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Screening and analysis of polar pesticides in environmental monitoring programmes by coupled-column liquid chromatography and gas chromatography–mass spectrometry

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Abstract

Screening and analysis of polar pesticides based on coupled-column reversed-phase liquid chromatography (LC–LC) and GC– or LC–MS is a powerful tool in the execution of environmental monitoring programmes. This paper presents a unified approach utilising LC–LC screening followed by GC–MS confirmation. As polar pesticides are not generally amenable to GC a widely applicable derivation technique is used. The results demonstrate that the proposed LC and MS techniques are capable of analysing a wide range of polar pesticides down to levels of 0.1 μg/l (EU limit for drinking water). LC switching techniques for group analysis or individual compounds rely on the reversed-phase retention and the UV detectability of the pesticides in combination with the choice of the LC columns. Fast miniaturised derivatization prior to GC–MS forms an integral part in the proposed strategy. In order to avoid extraction losses, derivation in the aqueous sample, preferably with electrophoric reagents with enhanced sensitivity in GC–NICI-MS are employed where possible. In this communication, method development and validation fitting in the strategy are evaluated and the results of the combined approach are discussed.

Keywords: Environmental analysis; Water analysis; Coupled columns; Derivatization, GC; Pesticides

1. Introduction

The Dutch National Institute of Public Health and the Environment (RIVM) is a centre of knowledge in service of the Dutch government. One of the major tasks of the RIVM is to diagnose and forecast the state of the environment as influenced by e.g., chemical factors. Within this task the measurement of contaminants in all environmental compartments e.g., soil, groundwater, surface water air and food, plays an important role. Monitoring programmes are aimed at selected pollutants and carried out with a specific sampling strategy.

The use of pesticides in the Netherlands is probably amongst the highest in the world in terms of mass of active ingredient per unit area. The intensive use of pesticides in agri- and horticulture, along railway tracks and roads, as well as in households and in cooling systems may result in impoverishment of biodiversity, pollution of ground and surface water (including coastal and marine waters) and risks to human health from direct exposure or through residues in food and drinking water. Hence, the availability of reliable data on the occurrence of pesticide residues is essential for the proper assessment of these risks.

In this framework our laboratory carries out monitoring programs dealing with a large variety of

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pesticides in a number of matrices. These programs can range from field studies, with known use of a pesticide, for instance to evaluate leaching to groundwater, indicative survey programmes based on use-patterns combined with physicochemical properties, to indicative screening programmes for a wide range of pesticides in various matrices.

Pesticide science is a very dynamic field in which several trends in product development and use patterns have emerged. For example, there has been a clear shift from the use of non-polar persistent "long-life" pesticides such as organochlorine compounds that accumulate in the lipid fraction of the human food chain, to that of more polar and readily degradable "short-life" pesticides such as N-methylcarbamate insecticides. Furthermore, new pesticides such as sulphonyl urea herbicides with higher biological activity and, therefore, lower dose rates of application have replaced older pesticides such as phenyl urea herbicides. Hence, analytical methodology has to be adapted continuously, requiring almost always efforts in analytical method development. In fact, the time spent on method development often exceeds the time spent on the analysis of the samples themselves. Hence, especially from the efficiency point of view the availability of a unified method development approach is highly desired in this field of analysis.

In this communication, our strategy for the analysis of polar pesticides in environmental water samples is discussed. It includes the use of robust screening methods based on coupled-column liquid chromatography obtained through a method development strategy [1–5]. Screening may be followed by selective analytical methods based on GC–MS. As most polar pesticides are not amenable to GC, derivatization techniques are used. Aqueous derivatization methods in combination with sensitive ionisation techniques such as electron-capture negative ion chemical ionisation (ECNICI) MS are preferred. Furthermore the use of measurement strategies rather than dedicated methods also has its implications for the statistical evaluation of the data. Stand-alone procedures are often validated during method development; their quality is assured during the actual analyses of samples. In the application of strategies the first step is to prove that the approach is generally valid for specific sets of samples. In our approach two sets of data are available originating from the screening and the confirmation method requiring statistical comparison.

On the basis of our work (1993–1995) concerning the determination of polar pesticides in environmental water samples both LC and MS based methodologies will be reviewed and discussed.

2. Survey programmes

During the period 1993–1995 ten monitoring programmes have been carried out involving the determination of polar pesticides in environmental water samples taken from various locations in the Netherlands.

In four studies ETU was determined in surface water (65 samples, 5 locations), ground water (762 samples, 41 locations and 64 samples, 16 locations), drinking water (31 samples, 14 locations) and raw drinking water (70 samples, 35 locations). In the latter study metamitron was also analysed. Pentachlorophenol was assayed in raw drinking water (70 samples, 35 locations). Phenylurea herbicides (monolinuron, metobromuron and diuron) were analysed in drinking water (see ETU) and raw drinking water (40 samples, 3 locations). In the latter study also carbendazim and chlorophenoxy acids (2,4-D, MCPA, MCPB and MCPP) were analysed. During 1993–1995, each year rain water at one location was sampled once a month and analysed on chlorophenoxy acids and bentazone.

The period of sampling of the various projects ranged from 3 to 52 weeks.

