Identification of herbicides in river water using on-line trace enrichment combined with column liquid chromatography–Fourier-transform infrared spectrometry

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Abstract

Trace enrichment of herbicides on a 10×2.0 mm I.D. precolumn packed with Polygosil C_{18} material, was combined on-line with column liquid chromatography–Fourier-transform infrared spectrometry (LC–FT-IR). The isocratic LC separation was carried out on a 200×2.1 mm I.D Rosil C_{18} column using acetonitrile–phosphate buffer as eluent. The LC–FT-IR coupling was based on post-column on-line liquid–liquid extraction and solvent elimination, followed by FT-IR microscopy. The feasibility of the complete system was demonstrated by analysing river water spiked with triazines and phenylureas at the low-μg/l level. Identifiable spectra were obtained for all analytes, which included several isomers. Using 50–100 ml water samples, the identification limit for the herbicides typically was 1 μg/l. The usefulness of six spectral library search algorithms to correctly identify the recorded spectra was tested. A Peak-Search routine which is based on the matching of peak frequencies alone was found to be the most suitable to identify the analytes at the trace level.

1. Introduction

In divergent fields of interest such as biomedical, pharmaceutical, environmental and polymer chemistry, there frequently is a need to characterize small amounts of sample constituents with a high level of confidence. Today, the on-line combinations of both gas chromatography (GC) and column liquid chromatography (LC) with mass spectrometry (MS), are considered the most powerful tools for analyte characterization. Nevertheless, complementary information is sometimes required, for instance to confirm MS-based identifications, or to discriminate between molecular structures with identical mass. Fourier-transform infrared spectrometry (FT-IR) is very useful for the characterization of functional groups, has strong "fingerprinting" capabilities, which are especially suitable for the differentiation of structural isomers and, thus, is a good alternative and complementary identification method [1]. Presently, the combination of GC and FT-IR is a well established technique, and various systems are commercially

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available [2,3]. LC–FT-IR, although still in its infancy, is rapidly being recognized as a feasible and rewarding technique [4,5], as becomes evident from the introduction of the first commercial interfaces [6].

Coupling of LC and FT-IR is most effectively accomplished by solvent elimination prior to IR detection. This involves the use of an interface in which the eluent is evaporated and the analytes are deposited on a substrate suitable for FT-IR detection. In the literature several of such solvent-elimination interfaces are described for both normal- and reversed-phase LC [7–13]. Various real-life applications have been reported such as the profiling of steroids [13] and their impurities [14], protein conformational studies [15,16], isomer-specific analysis of organic-synthetic mixtures [17] and polymer additive analysis [18,19]. Still, although the mass sensitivity for micro-LC–FT-IR is quite favourable (0.5–10 ng) [9,10,12], analyte detectability of LC–FT-IR in terms of concentration units leaves much to be desired. The minimal concentrations that can be detected by most LC–FT-IR methods [7–19] are in the (high) mg/l range, which is insufficient to meet current demands of, especially, environmental analysis.

In a recent paper, we showed that considerably improved concentration detection limits can be achieved with a solvent-elimination based LC–FT-IR system that utilizes on-line post-column liquid–liquid extraction (LLE) [20]. This system can handle aqueous eluents containing non-volatile buffers at flow-rates up to 0.2 ml/min, and allows the use of 2.0 mm I.D. analytical columns and conventional LC equipment. By trapping the analytes at the top of the LC column (on-column focusing), we could inject relatively large volumes, which resulted in minimal identifiable concentrations of 200–400 μg/l. However, trace analysis requires much better analyte detectability. In general, trace-analytical procedures include a trace-enrichment step prior to the chromatographic separation. For aqueous samples, on-line solid-phase extraction (SPE) can be used to preconcentrate the analytes of interest. Today, this technique is being applied routinely for the determination of organic micropollutants in environmental waters by LC–diode-array UV detection as well as LC–MS [21,22].

The present study is directed at the application of on-line trace enrichment in LC–FT-IR. Using the determination of triazines and phenylureas in river water as an example, we examined whether (i) on-line SPE can be incorporated in the existing LC–LLE–FT-IR system [20], and (ii) the SPE approach allows FT-IR identification of compounds at the desired low-μg/l level. We also tested the usefulness of several search algorithms of a commercial library search programme for the correct identification of the herbicide spectra recorded in this study.

2. Experimental

2.1. Chromatographic instrumentation and procedures

Stainless-steel precolumns (10×2.0 mm I.D) were slurry packed manually with 10 μm Polygosil 100 C_{18} (Macherey–Nagel, Düren, Germany) and installed in a home-made leak-tight holder. A Gilson (Villiers-le-Bel, France) Model 302 pump was used to load the aqueous sample onto the precolumn and to clean and condition the sorbent with methanol and HPLC-grade water. For eluent delivery a Gilson Model 302 pump equipped with a Gilson 802c manometric module and an Applied Biosystems (Foster City, CA, USA) Model 400 pump were used. Separations were carried out on a 200×2.1 mm I.D. column packed with 5 μm Rosil C18 (Research Separations Laboratories, Eke, Belgium). Acetonitrile-0.01 M potassium phosphate buffer (pH 7) (40:60, v/v) was used as eluent. Three home-made six-port injection valves were used to allow efficient column-switching (see below). For direct injections a valve equipped with a loop of 160 μl was used. On-line detection was performed with a Kratos Analytical (Ramsey, NJ, USA) Spectroflow 757 absorbance detector, which was connected to a Varian (Walnut Creek, CA, USA) Model 4400 integrator.

