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Journal of Chromatography A, 740 (1996) 83–98

JOURNAL OF CHROMATOGRAPHY A

Rapid method for the analysis of a variety of chemical classes of pesticides in surface and ground waters by off-line solid-phase extraction and gas chromatography–ion trap mass spectrometry.

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Received 23 August 1995; revised 23 January 1996; accepted 24 January 1996

Abstract

A rapid multiresidue method for the analysis, at trace levels, of 96 target analytes in field water samples has been developed. Pesticide parent compounds, from a variety of chemical and biological classes, as well as some of their major conversion products, were included in the target analyte list. Analytes were extracted from 1-l filtered water samples by off-line solid-phase extraction (SPE) on three tandem Sep-Pak C_{18} cartridges. The sorbed analytes eluted with ethyl acetate were directly analysed by gas chromatography–ion trap mass spectrometry (GC–IT-MS), the mass spectrometer operated in the electron impact (EI) ionisation mode. The mean recoveries, at the 0.5 μg/l fortification level, for two-thirds of the analytes ranged from 75 to 120%; the recoveries for less than one third of the analytes ranged from 50 to 75% and the recoveries for the 10 relatively most polar analytes ranged from 12 to 50%.

The limit of detection (LOD) of the method for 69 analytes was better than 0.01 μg/l; the LOD for eighteen analytes was better than 0.05 μg/l; for captan, carbofenothion, decamethrin, demeton-S-methyl sulphone, fensulfothion, deisopropyl-atrazine and metamitron, the LOD was 0.1 μg/l and for chloridazon and tetradifon, it was 0.5 μg/l. Identification, in full scan mode, was made at $S/N \geq 3$. Quantification, for the majority of the analytes, was made at the base mass. The system was evaluated for monitoring pesticides in surface and ground water samples of Macedonia, Greece.

Keywords: Water analysis; Environmental analysis; Pesticides

1. Introduction

The occurrence of pesticides, parent compounds and their conversion products, in aquatic systems is of major concern world-wide. The EU directive 80/779 [1], concerning the quality of water intended for human consumption, defined the 0.1 μg/l level as the maximum permissible residue level (MRL) for individual pesticides and 0.5 μg/l for the total pesticides present. Therefore, reliable analytical methods for monitoring pesticides and their conversion products at these levels in aquatic systems, under the regional conditions of member states, are needed.

Nowadays, gas chromatography (GC) associated with mass spectrometry (MS) is routinely used for monitoring pesticides in aqueous samples [2–7], whereas the on-line coupling of liquid chromatog-

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0021-9673/96/$15.00
PII S0021-9673(96)00099-4

raphy with GC–MS, for on-line solid-phase extraction (SPE) and GC–MS analysis of pesticides in aqueous samples, is already at an advanced stage of development [8] and this innovation is expected to broaden the range of applications of GC–MS in environmental monitoring studies.

Among the GC–MS methods reported so far, for the analysis of pesticides in aqueous samples, GC associated with ion trap MS (GC–IT-MS) has been used only on two occasions [9,10], while already there has been increased interest in applications of GC–IT-MS in multiresidue methods of pesticide analysis in foods [11–15]. GC–IT-MS was used in a method developed for the analysis of atrazine, cyanazine, simazine, alachlor and metolachlor and two degradation products of atrazine and alachlor, respectively, in water [9]. In this work, it was demonstrated that, using GC–IT-MS, the MS operated in the electron impact (EI) ionisation mode, high sensitivity with a detection limit of 60 pg, based on full scan mode, could be easily achieved. In another study GC–IT-MS, the MS operated in the chemical ionisation (CI) mode, was used for the analysis of twenty pesticides in surface water samples [10]. In the latter case, the reported LOD values ranged from 1.0 to 0.005 μg/l level. Due to the limited data available to date on the performance of GC–IT-MS in trace analysis of pesticides in water, a study was undertaken to develop a multiresidue method for the GC–IT-MS analysis of a wide chemical range of pesticides in aqueous samples.

The pesticides included in the target analyte list of this method were selected from pesticides considered to be of environmental pollution concern in the Axios River basin, Greece. The selection was based on pesticide use patterns in this area and on the data derived from previous monitoring studies in surface and ground water systems of the basin, using GC associated with nitrogen–phosphorus detection, flame photometric detection and MS [16,17] and with high-performance liquid chromatography (HPLC)–diode array detection (DAD). A rapid multiresidue method for the reliable identification and determination, at trace levels, of most of the GC-amenable compounds of interest will be described here.