3. Coupled-column liquid chromatography (LC–LC)

3.1. General aspects

Reversed-phase liquid chromatography (RPLC) in combination with UV detection is an attractive technique for the analysis of polar pesticides in aqueous samples. It is robust, rugged and allows the direct injection of aqueous samples, without a need

for extraction, derivation or other sample manipulations. A relative disadvantage is that the sensitivity attainable with UV detection usually insufficient for trace analysis. Consequently, a sample preconcentration step is needed for sensitive analysis. The compatibility of the mobile phase system with aqueous samples allows on-line sample enrichment by large volume injections in combination with LC column switching techniques. Depending on the dimensions of the first column (C-1) used, two different approaches can be distinguished in coupled-column RPLC: (i); precolumn switching LC (PC–LC) using a small size column (or SPE cartridge) for fast sample enrichment and (ii); coupled-column LC (LC–LC) employing two full size separation columns. PC–LC is the most widely used variant [6–13], however, LC–LC has shown very useful in a number of dedicated applications [1–5,14–17].

The higher selectivity obtained by an efficient pre-separation of the polar analyte(s) and excessive amounts of interferences like salts and humic acids on the first separation column is an important advantage of LC–LC in comparison to PC–LC. This feature can be fully exploited in the analysis of the more polar pesticides which elute during sample introduction as shown in the analysis of ethylenethiourea (ETU) and chloroallylalcohol (CAAL) in surface and ground water [14–16]. These procedures are based on coupled systems with similar stationary phase systems, which can not always provide sufficient selectivity. For example, in environmental analysis the determination of acidic compounds with RPLC–UV is always severely hampered by the presence of humic and fulvic acids. In case of chlorophenoxy acids herbicides selectivity is improved by using selective sorbents for preconcentration, on-line [18,19] or off-line [20,21]. For herbicides with amphoteric properties such as glyphosate and glufosinate the combination of (i); precolumn derivation with 9-fluorenylmethoxycarbonyl (FMOC), (ii); coupled-column LC using ion-exchange chromatography coupled to reversed-phase, (iii); fluorescence detection and (iv); and large volume injections (1–2 ml) allowed the rapid determination of these analytes in environmental water samples [22,23].

A minor drawback of LC–LC compared to PC–LC is that the smaller injection volumes in LC–LC may adversely affect the sensitivity. However, LOD levels of below 0.1 μg/l can be obtained using 5 ml injection volumes on an LC–LC system for pesticides with sufficient UV-sensitivity (ε>15 000 l/mol cm); bentazone and the phenyl urea herbicides are examples of such pesticides [16]. Volumes below 10 ml can be processed easily by an autosampler (Fig. 1). An important advantage of this approach is the high sample throughput which can be achieved (up to 10 h^{-1}).

In the execution of larger monitoring programmes involving alternative groups of pesticides, on-line techniques are not always that advantageous. Organic extracts (concentrated) obtained by of off-line procedures, e.g., liquid-liquid extraction or solid-phase extraction (SPE), may be stored over a longer period of time so that they can be analysed as batches. Furthermore, the assay of other pesticides in the same sample extracts is possible using e.g., gaschromatographic techniques.

3.2. LC–LC methods

Concerning the determination of analytes involved in the studies mentioned above coupled-column RPLC–UV methods were used for analysis of ETU [14] and chlorophenoxy acid herbicides [19]. For bentazone and phenylurea herbicides fast single residue methods using the analyser (see Fig. 1) were available [16]. However, the low frequency of sample collections of the monitoring programmes

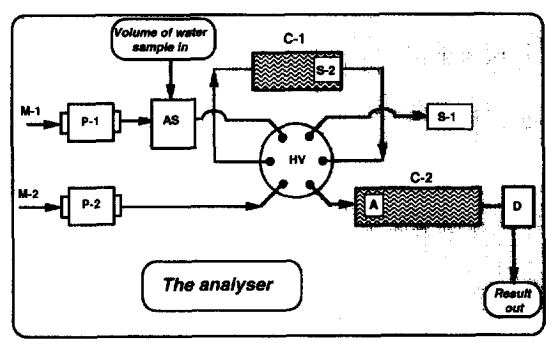

Fig. 1. Schematic presentation of coupled-column RPLC–UV analyser. AS, autosampler; C-1 and C-2, first and second separation column; P-1 and P-2, LC-pumps; HV, high pressure valve; M-1 and M-2, mobile phases; S-1, S-2, interferences; A, target analyte; D, UV-detector.

involved made an off-line concentration procedure, providing sample extracts in organic solvents more attractive. LC–LC methods for the analysis of the other pesticides, viz., metamitron, pentachlorophenol and carbendazim were developed according to previously reported guidelines [1–5]. The developed and modified procedures are described below.

3.2.1. Metamitron.

This compound was assayed directly in the water samples by means of the RPLC-UV analyser (see Fig. 1). *LC–LC conditions*: Injection volume, 6 ml of sample; C-1 and C-2, 150×4.6 mm I.D. packed with 5 μm Hypersil ODS; M-1, acetonitrile–0.1% ammonia in water (25:75; v/v); M-2, acetonitrile–0.1% ammonia in water (30:70; v/v); cleanup volume, 10.4 ml; transfer volume, 0.10 ml; UV detection, 310 nm.

3.2.2. Pentachlorophenol.

Sample pretreatment: Add 500 μl of 0.1 *M* HCl to a water sample of 50 ml and percolate it through a 100-mg C_{18} SPE-cartridge. Dry the cartridge by passing air for 30 min. Desorb the analyte from the cartridge into a calibrated tube with 1 ml of acetone. Evaporate the extract to dryness with nitrogen and redissolve the residue by adding 500 μl acetonitrile and 500 μl water, respectively. *LC–LC conditions*: Injection volume, 150 μl of extract; C-1, 50×4.6 mm I.D. packed with 3 μm Microspher C_{18}; C-2, 100×4.6 mm I.D. packed with 3 μm Microspher C_{18}; M-1, acetonitrile–0.03 *M* phosphate buffer pH 3.1 (55:45, v/v); M-2, acetonitrile–0.03 *M* phosphate buffer pH 3.1 (70:30, v/v); cleanup volume, 3.55 ml; transfer volume, 0.30 ml; UV detection, 212 nm.