A schematic of the total analytical set-up is shown in Fig. 1. During analysis, pumps P2, P3 and P4 were running continuously, each at a flow-rate of 0.2 ml/min. The SPE–LC procedure was as follows. Using pump P1, the C_{18} precolumn was flushed at 4 ml/min with successively 10 ml methanol and 10 ml
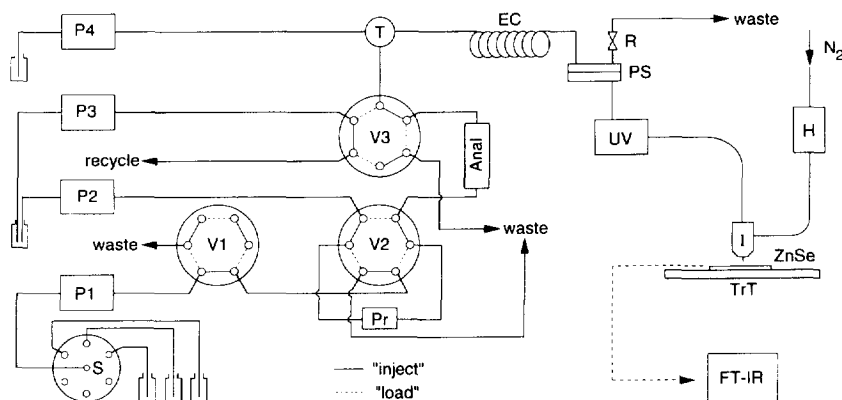
Fig. 1. Set-up of on-line SPE–LC–LLE–FT-IR system; P1: pump delivering methanol, HPLC water and aqueous samples; P2, P3: eluent pumps; P4: organic phase pump; S, solvent selection valve; V1–V3: six-port injection valves; Pr: precolumn; Anal: analytical column; T: T-piece; EC: extraction coil; PS: phase separator; R: restrictor; UV: UV absorbance detector; I: spray-jet assembly interface; H: nitrogen gas heater; ZnSe: zinc selenide deposition window; TrT: translation table; FT-IR: FT-IR microscope.

HPLC-grade water. Subsequently, the aqueous sample (10–100 ml) was loaded onto the precolumn at 2 or 4 ml/min. Prior to desorption of the precolumn by eluent pump P2, valve V3 was switched to the "load" position, so that the column effluent was temporarily sent to waste; at the same time pump P3 assured an uninterrupted supply of eluent to the phase segmentor, T. Next, by switching valve V2 to the "inject" position, the compounds trapped on the precolumn were desorbed with eluent (backflush mode) and transferred on-line to the C_{18} analytical column for LC separation. After 5.0 min, valve V3 was switched to "inject", directing the column effluent to the T-piece again, and thus starting the actual liquid–liquid extraction of the LC chromatogram.

2.2. On-line extraction and phase separation

The organic phase, dichloromethane, was delivered by an Applied Biosystems Model 400 pump (P4) and added to the aqueous LC effluent via a 0.25 mm I.D. T-piece. The resulting segmented stream was led to a 1.5 m×1.1 mm I.D. stainless-steel extraction coil (helix diameter, 50 mm). After extraction the phases were continuously separated using a home-made sandwich-type phase separator [23]. Restriction capillaries (I.D., 0.12–0.25 mm; length, 100–300 mm) connected to the aqueous

separator outlet (waste) were used to regulate the amount of organic phase directed to the evaporation interface.

2.3. Interface

After phase separation, the organic stream was led to the spray-jet assembly interface via the absorbance detector and a 300×0.23 mm I.D. fused-silica capillary which was connected to a stainless-steel needle. A heated nitrogen flow around the needle, which protruded through a nozzle, effected complete solvent evaporation and ensured deposition of the extracted compounds onto a zinc selenide (ZnSe) window which was moved by a modified Camag (Muttenz, Switzerland) Linomat III translation table. A ventilation duct connected to a fume hood was placed above the interface to sweep away the generated solvent vapour and kept the dichloromethane concentration in the laboratory air at an acceptably low level. In this study the interface conditions typically were: needle-protrusion distance, 0.8 mm; needle-to-substrate distance, 0.8 mm; nitrogen gas pressure, 7–8 bar; nitrogen gas temperature, 150°C; table speed, 1.6 mm/min. After deposition, the ZnSe substrate holding the immobilized analytes was transferred to the FT-IR microscope beam area for the recording of IR spectra.

2.4. FT-IR

FT-IR transmission measurements were carried out on a Bruker (Karlsruhe, Germany) IFS-55 spectrometer equipped with a Bruker A590 FT-IR microscope containing a narrow-range mercury cadmium telluride (MCT) detector. A computer-controlled Bruker microscope X–Y stepper table was used to position the ZnSe window in the focused IR beam. IR scanning of the deposited chromatogram was performed by the sample-mapping routine of the Bruker Opus 2.0 software, using a microscope aperture of typically 150 μm×150 μm. First, reference interferograms were recorded at a blank spot at the start of the deposition trace. Next, the table was moved stepwise along the trace with a step width of 100 μm. At each position a spectrum was recorded by co-adding 8 scans at 8 cm^{-1} optical resolution. Simultaneously, the acquired spectra were shown on the computer screen in a stacked-plot representation. The analysis time per step was 1.7 s; the total time of analysis for a 5-cm deposition trace (i.e. a 30-min chromatogram) was about 14 min. After the IR scanning of the trace was completed, Gram–Schmidt and/or spectral window chromatograms were constructed by the software. In the latter case, for each spectrum the integrated absorbance signal in a defined spectral region was determined (total absorbance). In order to obtain high-quality spectra of individual analytes, re-measurements were performed at peak maxima. For this purpose, the ZnSe window was moved to the proper position and the microscope aperture was adjusted to match the spot width. Subsequently, a spectrum was recorded with 250 scans co-added at 4 cm^{-1} optical resolution (acquisition time, 55 s). The spectra were baseline corrected and, if present, the bands arising from atmospheric carbon dioxide were eliminated.

2.5. Spectral library search

The spectra obtained during this study were searched against a library which contained 391 spectra (KBr disc; stored at 4 cm^{-1} data point resolution) of miscellaneous pesticides including 17 triazines and 17 phenylureas. These spectra were baseline corrected and normalized to 2.0 absorbance units for the strongest band, prior to library storage.