2. Experimental

2.1. Materials

All solvents used (ethyl acetate, hexane, acetone, toluene and methanol) were of pesticide residue grade and were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulfate and sodium chloride were of proanalysis grade (Merck). Sep-Pak C_{18} cartridges were purchased from Millipore (Milford, MA, USA). Membrane filters (0.45 μm) were purchased from Schleicher and Schuell (Dassel, Germany). Pesticide analytical standard materials were purchased from Promochem (Augsburg, Germany), Chem Servis (West Chester, PA, USA), Riedel-de Haen (Seelze-Hannover, Germany) and from Alltech (Deerfield, IL, USA). Analytical standards of atrazine, deisopropylatrazine (G-28), deethylatrazine (G-30) and of metolachlor were donated by CIBA (Basel, Switzerland).

2.2. Instrumentation

A Tracker/Magnum ion trap mass spectrometer (Finnigan Mat, San Jose, CA, USA), associated with a Varian (Varian Instruments, Sunnyvale, CA, USA) Model 3300 gas chromatograph, was used. The data system was operated on an IBM-compatible computer that was connected to a Laserjet IIIP printer (Hewlett-Packard, Palo Alto, CA, USA). The operating conditions of the GC–IT-MS system were as follows: (a) gas chromatograph. A split–splitless injector operated in the splitless mode was used under isothermal conditions at 230°C. A 1.5 m×0.25 mm I.D. guard column of deactivated fused silica (Alltech) was inserted between the injector and the analytical column, a 30 m×0.25 mm I.D. DB-5-MS and with 0.25 μm film thickness (JW Scientific, Folsom, CA, USA). The guard column was replaced periodically, depending upon the type of samples analysed. The oven was temperature-programmed from 80°C (held for 1 min at 80°C) to 200°C at 6°C/min, held for 3 min at 200°C and then increased from 200 to 260°C at 6°C/min (held for 10 min at 260°C). The total run time was 54 min. Helium was used as the carrier gas and gave a column head pressure of 7.3 p.s.i. (30 cm/s; 1 p.s.i.=6894.76 Pa).

Injections (2 μl) were made either manually or by use of an autosampler, Model A200S (Finnigan Mat). (b) Mass Spectrometer. The IT-MS system was operated in the EI ionisation mode. The filament emission current was 28 μA, the multiplier voltage was 1750 V, the axial modulation amplitude was 3.6 V and the electron multiplier gain was $1 \cdot 10^5$. The scan range was set to 50–450 u at 1 scan/s. The transfer line and the manifold temperatures were set at 250 and 220°C, respectively.

2.3. Methods

2.3.1. Calibration solutions

Stock solutions of individual pesticides, at a concentration of 1 mg/ml in ethyl acetate, were stored in a deep freeze (−23°C). Five mixed working pesticide standard solutions, made in ethyl acetate, were serially diluted with ethyl acetate to give working standard solutions of 5.0, 2.5, 1.0, 0.5, 0.25, 0.1 and 0.05 μg/ml, respectively. The latter solutions were used for the construction of calibration curves and the preparation of the fortified water samples needed for the recovery studies. The working standard solutions were stored refrigerated and renewed approximately every two months.

2.3.2. Preparation of fortified and field water samples

Pesticides were extracted from water by SPE using three tandem Sep-Pak C_{18} cartridges. Water samples were filtered through 0.45-μm membrane filters in an all-glass filtration apparatus and 1-l aliquots were introduced by suction onto the Sep-Pak cartridges at a rate of 20 ml/min. Analytes were eluted with 30 ml of ethyl acetate. The eluent was collected into a round-bottomed flask through a funnel plugged with a small piece of glass wool and containing a small portion (1 g) of anhydrous sodium sulfate that had been washed previously with 30 ml of hexane followed by 30 ml of ethyl acetate. The funnel was rinsed with an additional 15 ml of ethyl acetate and the rinses were combined with the eluent in the same flask. Toluene (1 ml) was added in each flask and the eluent was concentrated to a small volume by use of a rotary evaporator operated under reduced pressure. The extract that had been transferred into a centrifuge tube was concentrated to dryness by use of a nitrogen stream. The residue was dissolved in 100 μl of ethyl acetate and was analysed by GC–IT-MS.

Before use, new Sep-Pak cartridges were conditioned by elution with methanol (30 ml) and water (30 ml). These cartridges were reusable. After each sample SPE, the cartridges were washed in the backflush mode with ethyl acetate, methanol and water. The same set of cartridges was used for the processing of ten–fifteen samples.