3.2.3. Phenylurea herbicides.

Sample pretreatment: Extract 500 ml of water sample with dichloromethane (100, 50 and 50 ml). Dry organic extract over sodium sulphate and evaporate with Kuderna Danish. Redissolve the residue by adding 200 μl methanol and 1800 μl water, respectively. *LC–LC conditions*: Injection volume, 100 μl of extract; C-1, 50×4.6 mm I.D. packed with 3 μm Microspher C_{18}; C-2, 150×4.6 mm I.D. packed with 5 μm C_{18} Suplex pKb; M-1, acetonitrile–water (35:65, v/v); M-2, acetonitrile–water (40:60, v/v); cleanup volume, 2.37 ml; transfer volume, 1.05 ml; UV detection, 244 nm.

3.2.4. Carbendazim.

Sample pretreatment: Concentrate 100 ml of water sample to about 1 ml with rotating film evaporator at 70°C. Transfer residue to calibrated tube. Add 0.5 ml water and 0.2 ml acetonitrile to the evaporator bulb, swirl and place it into an ultrasonic bath for 2 min. Transfer the content to the tube and the adjust the volume to 2.0 ml with water containing 0.5% of ammonia. *LC–LC conditions*: Injection volume, 100 μl of extract; C-1, 60×4.6 mm I.D. packed with 5 μm Hypersil ODS; C-2, 150x×4.6 mm I.D. packed with 5 μm Hypersil ODS; M-1, acetonitrile–0.5% ammonia in water (10:90; v/v); M-2, acetonitrile–0.5% ammonia in water (20:80; v/v); cleanup volume, 4.0 ml; transfer volume, 1.2 ml; UV detection, 280 nm.

3.2.5. Bentazone.

Sample pretreatment: Add 200 μl trifluoroacetic acid to a water sample of 200 ml and percolate it through a 300-mg C_{18} SPE-cartridge. Dry the cartridge by passing air for 30 min. Desorb the analyte from the cartridge into a calibrated tube with 2 ml of acetone. Evaporate the extract to dryness with nitrogen and redissolve the residue by adding 400 μl methanol and 1600 μl 0.1% phosphoric acid in water, respectively. *LC–LC conditions*: Injection volume, 200 μl of extract; C-1, 50×4.6 mm I.D. packed with 3 μm Microspher C_{18}; C-2, 100×4.6 mm I.D. packed with 3 μm Microspher C_{18}; M-1, methanol–0.03 *M* phosphate buffer pH 2.4 (50:50, v/v); M-2, methanol–0.03 *M* phosphate buffer pH 2.8 (50:50, v/v); cleanup volume, 2.40 ml; transfer volume, 0.55 ml; UV detection, 220 nm.

All methods have LODs of at least of 0.1 μg/l; recovery ranges between 70–100% and R.S.D. ranges between 2–10% were obtained for spiked drinking water samples made at two levels (n~4 determinations at approx. 0.2 and 1 μg/l).

Residues of pentachlorophenol (PCP), metamitron and carbendazim above the 0.1 μg/l were not found

with the RPLC–UV methods. Therefore no confirmation analyses were carried out.

As mentioned before, the applicability of LC techniques is limited in case of acidic compounds, especially if a large number of compounds is to be analysed. Despite the selectivity of both off-line and on-line sample pre-treatment, the separation obtained for chlorophenoxy acids and sample interferences was not always sufficient. Hence, confirmation of identity and level was performed with GC–MS [24].

In three out of 40 raw drinking water samples analysed with RPLC–UV for three phenyl urea herbicides, monolinuron was found at levels near 0.1 μg/l (see example in Fig. 2). These results were not confirmed by LC–electrospray MS analysis and were classified as false positive.

As mentioned above, many of our studies involve the determination of ETU, the main metabolite of ethylenebisdithiocarbamate fungicides like zineb and maneb. Therefore an evaluation of data found with this method, compared with the data of the GC–MS method [25] is given in section 4.

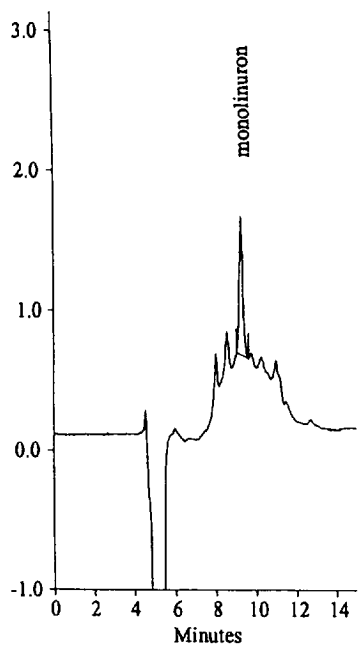

Fig. 2. Coupled-column RPLC–UV (244 nm) of an extract of a raw drinking water sample indicating a monolinuron residue of 0.1 μg/l. Experimental and instrumental condition, see Table 1.