The analyte spectra were treated identically before library search. The spectral search region was 3500–700 cm^{-1}. The IR Search Master 3.04 (Biorad, Sadtler Division, Philadelphia, PA, USA) library programme was used; it comprised the full-spectrum algorithms Absolute Value, Euclidean Distance, First Derivative Absolute Value and First Derivatives Least Squares, and the peak-matching algorithms Peak Search and Peak Search Intensity Ignored. In essence, the full spectrum routines for each reference spectrum compute the total sum of absolute or squared differences between corresponding data points of the analyte spectrum (or its first derivative) and the reference spectrum (or its first derivative). On the basis of this summation, the algorithm produces a Hit Quality Index (HQI) (exact calculation unknown) for each reference spectrum. The HQI ranges between 0 and 999; the larger the numerical value of HQI, the better is the mathematical match of the spectra. The Peak Search routines are peak-matching procedures which basically measure how well the absorption band frequencies in the analyte spectrum match those in the reference spectra. In this study, the "Search Tolerance", which defines the maximum allowed difference between a matching query and library peak, was 5 cm^{-1}. A peak table (band frequencies with intensities) was produced for each analyte spectrum by the Peak Picking routine of the Search Master software. Peaks smaller than 8% of the highest peak or smaller than two times the spectral noise were not included in the peak table. In the Peak Search algorithm, band intensity information is included: matching of two peaks requires the relative intensity of the reference peak to be equal to or larger than the relative intensity of the query peak. Under "Intensity Ignored" conditions, the intensity of the peaks is not considered during evaluation. The Peak Search results are expressed as an HQI.

2.6. Materials

Acetonitrile, methanol, water, dichloromethane (all HPLC-grade) and potassium hydrogen phosphates (analytical-grade) were obtained from J.T. Baker (Deventer, Netherlands). Herbicides (96–99% purity) were purchased from Riedel-de-Haën (Seelze, Germany). Stock solutions of each of the phenyl-

ureas (500 μg/ml) and triazines (200 μg/ml; except simazine, 100 μg/ml) were prepared in acetonitrile. Two mixtures were prepared from these solutions: a phenylurea mixture containing monuron, chlortoluron, diuron, metobromuron, linuron and chlorbromuron, and a triazine mixture containing simazine, atrazine, sebutylazine, propazine and terbutylazine. The mixtures were diluted with phosphate buffer to the desired concentration for either standard injection or spiking of water. River water was collected from the river Meuse (Keizersveer, Netherlands) and filtered over a 0.45-μm filter (Schleicher & Schüll, Dassel, Germany) before use. A 60×30×1.5-mm ZnSe window (Biorad, Düsseldorf, Germany) was used as deposition medium.

3. Results and discussion

To study the potential of on-line trace enrichment in LC–FT-IR, analyte recoveries on the C_{18}-bonded silica precolumn were determined, and an experimental set-up was designed which allowed the analysis of river water samples. Subsequently, the total SPE–LC–LLE—FT-IR system was evaluated with emphasis on analyte detectability (in concentration units), and the relative merits of several library search algorithms for the correct assignment of the trace-level FT-IR spectra obtained with the current method were compared.

3.1. On-line trace enrichment

Substantial trace enrichment is required to obtain LC–FT-IR identification limits in the low-μg/l range. Considering the sensitivity achieved in a previous LC–FT-IR study in which 150-μl injections were used [20], preconcentration of sample volumes of at least 15 ml were thought necessary to attain the stated end. In the present study, a C_{18}-bonded silica precolumn (10×2 mm I.D.) was used for analyte trapping. Phenylurea and triazine herbicides, which are well-known micropollutants in surface water, were chosen as test compounds. Among the triazines, there were three isomers, viz. sebutylazine (2-sec.-butylamino-4-chloro-6-ethylamino-1,3,5-triazine), terbutylazine (2-tert.-butyl-amino-4-chloro-6-ethylamino-1,3,5-triazine) and

propazine (2,6-bis(isopropylamino)-4-chloro-1,3,5-triazine).

The recoveries of all test compounds were measured as a function of sample volume (Table 1). For this purpose, river water samples were spiked (5 μg/l) either with the phenylurea or with the triazine mixture. These samples were analyzed by SPE–LC–UV using three sampling volumes (20, 50 and 100 μl), and the analyte recoveries determined by comparing the peak areas obtained with those of 160-μl standard injections of a phenylurea or triazine mixture (2 mg/l in buffer). In Table 1 both the phenylureas and triazines are listed according their order of elution during LC–UV analysis.

For the 20-ml preconcentration the recoveries obtained were essentially quantitative for all compounds except monuron. When using higher sampling volumes, the recovery of several herbicides decreased. The strongest reduction was observed for the early eluting (i.e. the most polar) analytes. Therefore, the recovery decrease can be attributed to breakthrough as a result of insufficient retention on the precolumn. Despite the lower recovery, however, for most analytes still enrichment was more favourable when 50, or even 100, ml were preconcentrated. For instance, the amount of metobromuron recovered

Table 1
Recovery data after preconcentration of different volumes of herbicide-spiked river water (5 μg/l) on a home-made 10×2 mm I.D. C_{18}-bonded silica precolumn[a]

Compound	Analyte recovery (%; $n=2$) at sample volume of:		
	20 ml	50 ml	100 ml
Phenylureas			
Monuron	51	21	10
Chlortoluron	99	74	36
Diuron	98	85	44
Metobromuron	99	62	30
Linuron	96	95	78
Chlorbromuron	96	100	100
Triazines			
Simazine	87	35	17
Atrazine	99	90	40
Sebutylazine	99	99	90
Propazine	98	100	93
Terbutylazine	98	102	88

[a] Recovery calculations based on peak area in LC with UV detection at 250 nm (phenylureas) and 222 nm (triazines). For further details, see Section 2 and Section 3.1.

in the 20-ml experiment was 99 ng (recovery, 99%), while 155 ng was recovered with the 50-ml pre-concentration (recovery, 62%). This gain in mass (though not in efficiency) is important because the final LC–FT-IR detection limits will mainly depend on the mass of analyte deposited on the ZnSe window. In other words, assuming that matrix compounds do not interfere, preconcentration of relatively large volumes may be rewarding, particularly when very low concentrations have to be analyzed.