Laboratory-distilled water (pH 5.8), filtered through a 0.45-μm membrane filter, was used for the recovery studies. Aliquots (1 l), fortified with 100 μl of the appropriate working standard solution to give samples fortified at the 0.5, 0.1, 0.05 0.01 and 0.005 μg/l level, were extracted, as described above. Control samples were processed after the addition of 100 μl of ethyl acetate. In order to evaluate the overall performance of the extraction set-up, fortified samples (1 l) were also extracted by liquid–liquid partition (LLE) with 2×60 ml of dichloromethane, after the addition of 50 g of sodium chloride. The dichloromethane extracts that had been dried over anhydrous sodium sulfate and concentrated to a small volume, as described above, were analysed by the GC–IT-MS system.

2.3.3. Identification and quantification of the target analytes

The system was calibrated first by injecting 2-μl aliquots of each working standard solution to produce a seven-point calibration curve and respective search files for all the analytes. Analyte search and identification was made by use of the automatic search and identification menu of the Magnum Data System. Analytes were identified by comparing EI mass spectra (sixteen main ions) with known spectra of analytes stored in the search files. All analytes identified at a pre-determined minimum spectral fit and $S/N \geq 3$ were listed and quantitated by use of the external standard calibration curves. Calibration curves were linear in the working range of 0.1 to 10 ng and the linearity was checked periodically by injecting working mix standard solutions. The corre-

lation coefficients of the calibration curves were usually higher than 0.999.

3. Results and discussion

3.1. General considerations

A wide chemical range of pesticides (triazines, chloro-acetanilides, dinitro-anilines, organophosphates, pyrethroids, organochlorines and miscellaneous other chemical classes), including also some of their major conversion products, comprised the target analyte list of this project. The list of the common names and the respective chemical classification of the compounds mentioned in the text is presented in Table 1.

Among the target analytes, a substantial number of the so-called "conventional" organochlorine pesticides was also included. In recent years, the environmental, and thus the analytical, interest in these compounds was diminished, because their use has been banned in most countries. Among these pesticides, lindane (γ-BHC), dicofol, tetradifon and endosulfan are still used in Greece. However, since the entire group of the conventional organochlorines is still used or manufactured in other Balkan countries to the north of Greece, residues of these compounds are found in rivers originating from these countries and in aquatic systems where these rivers are discharged [16,17].

Preliminary data concerning the applicability of the method for the analysis of the organochlorines showed that among the 22 compounds included in the list, the system failed to discriminate chloropropylate from chlorobenzilate, as both compounds were eluted with the same retention time and exhibited the same mass spectrum. Apparently, both compounds decomposed to the same product at the GC injection port. This product was present as the molecular ion (m/z 251) in the IT-EI-MS spectra of chlorobenzilate and chloropropylate derived from M-$C(O)OCH_2CH_3$ and M-$C(O)OCH(CH_3)_2$, respectively (data not shown).

The carbamate pesticides as a group, due to their thermal instability, are considered as non-GC amenable compounds and therefore HPLC methods are preferred for their analysis [19,20]. However, GC-based methods, under certain conditions, are used for the analysis of some carbamates [21,22]. Preliminary work on the chromatographic behaviour of carbaryl, carbofuran, 3-hydroxy-carbofuran and molinate showed that the N-methyl carbamates decomposed under the GC conditions employed here. By comparing the total ion currents of the respective chromatographic peaks, it appeared that 30% of both carbaryl and carbofuran decomposed to naphthol and benzofuranol, respectively, while 80% of 3-hydroxy-carbofuran decomposed to the respective hydroxy-benzofuranol (data not shown). Only molinate, a thiocarbamoyl pesticide, was eluted in the form of a single peak. Therefore, among the N-methylcarbamates intended to be analysed, only carbofuran was included in the target analyte list of this method, because, as will be seen later, in spite of the documented degradation under GC conditions, the precision of the method for the analysis of carbofuran was better than the lowest acceptable level (R.S.D.$<$20%).

Most of the phenylurea herbicides are also non-GC amenable compounds, due to thermal instability, and thus HPLC-utilising methods are preferred for their analysis [20]. All the phenylureas investigated in this study, except fluometuron, were degraded under GC conditions. Judging from the total ion currents of the respective chromatographic peaks (data not shown), their stability under GC conditions decreased in the order of fluometuron$\gg$monolinuron$>$metobromuron$>$linuron$>$monuron, chlortoluron$\gg$diuron. The major degradation product of these ureas was the substituted phenyl isocyanate ion, while linuron and diuron were further degraded to produce 3,4-dichlorobenzenamine (data not shown). Amongst the phenylureas intended to be included in the target analyte list, only fluometuron, monolinuron, metobromuron and linuron were included, since, for these compounds, in spite of their degradation under GC conditions, the precision of analysis was not significantly effected.