4. LC- and GC–MS

4.1. General aspects

Although LC–MS techniques have been improved in terms of reliability and sensitivity, chemical derivation/gas chromatography/mass spectrometry often has been the preferred method for pesticide trace analysis in our laboratories. In several applications, derivation provided an excellent means to convert the polar compound into a suitable form for their subsequent sensitive analysis by electron capture negative ion chemical ionisation (ECNICI) MS. This occurs by the incorporation of high electron affinity groups (electrophores). In addition, a general feature of GC is its higher separation efficiency compared to LC, resulting in more narrow peaks in GC compared to normal bore LC, thus rendering an increased mass flux in the detector. A serious drawback however of GC and GC–MS techniques that they are less generally applicable than LC. In the daily pesticide analysis practice, meaningful use of GC requires knowledge of effective derivation methods. In particular, if complete derivation cannot be obtained in a single reaction, e.g., if different functional groups are present, it may lose a significant deal of its attractiveness. Another disadvantage of derivation/GC is the need of a prior extraction of the compounds of interest from the aqueous (environmental) sample, whereas LC permits their direct analysis thus circumventing extraction losses.

Up to now, at least three different LC–MS techniques have been used in pesticide analysis, all three having their specific merits. Individually, they cover only a limited range of the group as a whole. Particle beam LC–MS has been satisfactorily applied to the analysis of rather non-polar pesticides like carbamates [26]. Thermospray permits the analysis of slightly more polar pesticides [27], and electrospray (ESI) is the ideal technique for really polar compounds but applications to pesticides are scarce. Positive and negative ion ESI–MS gives highest sensitivities for basic and acidic compounds, respectively, whereas neutral compounds are in general insensitive [28]. For the latter, atmospheric pressure chemical ionisation (APCI) often optionally available to the ESI source may be a good alternative [29].

Ion-yields (sensitivity) in APCI may strongly depend on the compound's nature.

4.2. LC–MS and GC–MS methods

As discussed already, a major drawback of GC and GC–MS in pesticide analysis is the need for extraction of the pesticide and to carry out a suitable derivation reaction. Both steps may be laborious to perform and may suffer from non-quantitative yields. Therefore, derivation techniques in the aqueous sample would be convenient and preferred where possible. We have used successfully two of such techniques: (i); aqueous acetylation and (ii); Phase Transfer Catalysed Alkylation (PTC).

Acetylation with anhydrides (R-CO-O-CO-R) [30] and acid chlorides (R-CO-Cl) [31,32] proceeds rapidly and quantitatively for amines and phenols in aqueous samples at increased pH, resulting in less polar and more readily to extract products. In addition, using a electrophoric reagent, the attached electrophore will provide an increased sensitivity with electron-capture based detectors including GC-ECNICI-MS [24,25].

PTC is applicable to a large number of compounds. An overview on the basis of functional groups is given in Table 1. Pentafluorobenzoylchloride (PFB-Cl), 3,5-*bis*(trifluromethyl)-benzoylchloride (BTFMBCl) or even perfluoroacetic anhydrides are suitable reagents for this purpose.

In the PTC technique, extraction and reaction take place simultaneously for compounds with acidic functional groups. It can be performed in a biphasic system comprising the aqueous sample at elevated pH (≥9, dissociation of the acid) and a non-water miscible solvent (e.g., dichloromethane) in the presence of a suitable complexing agent (tertiary alkylammonium salts, crownethers). The resulting bulkier pesticide complex will be transferred to the organic phase, where the reaction takes place. Alkylbromides are most suitable reagents, particularly the electrophoric pentafluorobenzylbromide (PFB-Br and variants like 3,5 trifluoromethylbenzylbromide, BTFMB-Br) yield EC and ECNICI sensitive derivatives. Because the reaction proceeds slowly, the half-life of the reagent should be long enough in water (low hydrolysis rate) to ensure completion of the reaction. PTC was successfully applied to the analysis of phenoxy acids and other carboxylic pesticides [24,25]. Yields were essentially quantitative in all cases. The PTC technique was successfully applied to Bentazone (reaction replaces the acidic proton in the NH-SO_2 moiety) and ethylenethiourea (ETU) [25]. For ETU one or two protons were replaced by the BFTMB group. Due to keto-thiol tautomerism, three different forms were formed comprising a minor quantity of the mono N-BTFMB derivative and two *bis*-BTFMB derivatives, i.e., the N, N (major) and the N,S derivative, respectively. In trace samples (≤5 μg/l), quantification was performed at the abundant [M-BTFMB=M-227] anion of the N,N derivative and in high-level samples at this ion of the N,S isomer. This gave a larger linearity range of the assay as high level samples were analysed on a minor component in the reaction. The detection limit was 0.05 μg/l with recoveries ranging between 60 and 110% in low-level samples. The presence of more than one derivative causes, on one hand, a lower detection limit due to a spread of the response over more than one peak but, on the other hand, may provide additional evidence for a positive identification in unknown samples: peaks should be present in a correct abundance ratio.

Under ECNICI conditions, PFB and BTFMB esters readily lose their alkyl moiety under the formation of a relatively stable carboxylic anion at M-181 or M-227, respectively. Commonly, this ion dominates the ECNICI spectra, often being the only peak. Depending on their nature, additional fragmentation can be induced by analysis at elevated ion source temperatures [33]. PFB esters are extremely sensitive to ECNICI. Starting with a few ml sample

Table 1
Applied derivation techniques for pesticides in aqueous samples prior to GC–MS

Functional group	Derivation technique[a]	Pesticide(s)	Ref.
R–NH_2	Ac	Amitrole	[b]
Ph–OH	Ac	Phenols	[b]
R–COOH	PTC	Phenoxy	24
–SO_2–NH–	PTC	Bentazone	24
–SH	PTC	ETU	25

[a]Ac, aqueous acylation; PTC, phase transfer catalyses. Ac and PTC can be applied consecutively, perferably in the given order.
[b]Internal procedure (not published).

volumes, typical detection limits were at the low ng/l level. A drawback of the method is the formation of several by-products during the reaction which may adversely affect the quantification. Background reduction by use of increased MS resolution can be helpful to lower detection limits in real samples to the sub ng/l level.