3.2. Post-column on-line extraction

In the post-column liquid–liquid extraction (LLE) set-up, continuous extraction is performed by introducing dichloromethane to the aqueous LC effluent. The two liquid phases are separated by a sandwich-type phase separator; the aqueous phase is sent to waste, and a large proportion (i.e. 80–90%) of the organic phase in which the extracted analytes are dissolved, is led to the solvent elimination interface. The design and characteristics of the LC–LLE–FT-IR system have been described in detail elsewhere [20]. High extraction yields (above 90%) were found for medium-polar compounds such as phenylureas and triazines [20].

In order to study the feasibility of on-line SPE–LC–LLE, herbicide-spiked HPLC-grade and river water samples were analyzed by SPE–LC–LLE–UV. The analysis of the spiked HPLC-grade water did not cause any problem. The performance of the extraction module was not disturbed by the column-switching procedures and both the trace enrichment achieved and the chromatographic resolution were fully maintained during the extraction procedure. However, upon desorption of a precolumn loaded with river water, a strong disturbance of the UV detector signal was observed in the early part of the on-line extracted chromatogram. Auto-zeroing the detector was necessary to obtain a proper baseline which, nevertheless, showed considerable drift along the whole chromatogram. At the same time, the flow from the organic outlet of the phase separator fluctuated markedly. Apparently, early eluting sample constituents, probably humic substances, strongly affected the performance of the phase separator. Humic substances are commonly present in surface

water at relatively high concentrations and are known to be concentrated to some extent on a hydrophobic precolumn. Under the LC conditions applied (40% acetonitrile; pH 7), the main part of the humic compounds eluted as a rather narrow band in the $t_R = 2$–5 min interval, as is illustrated by the SPE–LC–UV analysis of a triazine-spiked river water sample (Fig. 2). To prevent interferences, a valve (V3 in Fig. 1) was inserted between the analytical column outlet and the phase segmentor. This enabled us to switch the humic substance band to waste before starting the extraction of the LC effluent. The use of an additional pump (P3) ensured an uninterrupted supply of eluent to the extraction system. With this configuration, the phase separator showed constant performance over a long period of time, and an interference-free analysis of river water samples could be accomplished. On the basis of this experience, in the SPE–LC–LLE–FT-IR experiments described below, the first 5 min of the LC separation were always discarded.
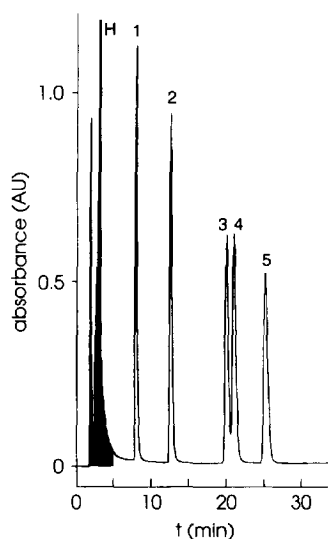

Fig. 2. SPE–LC–UV of 20 ml river Meuse water spiked with five triazines (30 μg/l each); UV detection at 263 nm. For further conditions, see Section 2. Peaks: H=humic substances; 1= simazine; 2=atrazine; 3=sebutylazine; 4=propazine and 5= terbutylazine. The first 5 min of the chromatogram (shown in black) are discarded during FT-IR analysis (for explanation, see Section 3.2).

3.3. SPE–LC–LLE–FT-IR

For SPE–LC–LLE–FT-IR the set-up depicted in Fig. 1 was used. The interface conditions (see Section 2) were the same as those used in a previous study involving on-line extraction [20]. During the immobilization of on-line extracted chromatograms of river water on ZnSe, a weak and continuous deposit was always observed in the first part of the chromatogram, most likely due to a small, late eluting fraction of humic substances. Fortunately, in all cases this deposit was too small to cause spectral interferences. In order to examine the total system, river water samples spiked with either triazines or phenylureas were analyzed.

For the preconcentrated triazine-spiked samples homogeneous analyte spots (typical width 0.1 mm) were obtained. Fig. 3A shows the Gram–Schmidt reconstructed FT-IR chromatogram of 20 ml river Meuse water spiked with 30 μg/l of triazines. It is evident that the resolution of the LC separation (see Fig. 2) is maintained during the deposition procedure. High-quality spectra were obtained for each individual triazine by FT-IR measurement of the centres of the deposited spots.

To determine the minimum identifiable concentrations of the triazines, low-level (6 and 2 μg/l) spiked river water samples were analyzed; simultaneously the sample volume was increased to 50–100 ml. Furthermore, for the construction of the FT-IR chromatograms, total absorbance in a specific spectral window (1650–1500 cm^{-1}) was preferred to Gram–Schmidt representation, because the former approach showed a more favourable signal-to-noise ratio. The loading of 50 ml of the 6 μg/l sample was sufficient to achieve a clear FT-IR detection of all spiked triazines (Fig. 3B); at the 2 μg/l level, preconcentration of 100 ml was required. In the resulting chromatogram, the five triazine peaks can indeed be discerned (Fig. 3C), although in this instance, atrazine (peak No. 2) and particularly simazine (peak No. 1), clearly suffer from a poor recovery (cf. Table 1). Still, even at this low level, pure and identifiable spectra could be recorded for every triazine (Fig. 4). This was accomplished by "post-run" recording of spectra at positions of maximum analyte concentration using 250 scans per
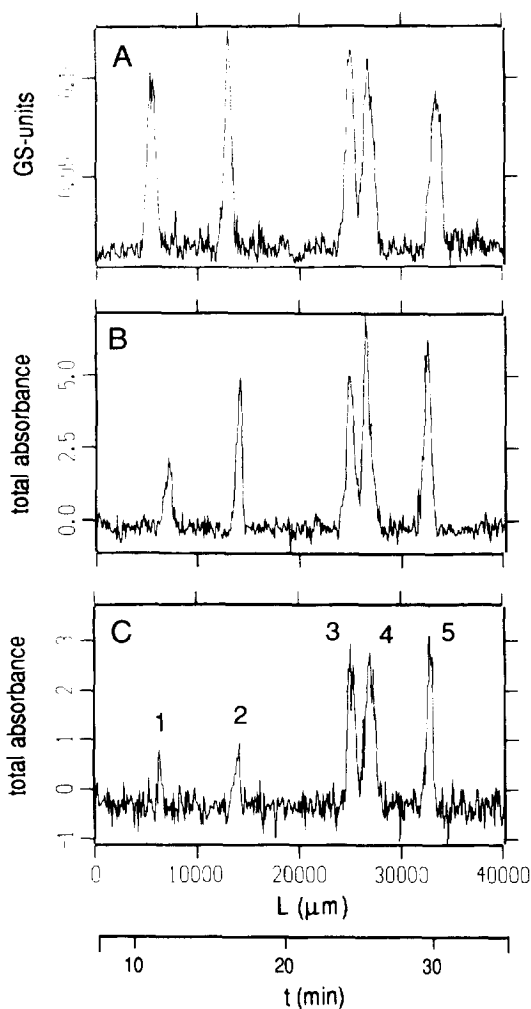

