is a co-eluting peak. A standard addition made the first peak increase with good linearity. We tried to eliminate the second part of the peak for the spectrum recording, but no confirmation was obtained. Confirmation of the compound has to be carried out with a second on-line analysis in which the nature of the analytical column is changed to a cyano-bonded type, as shown by Di Corcia and Marchetti [3]. The coupling of on-line trace enrichment and LC coupled to mass spectrometry is now being developed and has been applied to the trace-level determination of phenylureas [14].

3.4. Detection limits

In drinking waters

From Fig. 4, representing the handling of 150 ml of drinking water spiked at the 0.3 μg/l level, it is obvious that detection limits depend on the UV properties of analytes, but are lower than 0.1

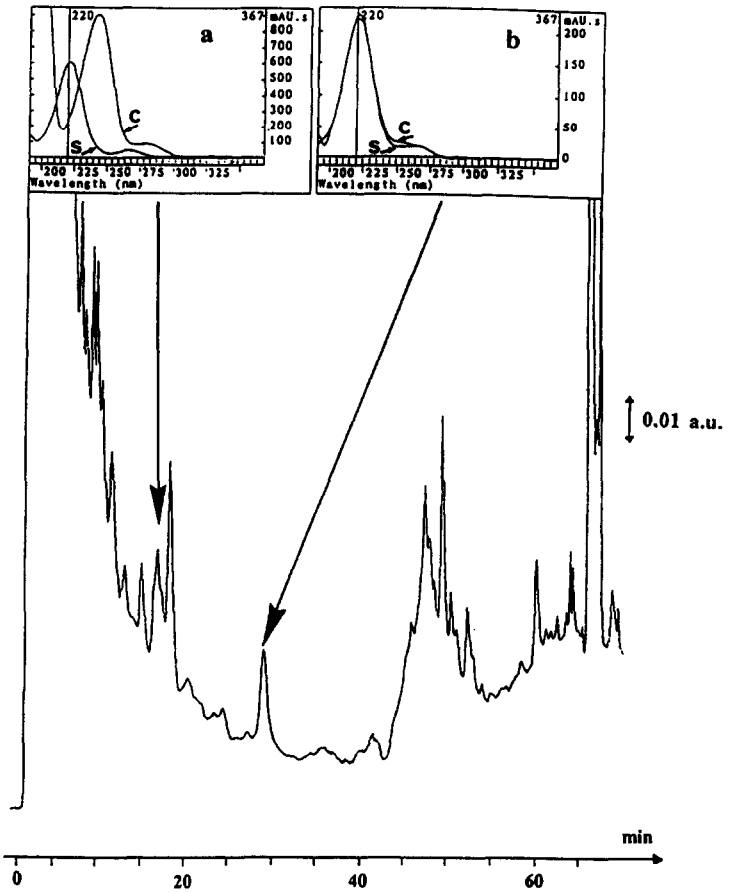

Fig. 5. On-line analysis at 150 ml of Seine river water sample (non-spiked) and quantification methods; experimental conditions as in Fig. 2. Comparison of standard spectra and unknown peak spectra for (a) simazine and (b) atrazine. S refers to the standard and C to the peak of the unknown compound.

μg/l for most of the compounds. This level is required for the control of drinking water in European countries and the handling of 150 ml is sufficient. Now, if quantification is required, a lower detection limit has to be obtained and this is easily done with drinking water by increasing the sample volume. A sample volume of 300 ml was tried, as each compound having a retention time higher than that of simazine or methoxuron has a breakthrough volume higher than 300 ml. Fig. 6 shows the on-line analyses of 300 ml of drinking water spiked with (a) triazines and some of their degradation products or (b) phenylurea herbicides. In each group of herbicides, a wide range of polarity is covered from the first to the last eluted peaks. These chromatograms show that the detection limits are from 5 to 30 ng/l, depending on the analytes. Similar detection limits have been obtained in various ground waters.