An advantage of LC–LC followed by GC–MS confirmation over LC and LC–MS is the greater independence of the analysis and confirmation technique.

5. Evaluation of analytical data

5.1. General aspects of validation

Calibration is an important part of analysis since it determines the relation between instrument response and the concentration for all relevant sample matrix combinations. Calibration and validation should be representative of the variety of samples and the range of experimental conditions.

The experimental conditions can be covered by mixing the measurement sequence of real samples with the calibration/validation samples. The deviations between the results of the samples of known composition are then a reasonable estimation of deviations, accidental or due to changes in experimental conditions, in the real samples.

To cover the variety of samples the influence on the performance can be tested on representatives of classes of samples that are to be studied. The number of classes is a balance between assumptions on which samples should have similar influences and the amount of effort.

The actual calibration process determines a mathematical relation between concentration and response. This relation is usually calculated using least squares linear regression [34] over the whole concentration range, but this makes the incorrect assumption of homoscedasticity (i.e., error in response independent of concentration). With this illegal assumption calibration usually provides unrealistic high standard deviations for concentrations near the limit of determination (LOD) and, consequently, the calculated LOD will be unnecessary high. This is an important drawback in pesticide residue analysis since a low LOD is often required by the objective of the study. A "solution" to obtain a more realistic, and lower, standard deviation at low concentrations can be to perform separate experiments at a level near the LOD. However, the necessity for this separate experiment clearly indicates the inadequacy of the assumption of homoscedasticity.

Calibration and validation are illustrated below using as an example the analysis of ETU in ground water carried out two different methods of analysis. The first method uses coupled-column RPLC–UV for separation and detection [14] (see Fig. 3). The second method consists of GC–MS analysis on two ETU derivatives [25] (see Fig. 4).

5.2. LC–LC method

To validate the performance of the method during this study an extensive number of samples (21) with

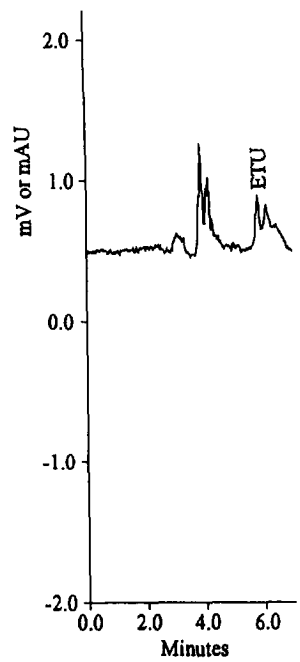

Fig. 3. Coupled-column RPLC–UV (230 nm) of an extract of a ground water sample containing 0.08 μg/l ETU. Injection of 100 μl of aqueous extract obtained after concentration–extraction procedure [14]. M-1 and M-2, acetonitrile–0.1% ammonia in water (1:99; v/v) with flow-rate of 1 ml/min; C-1 and C-2, 150×4.6 mm I.D. packed with 5 μm Hypersil ODS; cleanup volume, 2.85 ml; transfer volume, 0.50 ml.