Fig. 3. SPE–LC–LLE–FT-IR chromatograms of river Meuse water spiked with five triazines: (A) 20 ml (30 μg/l), (B) 50 ml (6 μg/l) and (C) 100 ml (2 μg/l) sample. Chromatogram representation, (A) Gram–Schmidt or (B and C) spectral window (1650–1500 cm^{-1}). For further conditions, see Section 2; for peak assignment, see Fig. 2.

spectrum. The triazine spectra of the three isomers (peaks No. 3, 4 and 5) are, of course, closely similar, but can be distinguished on the basis of differences in the 1500–1000 cm^{-1} region. Moreover, using the Peak-Search-Intensity-Ignored library search algorithm, the 2 μg/l spectra were correctly library-identified in all but one case. The rather weak
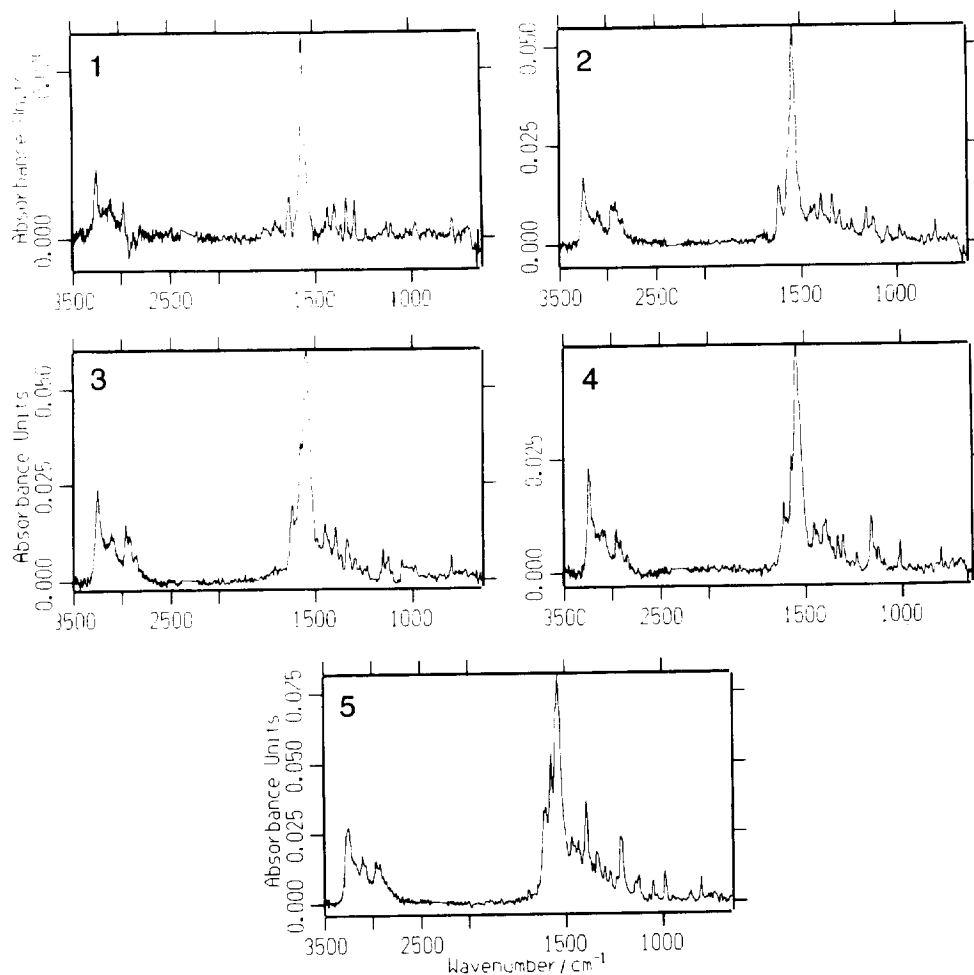
Fig. 4. FT-IR spectra recorded during SPE–LC–LLE–FT-IR of 100 ml river Meuse water spiked with five triazines (spike, 2 μg/l). Spectra 1–5 correspond with LC peaks 1–5 in Fig. 3C. For conditions, see Section 2 and Section 3.3.

spectrum of LC peak No. 1 was attributed to atrazine by the library search programme, while the target compound (simazine) came out second best. The spectra of Fig. 4 indicate that with a 100-ml sample volume, the limit of identification for triazines is 1–2 μg/l.