In surface water

The Seine river in Paris can be taken as providing convenient samples for studying detection limits in surface water. Fig. 2, which compared drinking and river waters spiked with 0.3 μg/l of the multi-residue solution, showed that the detection limits for some analytes should be of the same order than those obtained in drinking waters. Fig. 7a and b represent the on-line

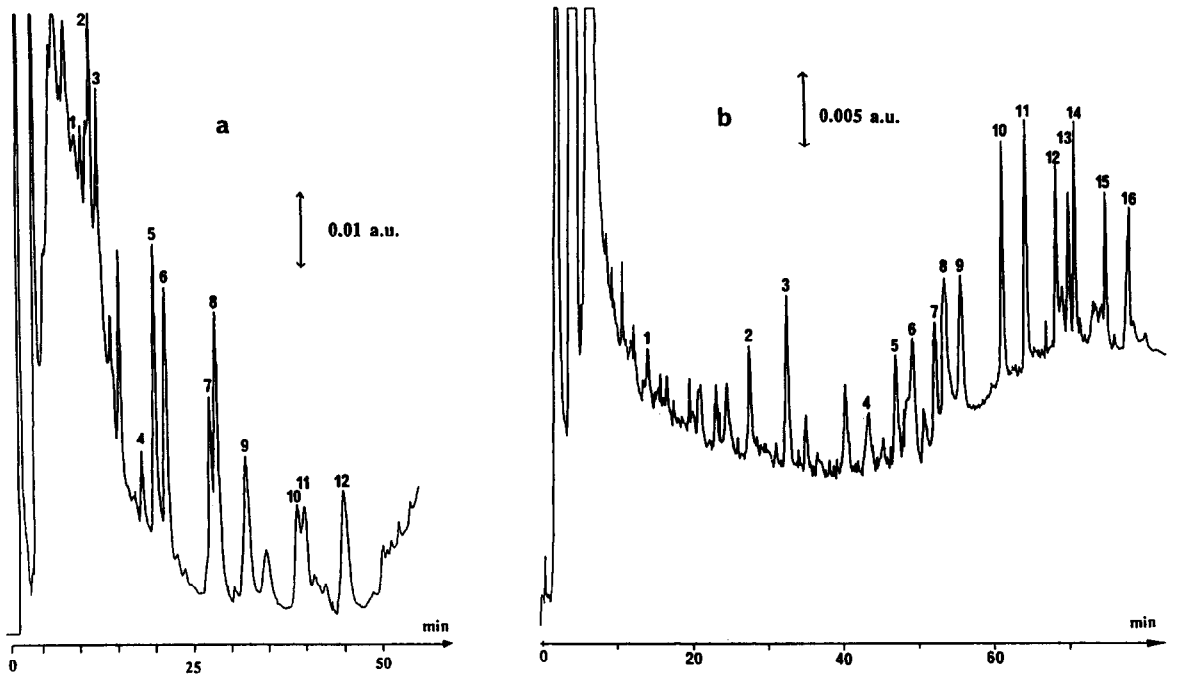

Fig. 6. Preconcentration on PLRP-S of 300 ml of drinking water spiked at 0.1 μg/l with (a) triazines and (b) phenylureas. Triazines: 1 = deisopropylatrazine; (2) = hydroxyatrazine; 3 = deethylatrazine; 4 = hexazinone; 5 = simazine; 6 = cyanazine; 7 = simetryne; 8 = atrazine; 9 = prometon; 10 = sebutylazine; 11 = propazine; 13 = terbutylazine. Analytical column, Varian ODS (25 × 0.46 cm I.D.); flow-rate, 1 ml/min; acetonitrile gradient with phosphate buffer at pH 7, gradient 15 to 30% acetonitrile from 0 to 9 min, 30 to 34% from 9 to 16 min, 34 to 40% from 16 to 45 min and 40 to 60% from 45 to 55 min; detection at 220 nm; zero offset, −30%; attenuation, 16. Phenylureas: 1 = fenuron; 2 = methoxuron; 3 = monuron; 4 = methabenzthiazuron; 5 = chlortoluron; 6 = fluometuron; 7 = monolinuron; 8 = isoproturon; 9 = diuron; 10 = difenoxuron; 11 = buturon; 12 = linuron; 13 = chloroxuron; 14 = chlorbromuron; 15 = diflubenzuron; 16 = neburon. Acetonitrile gradient with phosphate buffer at pH 7: 20 to 35% acetonitrile from 0 to 52 min, 35 to 70% from 52 to 77 min; detection at 249 nm; zero offset, 5%; attenuation, 8.

analysis of 150 ml of river waters spiked with 0.1 μg/l of the triazine mixture and of the phenylurea mixture, respectively. The degradation products of atrazine are not visible owing to the broad interfering peak due to the low attenuation of the detector and peak 5 is high owing to the presence of atrazine in the raw sample. The sample analysed in Fig. 7b contained 1.2 μg/l of diuron, which was identified and measured by the MCA method and the standard addition method. Other compounds were not present in the raw samples and the two chromatograms in Fig. 7 show that detection limits of 0.1 μg/l can be reached for river waters without any clean-up. This excellent result is due to (i) the efficient coupling of the precolumn with the analytical column provided by the Prospekt device, (ii) the low sample volume which lowers the matrix interferences and (iii) the shape of the analytical gradient which can decrease the interfering peaks. The sample volume was increased to 300 ml, but it was impossible to obtain a chromatogram at the same attenuation of detection, which had to be increased by a factor of 2. The result is that the same information was obtained with a higher interfering peak.