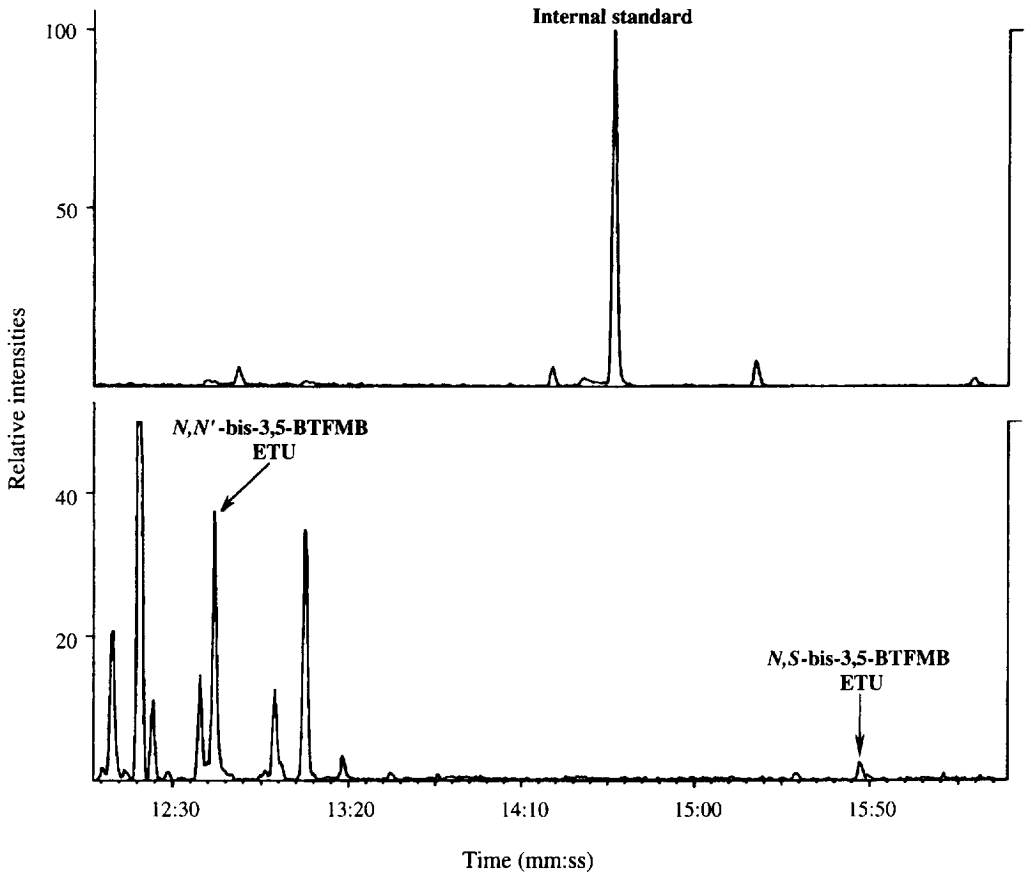

Fig. 4. Example of GC–MS analysis of a surface water spiked with ETU at a level of 0.13 μg/l. Structure of the deviations are indicated at the peaks.

known composition were analysed in the same batches as the "real" samples. Therefore the relevant experimental conditions were covered by these calibration samples. The calibration samples were made with tap water (18) and with ground water (3). The number of ground water calibration samples was relatively small since only ground water samples without ETU can be used for this purpose. To know which samples have no measurable amount of ETU, a part of the samples should be analysed first.

The results of the calibration samples are used for the calibration of the results of the real samples. This implies that all results are automatically corrected for losses during the analytical procedure. The average recovery of the calibration samples is about 70%. Based on these recoveries also a variance function can be estimated using the Calwer program [35]. The estimated variance as function of the concentration (conc.) reads:

$$s^2_{\mathrm{HPLC}} = (0.01)^2 + (0.05 \cdot \mathrm{conc.})^2$$

This implies that the standard deviation of reproducibility within these series of experiments is about 0.01 μg/l for small concentrations (≤ 1 μg/l) and about 5% of the estimated concentration for high concentrations (≥ 10 μg/l). The standard deviation for low concentrations is in good agreement with the LOD of 0.05 μg/l which was estimated on the basis of the chromatograms.

However, when ordinary lest squares regression is applied on this data a standard deviation of 0.14 mg/l is estimated providing a LOD of approximately 0.5 μg/l which is about a factor of 10 too high.

An objective statistical comparison between the unweigthed regression and weighted regression with variance model described above can be obtained using the logarithmic likelihood [36]. The model with the maximum (logarithmic) likelihood has the best fit with the data. For this data the unweighted regression has a logarithmic likelihood of 38.2 and the mixed variance model a value of 59.9. Since a difference of 2 is already significant these values show that the unweighted regression really gives a highly inferior fit.

5.3. GC–MS method

To validate the performance of the GC–MS method 19 spiked demineralised water samples were analysed in the same batches as the "real" samples. In addition, 6 ground water samples spiked at two levels (0.5 and 5.0 μg/l) were analysed according to the procedure. The results of this calibration experiments were confirmed by recovery experiments made to ground water samples; 6 ground water samples spiked at levels of 0.5 and 5.0 μg/l provided a mean recovery of 88% with a standard deviation of 10%.

The calibration procedure implicitly corrects for losses in the analytical procedure. Using the calibration results for the GC–MS method (based on the N,S- isomer) a variance function is estimated as:

$$s^2_{GCMS} = (0.03)^2 + (0.10 \cdot \text{conc.})^2$$

5.4. General aspects of method comparison

The necessity for a quantitative comparison between the results of the two different methods depends on the objective of the study. For instance in regulatory practice, when the main objective is to determine whether the concentration in a sample exceeds a legal critical level, a confirmation for values below this level might not be useful at all while for values far above this level the confirmation of the identity of the component is the crucial one. Far above the critical level the requirement that results should be in the same order of magnitude is assumed to be sufficient and only the HPLC data are reported, with their uncertainty, including a statement that the data are "confirmed by mass spectrometry". For these studies a more quantitative estimate of both levels and precision is only required for the concentration range around the critical level.

In other types of studies samples are analysed in order to get a general view for instance in trends or geographic contour plots. In general this implies that the analytical data are used as input into mathematical/statistical models which require estimates on the uncertainties on all levels. Therefore the results of both method validation and method comparison should preferably yield this information. An example of a quantitative method comparison is shown below for the ETU analysis.

5.5. Method comparison HPLC versus GC–MS

An alternative approach for the comparison of two different methods is the analysis of a number of real samples with both methods. In this example about 64 samples were analysed with both the HPLC and the GC–MS method. The data of the comparison are shown in Fig. 5, the larger deviations for concentrations below 1 μg/l this concentration range is enlarged in the upper left corner. The latter were actually measured in duplicate because of the relatively high standard deviations of this method. To compare the results with a regression like technique assumptions/knowledge about the spread in both methods is necessary. Since the repeatability of the HPLC method is much better the spread in these results is assumed to be negligible in comparison with the GC–MS method.