The repeatability of the total procedure was tested by the analysis (n=5) of 50 ml river water spiked with sebutylazine and propazine at the 6 μg/l level. Fig. 5 shows the relevant time interval of the FT-IR spectral window chromatograms. The resolution of the two isomers is seen to be the same in each run, allowing the post-run acquisition of pure and identifi-

able spectra for both triazines. The relative standard deviations (R.S.D.s) of the peak heights were 15–20%, which is quite satisfactory for a procedure which should provide identification rather than quantification. Since simultaneous SPE–LC–LLE–UV analysis revealed peak-height R.S.D.s of less than 5%, the total variation is probably mainly due to small differences in spot characteristics such as size, thickness and morphology, induced by the deposition procedure. The back pressure of the precolumn gradually increased (from 2 to 20 bar) during ten consecutive 50-ml runs, however, no negative influence on the system performance was observed.
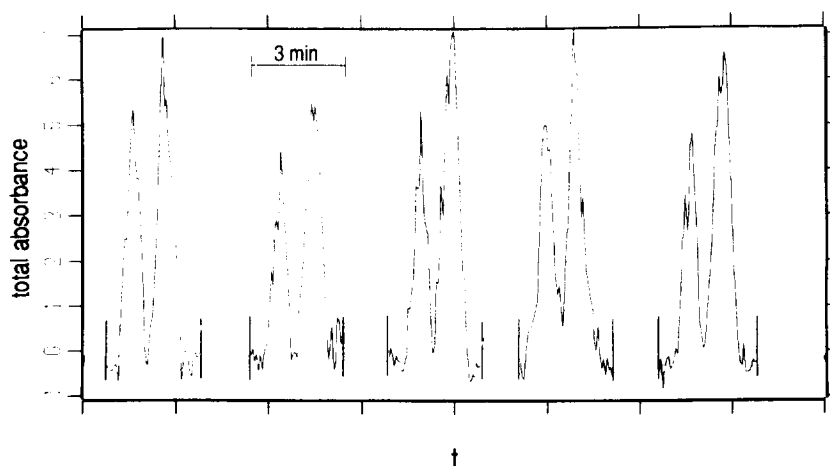
Fig. 5. Repeated SPE–LC–LLE–FT-IR analysis ($n=5$) of 50 ml river Meuse water spiked with 6 μg/l of sebutylazine and propazine, showing the 23.5–26.5 min retention time interval (including LLE time). Spectral window, 1650–1500 cm^{-1}. For further conditions, see Section 2.

Nevertheless, to be on the safe side, after every ten river water analyses the precolumn was repacked.

Phenylurea-spiked river water was also analysed. Fig. 6A shows the SPE–LC–LLE–UV chromatogram of a 20-ml sample spiked at the 24 μg/l level with six phenylurea herbicides. The analytes could clearly be recognized in the corresponding FT-IR chromatogram (Fig. 6B). However, their peaks were rather irregularly shaped and some were even split. These peak-shape discontinuities were caused by the spatial inhomogeneity of the phenylurea spots (width 0.2–0.3 mm); visual microscopic inspection revealed the presence of many small, isolated domains (typical size 70×70 μm). By aperturing down to one or two of these domains using the FT-IR microscope, high-quality spectra could be recorded for every phenylurea. These spectra were rather intense, with band maxima of over 0.2 absorbance units, indicating that the analytes were concentrated within these isolated regions.

In SPE–LC–LLE–FT-IR analysis of 5 μg/l spiked samples (50 ml), the phenylurea spot sizes were found to be similar to those observed in the 24 μg/l experiment, but now the spots showed fewer and smaller analyte domains. As a consequence, IR scanning of the deposition trace did not yield a proper FT-IR chromatogram from which all phenylurea peaks could be recognized reliably. Neverthe-
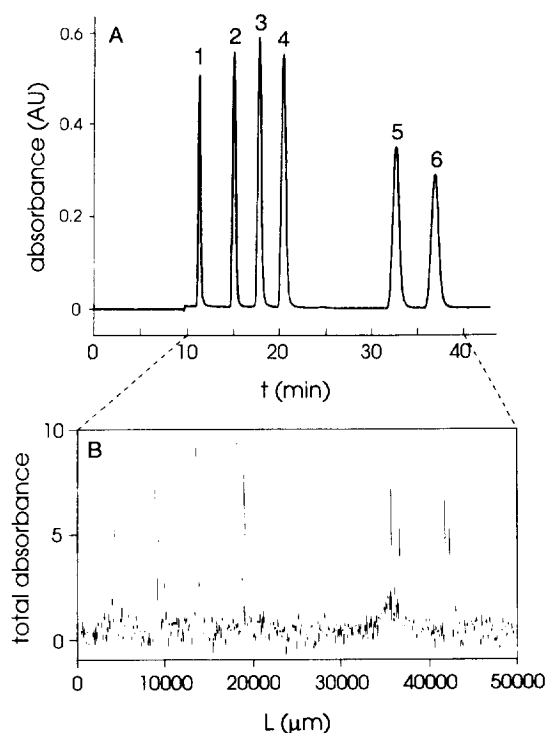

Fig. 6. (a) SPE–LC–LLE–UV and (b) SPE–LC–LLE–FT-IR of 20 ml river Meuse water spiked with six phenylureas (24 μg/l each); UV detection at 250 nm; IR spectral window, 1700–1600 cm^{-1}. Further conditions, see Section 2. Peaks: 1=monuron; 2=chlortoluron; 3=diuron; 4=metobromuron; 5=linuron and 6=chlorbromuron.

less, also at this low level good-quality spectra could be obtained from all analytes. This was accomplished by localizing the individual analyte spots on the ZnSe window on the basis of their retention times (established by UV detection), adjusting the microscope aperture to match the largest analyte domain(s) and recording the spectra using 250 scans. As an example, the spectra of diuron and linuron are presented in Fig. 7. These spectra indicate that using a sample volume of 50 ml the limit of identification for the phenylurea herbicides is approx. 1 μg/l. In a previous reported LC–FT-IR study involving on-line extraction [20], an identification limit of about 300 μg/l was found for the same compounds, using direct loop injection (150 μl). These data once more demonstrate the substantial gain in concentration detectability that can be achieved relatively simply by applying on-line SPE. In view of the recovery of linuron and chlorbromuron on C_{18} material (Table 1), the identification limits for these two compounds no doubt can be further improved by increasing the sample volume to 100 ml or more.
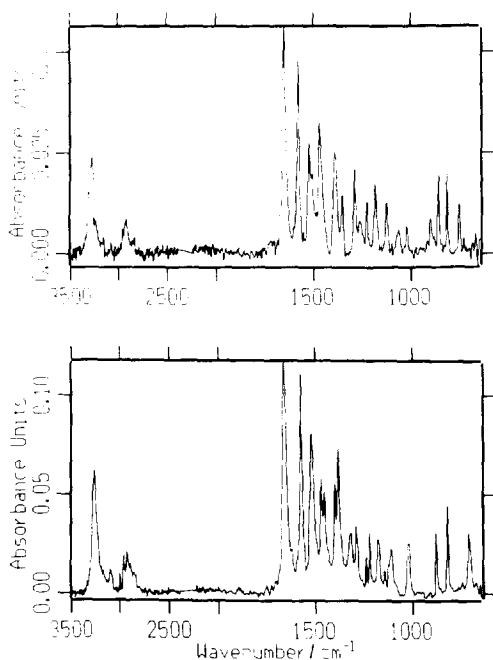