The results described above imply that the detection limits depend on the interfering peak shape and that a compromise should be found between low-level detection in river waters and the number of analytes and polarity range analysed. As the sample volume is low, analyses are automated and not too time consuming, so that

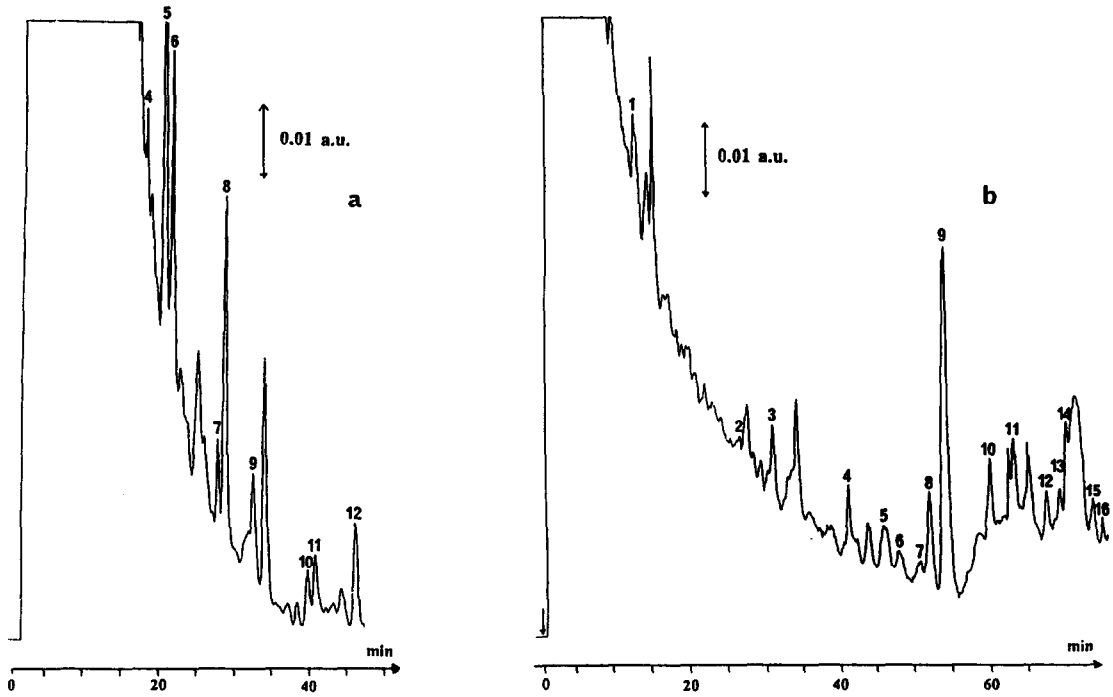

Fig. 7. Preconcentration on PLRP-S of 150 ml of surface water (Seine river) spiked at 0.1 μg/l with (a) triazines and (b) phenylureas. Experimental conditions and peaks as in Fig. 6. Triazines: detection at 220 nm; attenuation, 16; zero offset, −55%. Phenylureas: detection at 244 nm; attenuation, 16; zero offset, −15%.

it is alway possible to divide the polarity range into two groups and to perform two analyses.

4. Conclusions

On-line trace enrichment and LC analysis provide a powerful tool for the analysis of aqueous environmental samples. We have shown that, provided a good choice of the preconcentration parameters and a good selection of the analytical conditions are made, low-level determinations can be performed for many pesticides, even in highly contaminated surface waters and without any clean-up. Determinations below the 1 μg/l level can be achieved within an average R.S.D. of 15%. The weakest point is still the identification of compounds which cannot be confirmed only by a retention time and a UV spectrum for all the compounds. The use of a confirmation column is recommended. This problem could be solved by the development of efficient interfacing with mass spectrometry.

5. Acknowledgements

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