Then the comparison between the results of both methods can be performed in a similar method as the calculation of the calibration. A calibration model without intercept is used and a variance model is used for the weighted regression. Both the calibration and the variance model are estimated using the maximum likelihood method [36]. On average the results of the GC–MS method is about 94% of the HPLC results and a variance function is found as:

$$s^2_{samples} = (0.17)^2 + (0.13 \cdot \text{conc.})^2$$

Comparing the validation results of HPLC and the GC–MS method one expects a variance function which is the sum of the variance functions of the individual methods. Since the variance in the HPLC method is smaller than the GC–MS results the

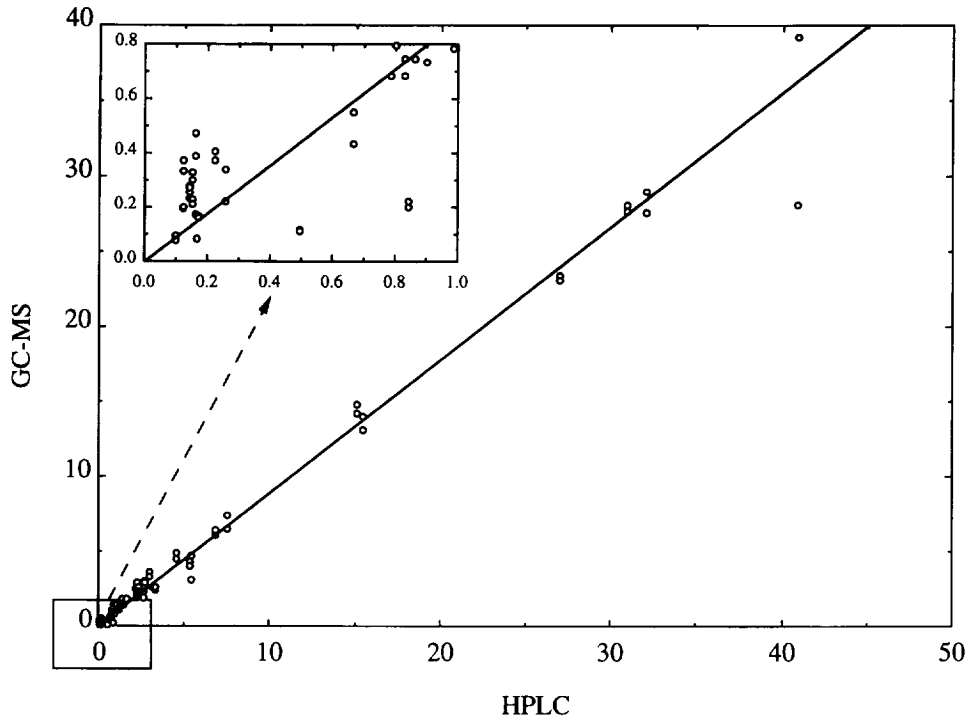

Fig. 5. Comparison of the results of the HPLC and the GC–MS method for the analyses of ETU in ground water samples.

combined standard deviation will be very close to the standard deviation of the GC–MS results.

The variance function for the real samples is quite similar to the data from the validation experiments for the high concentrations. This indicates that for these levels the validation strategy was sufficiently with respect to the real samples. For the low concentrations, however, the standard deviation is about a factor of 6 higher than was expected from the separate validations. The data, like chromatograms and spectra, obtained for samples which showed relatively large deviations between both methods, were studied thoroughly but showed no deviations from other samples. Due to the relative short period of stability of ETU it was not useful to reanalyse samples in order to answer this question.

5.6. Discussion

The validation of both individual methods shows that the precision of the HPLC–UV method is better than the precision of the GC–MS method.

For relatively high concentrations the deviation between the results of both methods, on the real samples was comparable with the deviation which was expected from the validation experiments. This implies that for these concentrations the validation strategy seems to be adequate. For low concentrations (≤ 1 μg/l) the deviations between the real samples is much larger than was expected from the validation results. This indicates that some results at low levels are effected by sources of variation which are apparently not present in the separate validation set up. Some of these sources of variation might be the (difference in) time delay between the collection and the analysis of the samples. Because of the stability of ETU this delay is always less than a week. But even within such a relatively small period of time changes may occur in the samples which have a large relative effect on low concentrations. Such a deviation may also attribute to the systematic difference of only 6% between two independent methods for analysis on these levels. Considering the relatively large deviations at low concentrations one

would like to know which method is to be considered as the most reliable with respect to the identification of the component. Considering the multidimensional information provided by GC–MS, this method is expected to have a smaller risk for misinterpretation of the identity. On the other hand one would expect the LC method to provide more adequate quantitative information. The latter is confirmed by the slightly better precision of the LC method, while the statement on the qualitative information requires additional information. This information is not available therefore a distinction between the selectivity of both techniques is not feasible on this data.

These considerations are a reflection of the attempts to continuously improve the quality of the analytical data. On the other hand, however, both the systematic deviation and the reproducibility are very satisfactory in comparison with the relative standard deviations for the analysis on these concentration levels, as predicted by Horwitz [36,37]. These predictions are 32%, 45% and 64% for concentrations of respectively 10, 1 and 0.1 μg/l. The relative standard deviations of Horwitz are derived from the results of interlaboratory comparisons, which is not very different from applying two independent methods in different departments from one laboratory.

6. Conclusion

The availability of a more or less standard approach for the analysis of pesticides in environmental samples has been very helpful in the performance of indicative surveys for the occurrence of pesticides during the last few years. LC–LC offers a reliable screening for the presence of target pesticides in a sample thus preventing false negatives, while GC–MS gives a reliable confirmation of the identity of the target compounds so that false positives are circumvented. With respect to the cost-effectiveness both procedures are simple and straightforward to perform with a high sample throughput, as well as applicable to a wide range of pesticides. In larger programmes with a relatively high number of positive samples the data of both procedures can be compared, yielding highly reliable data on the validity of the procedures.