Fig. 7. FT-IR spectra of diuron (top) and linuron (bottom) recorded during SPE–LC–LLE–FT-IR of 50 ml river Meuse water spiked with six phenylureas at 5 μg/l. For conditions, see Section 2 and Section 3.3.

3.4. Identification by library search

Today, large collections of digitally stored IR spectra are widely used in combination with commercially available library programmes, to automatically and quickly retrieve reference spectra that match or resemble the analyte spectrum. For a proper comparison of the sample and reference data, spectra should in principle have been recorded under identical conditions. Fortunately, studies from our group [9,14,17,20] and others [10,12,24,25] indicate that spectra obtained by solvent-elimination LC–FT-IR on ZnSe, are similar to conventional KBr spectra, so that existing correlation data and reference collections can be used. In trace analysis, the spectra obtained may well show a relatively high noise level which potentially obscures spectral features. Nevertheless, reliable spectrum identification often is extremely important, particularly in residue and environmental analysis. The IR Search Master library programme of Sadtler offers several search algorithms that can be used for spectral retrieval. The performance of such search procedures in the identification of different types of analytes can be quite different and may also depend on experimental conditions [26–28]. Therefore, we tested the usefulness of each of the Search Master algorithms for the identification of the low-level herbicide spectra acquired during the present SPE–LC–LLE–FT-IR study.

Library searches were carried out on 45 herbicide spectra obtained after the analysis of river water samples spiked with the triazine and phenylurea test mixtures at levels ranging from 2 to 50 μg/l. The spectral library consisted of 391 conventionally recorded pesticide spectra, including those of the analytes and several other triazines and phenylureas. The search algorithms and their operation are described in Section 2. Since a reliable search system should in the first instance be able to retrieve the reference spectrum of the analyte under search [27], the rank of the target compound in the library search hit list was used as the criterion for good search performance. The absolute Hit Quality Index (HQI) values were not used for this purpose, for two reasons. First, when applying the full-spectrum algorithms on our data set, the HQI values for the top library hits (true or false) were always above 900.

Secondly, since the various search algorithms are fundamentally different, between-algorithm comparison of HQI values is not valid.

The library search results on the 22 triazine- and 23 phenylurea spectra are summarized in Table 2. The First-Derivative routines and the Peak-Search-Intensity-Ignored routine showed the highest search efficiency. With the latter algorithm, 39 spectra were correctly identified and 6 were misclassified, of which only one was given a ranking worse than 5. Apparently, the matching of peak positions alone is a quite suitable criterion for the confirmation of the herbicides. Factors such as noise and broad non-specific spectral features presumably contribute only moderately to the result of the Peak-Search-Intensity-Ignored algorithm. Furthermore, the limited numbers of entrances listed in the library no doubt is advantageous here, because even when the peak table is small (e.g. for a weak spectrum) only few reference compounds will show a satisfactory match. The better performance of the derivative-type algorithms, in comparison with the other full-spectrum algorithms, is in line with other studies [26,28]; first-derivative routines are less sensitive to baseline effects and emphasize differences in peak position and shape, which helps to distinguish between similar compounds. On the whole, the Absolute Value and Euclidian Distance algorithms correctly recognized the class of herbicide (triazine or phenylurea); however, their ability to properly discriminate within a class was rather poor.

Closer analysis of the six identification errors by the Peak-Search-Intensity-Ignored algorithm revealed that two of them originated from trace-level (2–5 μg/l) spectra of simazine and monuron, which both show a low recovery in SPE (cf. Table 1). The four other incorrect assignments concerned chlorbromuron spectra present in the data set. Each of these spectra was attributed to linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) instead, which is structurally very similar to chlorbromuron (3-(4-bromo-3-chlorophenyl)-1-methoxy-1-methylurea). Visual comparison of the reference spectra also revealed a strong spectral resemblance: the fifteen most intense bands of chlorbromuron and linuron in the search region differ only 5 cm^{-1} or less, or in other words, they are regarded as matching. Hence, the spectral resolution is insufficient in this case. Indeed, in a previous LC–FT-IR study [20] in which 2 cm^{-1} optical resolution was used for both library and analyte spectra, chlorobromuron spectra were always appropriately recognized by the applied peak-frequency based library programme. Somewhat surprisingly, the linuron spectra of the present data set were always correctly identified by the Peak-Search-Intensity-Ignored algorithm.

Some strategies remain to further enhance the accuracy of the library search results. The signal-to-noise ratio of weak spectra can be increased by simply adding more scans per spectrum (for instance 1000 instead of 250). Reducing the spectral search range to the most discriminative region (e.g. the fingerprint region) may also improve the search results [29]. Another (powerful) strategy is to use the chromatographic retention as a spectral search pre-filter, which would immediately have eliminated the linuron/chlorbromuron ambiguity discussed above. This method is invariably used in SPE–LC–diode-array UV analysis of pesticides [30]. Obviously, it requires the knowledge of the retention times of all

Table 2
Library search results using several search algorithms on 45 herbicide spectra obtained after SPE–LC–LLE–FT-IR of spiked river water[a]

Search algorithm	Number of searches with target compound at:		
	Rank=1	1<Rank≤5	Rank>5
Absolute Value	21	7	17
Euclidean Distance	27	14	4
First Derivative Absolute Value	38	4	3
First Derivatives Least Squares	38	3	4
Peak Search	30	4	11
Peak Search Intensity Ignored	39	5	1

[a] Spike level, 2–50 μg/l; sample volume, 15–100 ml.

analytes but these can be expected to be known in target analysis. Further study of the potential of this approach seems indicated.