References

[1] E.A. Hogendoorn, C. Verschraagen, P. van Zoonen and U.A.Th. Brinkman, Anal. Chim. Acta, 268 (1992) 205–215.
[2] E.A. Hogendoorn, Strategies in Method Development for the Determination of Polar Pesticides with Coupled-Column Liquid Chromatography, Ph.D. Thesis, Vrije Univesiteit, Amsterdam, 1993.
[3] E.A. Hogendoorn, R. Hoogerbrugge, E. Dijkman, S.M. Gort, U.A.Th. Brinkman and P. van Zoonen, J. Chromatogr. Sci., 31 (1993) 433–439.
[4] S.M. Gort, E.A. Hogendoorn, E. Dijkman, P. van Zoonen and R. Hoogerbrugge, Chromatographia, 42 (1996) 17.
[5] E.A. Hogendoorn and P. van Zoonen, J. Chromatogr. A, 703 (1995) 149–166.
[6] U.A.Th. Brinkman, J. Chromatogr. A, 665 (1994) 217–231.
[7] U.A.Th. Brinkman, H. Lingeman and J. Slobodnik, LC·GC Int., 7 (1994) 157.
[8] V. Pichon and M.-C. Hennion, J. Chromatogr. A, 665 (1994) 269–281.
[9] J. Slobodnik, E.R. Brouwer, R.B. Geerdink, W.H. Mulder, H. Lingeman and U.A.Th. Brinkman, Anal. Chim. Acta, 268 (1992) 55.
[10] R. Reupert, I. Zube and E. Plöger, LC·GC Int., 5 (1992) 43.
[11] E.R. Brouwer. A.N.J. Hermans, H. Lingeman and U.A.Th. Brinkman, J. Chromatogr. A, 669 (1993) 45.
[12] S. Chiron, E. Martinez and D. Barceló, J. Chromatogr. A, 665 (1994) 283.
[13] V. Coquart and M.-C. Hennion, Chromatographia, 37 (1993) 392.
[14] E.A. Hogendoorn, P. van Zoonen and U.A.Th. Brinkman, Chromatographia, 31 (1991) 285–292.
[15] E.A. Hogendoorn, A.P.J.M. de Jong, P. van Zoonen and U.A.Th. Brinkman, J. Chromatogr., 511 (1990) 243–256.
[16] E.A. Hogendoorn, U.A.Th. Brinkman and P. van Zoonen, J. Chromatogr., 644 (1993) 307–314.
[17] W.A. Minnaard, J. Slobodnik, J.J. Vreuls, K.-P. Hupe and U.A.Th. Brinkman, J. Chromatogr. A, 696 (1995) 333.
[18] V. Coquart and M.-C. Hennion, Sci. Total Environ., 132 (1993) 349.
[19] J.V. Sancho-Llopis, F. Hernández-Hernández, E.A. Hogendoorn and P. van Zoonen, Anal. Chim. Acta, 283 (1993) 287–296.
[20] A. Di Corcia and M. Marchetti, Anal. Chem., 63 (1991) 580.
[21] A. Di Corcia, S. Marchese and R. Samperi, J. Chromatogr., 642 (1993) 163.
[22] J.V. Sancho, F.J. López, F. Hernández, E.A. Hogendoorn and P. van Zoonen, J. Chromatogr. A, 678 (1994) 59–67.
[23] J.V. Sancho, F. Hernández, F.J. López, E.A. Hogendoorn, E. Dijkman and P. van Zoonen, J. Chromatog. A, 737 (1996) 75.
[24] H.D. Meiring, G. den Engelsman and A.P.J.M. de Jong, J. Chromatogr., 644 (1993) 357.
[25] H.D. Meiring and A.P.J.M. de Jong, J. Chromatogr. A, 683 (1994) 157.
[26] RD Voyskner, J.T. Bursey, Anal. Chem., 56 (1984) 1582.

[27] M. Honing, D. Barcelo, B.L.M. van Laar, U.A.Th Brinkman, J. Mass Spectrom., submitted.
[28] R.D. Smith, J.A. Loo, R.R. Ogorzahk, M. Busman and H.R. Udseth, Mass Spectrometry Reviews, 10 (1991) 359.
[29] D.R. Doerge and S. Bajc, Rapid Communications in Mass Spectrom., 6 (1992) 663.
[30] N.O. Ahnfelt, P. Hartvig, Acta Pharm Suecica, 17 (1980) 307.
[31] O. Gyllenhaal, L. Johansson, J. Vessman, J. Chromatogr., 190 (1980) 347.
[32] A.P.J.M. de Jong. C.A. Cramers, J. Chromatogr., 276 (1983) 267.
[33] A.P.J.M. de Jong, R.M. Kok, C.A. Cramers, S.K. Wadman, J. Chromatogr., 382 (1986) 19.
[34] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte and L. Kaufman, Chemometrics: a textbook, Elsevier, Amsterdam, 1988.
[35] S.M. Gort and R. Hoogerbrugge, Chemom. Intell. Lab. Syst., 28 (1995) 193.
[36] ISO/CD 13752, Air Quality - Assessment of Accuracy or the Calibration Function of a Measurement Method under Field Conditions, International Organization for Standardization, Geneva, Switzerland,1995.
[37] W. Horwitz, Evaluation of analytical methods used for regulation of foods and drugs, Anal. Chem., 54 (1982) 67A–76A.