It should be added that in the present study the analyte reference spectra were known to be part of the library. This means that we were actually dealing here with the confirmation analysis of target compounds. When an unknown compound (reference spectrum not in library) would have to be searched, a peak matching procedure will not necessarily give optimum results. In such a situation it is probably more useful to apply an Euclidean Distance algorithm since it will yield hit compounds that are rather similar to the unknown, even if the latter compound is not actually in the library.

4. Conclusions

On-line trace enrichment in combination with LC–LLE–FT-IR offers a selective method for the characterization of medium-polar analytes such as phenylureas and triazines in water samples. This was demonstrated by the analysis of river water samples spiked with these analytes down to the 2–5 μg/l level. The integrated procedure yielded good-quality distinguishable IR spectra for all test compounds, which included three isomeric triazines. With sample volumes of 50–100 ml, the limit of identification of the analytes was about 1 μg/l, which is highly satisfactory for an LC–FT-IR based technique. For the least polar analytes of the test set, processing of higher sample volumes (100–250 ml) will probably effect a further improvement of the identification limits. Computer-assisted identification of the low-level herbicide spectra was carried out using a library of conventionally recorded pesticide spectra. A peak-matching type library search algorithm appeared to be most effective for the correct retrieval of the analyte reference spectra.

The present method appears to be a promising approach for the low-level confirmation/identification of medium-polar compounds in water samples, which would be particularly useful in combination with SPE–LC–diode-array UV detection. That is, after provisional identification and quantification of the analytes by the UV-based method, the compounds present above the alert level for surface water

(1 μg/l) could be unambiguously identified by the FT-IR based technique.

Acknowledgments

References

[1] K.S. Kalasinsky, B. Levine, M.L. Smith and G.E. Platoff, Jr., Crit. Rev. Anal. Chem., 23 (1993) 441.
[2] R. White, Chromatography/Fourier Transform Infrared Spectroscopy and its Applications, Marcel Dekker, New York, 1990.
[3] P. Jackson, G. Dent, D. Carter, D.J. Schofield, J.M. Chalmers, T. Visser and M. Vredenbregt, J. High Resolut. Chromatogr., 16 (1993) 515.
[4] C. Fujimoto and K. Jinno, Anal. Chem., 64 (1992) 476A.
[5] V.F. Kalasinsky and K.S. Kalasinsky, in G. Patonay (Ed.), HPLC Detection – Newer Methods, VCH Publishers, New York, 1992.
[6] LC-Transform Series 100, 300 and 400, manufactered by Lab Connections, Marlborough, MA, USA.
[7] J.J. Gagel and K. Biemann, Anal. Chem., 59 (1987) 1266.
[8] R.M. Robertson, J.A. de Haseth, J.D. Kirk and R.F. Browner, Appl. Spectrosc., 42 (1988) 1365.
[9] G.W. Somsen, R.J. van de Nesse, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst, T. Visser, P.R. Kootstra and A.P.J.M. de Jong, J. Chromatogr., 552 (1991) 635.
[10] A.J. Lange, P.R. Griffiths and D.J.J. Fraser, Anal. Chem., 63 (1991) 782.
[11] A.M. Robertson, D. Littlejohn, M. Brown and C.J. Dowle, J. Chromatogr., 588 (1991) 15.
[12] M.W. Raynor, K.D. Bartle and B.W. Cook, J. High Resolut. Chromatogr., 15 (1992) 361.
[13] J.L. Dwyer, A.E. Chapman and X. Liu, LC–GC, 13 (1995) 240.
[14] G.W. Somsen, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst and T. Visser, Appl. Spectrosc., 46 (1992) 1514.
[15] V.E. Turula and J.A. de Haseth, Appl. Spectrosc., 48 (1994) 1255.
[16] V.E. Turula and J.A. de Haseth, Anal. Chem., 68 (1996) 629.
[17] G.W. Somsen, L.P.P. van Stee, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst and T. Visser, Anal. Chim. Acta, 290 (1994) 269.
[18] J. Dwyer, Am. Lab., May (1994) 22.
[19] G.W. Somsen, E.J.E. Rozendom, C. Gooijer, U.A.Th. Brinkman and N.H. Velthorst, Analyst, 121 (1996) 1069.

[20] G.W. Somsen, E.W.J. Hooijschuur, C. Gooijer, U.A.Th. Brinkman, N.H. Velthorst and T. Visser, Anal. Chem., 68 (1996) 746.

[21] D. Barceló and M.-C. Hennion, Anal. Chim. Acta, 318 (1995) 1.

[22] U.A.Th. Brinkman, J. Chromatogr. A, 13 (1994) 373.

[23] C. de Ruiter, J.H. Wolf, U.A.Th. Brinkman and R.W. Frei, Anal. Chim. Acta, 192 (1987) 267.

[24] P.R. Griffiths and A.J. Lange, J. Chromatogr. Sci., 30 (1992) 93.

[25] A.J. Lange and P.R. Griffiths, Appl. Spectrosc., 47 (1993) 403.

[26] S.R. Lowry, D.A. Huppler and C.R. Anderson, J. Chem. Inf. Comput. Sci., 25 (1985) 235.

[27] M. Ruprecht and J.T. Clerc, J. Chem. Inf. Comput. Sci., 25 (1985) 241.

[28] W.M. Coleman III and B.M. Gordon, Appl. Spectrosc., 43 (1989) 998.

[29] M. Boruta, R.T. Carl, W.M. Grim III and F.J. Tontola, SPIE, 2089 (1993) 328.

[30] J. Slobodník, M.G.M. Groenewegen, E.R. Brouwer, H. Lingeman and U.A.Th. Brinkman, J. Chromatogr., 642 (1993) 259.