Pyrethroids are also considered as non-GC amenable compounds due to thermal instability [23], however, none of the four compounds (deltamethrin, fenvalerate and *cis/trans*-permethrin) included

Table 1
List of common names of compounds mentioned in the text

I. Organochlorines

Ia. Hexachlorocyclohexane isomers
α-BHC, β-BHC, γ-BHC or lindane, δ-BHC

Ib. DDT and related compounds
Chlorobenzilate, chloropropylate, dicofol, *o',p-* and *p',p*-DDE, DDD, DDT, methoxyclor, tetradifon

Ic. Cyclodienes
Aldrin, dieldrin, endrin, endosulfan I and II, endosulfan sulfate, heptachlor, heptachlor epoxide

II. Organophosphates

IIa. O,O-Dimethyl phosphorothionates
Azinphos methyl, chlorpyrifos methyl, cyanofos, dimethoate, fenitrothion, formothion, malathion, methacrifos, methidathion, parathion methyl, phenthoate, phosalone, phosmet, pirimiphos methyl

IIb. O,O-Dimethyl phosphates
Demeton-S-methyl sulphone, malaoxon, monocrotophos, mevinphos (*cis/trans*), paraoxon methyl, tetrachlorvinphos

IIc. O,O-Diethyl phosphorothionates
Azinphos ethyl, carbophenothion, chlorpyrifos ethyl, coumaphos, dialifos, diazinon, ethion, fensulfothion, parathion, pirimiphos ethyl, prothoate, pyrazophos, quinalphos, triazophos

IId. O,O-Diethyl phosphates
chlorfenvinphos, paraoxon

IIe. Miscellaneous organophosphates
Cadusafos, isofenphos

III. Triazines

IIIa. 2-Chloro-triazines
Atrazine, deethylatrazine, deisopropylatrazine, simazine, terbuthylazine

IIIb. 2-Thiomethyl-atrazines
Ametryne, desmetryne, prometryne, simetryne, terbutryne

IIIc. Other triazines
Metamitron, terbumeton

IV. Carbamates
Carbaryl, carbofuran, 3-hydroxycarbofuran and molinate

V. Phenylureas
Chlortoluron, diuron, fluometuron, linuron, metobromuron, monolinuron and monuron

VI. Pyrethroids
Deltamethrin or decamethrin, fenvalerate, *cis*-permethrin and *trans*-permethrin

VII. Miscellaneous chemical groups
Alachlor, captan, chloridazon, chlorothalonil, ethofumesate, fenpropimorph, flutriafol, metalaxyl, metolachlor, pendimethalin, procymidone, propachlor, propanil and trifluraline

among the target analytes, exhibited any thermostability-related problems. In addition to the analytes of the main chemical groups discussed above, a number of other important pesticides representing twelve different chemical classes (Group VII, Table 1) were also included.

3.2. Retention times and resolution between analytes

The data on the retention times (mean values) of the target analytes are presented in Table 2. The standard deviation (S.D.) of retention times, during consecutive chromatographic runs or during day-to-day operation of the GC–IT-MS system, was less than ±0.02 min (data not shown), except for the retention times of the pesticides chlorobenzilate, decamethrin and *trans*-permethrin, which exhibited mean retention times with S.D. values of ±0.08, ±0.07 and ±0.03 min, respectively. Higher deviations of retention times were usually corrected by replacing the guard column and the meticulous cleaning of the GC injection port.

The resolution between certain pairs of analytes was very poor under the chromatographic conditions employed here. However, by grouping the target analytes into five subgroups, optimisation of the GC system, to obtain baseline separation between the analytes of each subgroup, was possible and thus the accurate calibration of the mass spectrometer, for the operation of the automatic analyte identification and quantification menus of the Magnum Data System, was not effected. However, when field samples were analysed, there was always the possibility of poor resolution between analytes, or even co-elution of analytes with matrix components. Therefore, during analysis of field samples, auto-identification of analytes was always confirmed by manually observing the mass spectra of the data and comparing them with the respective spectra of the target analytes, stored in the library search files. In cases where poor resolution between target analytes or interference from adjacently eluting or co-eluting matrix components was suspected, the mass spectra of consecutive scans (every 1 s) of a certain chromatographic peak or co-eluting peaks, were retrieved and compared to respective spectra of target analytes stored in the search files.

3.3. Recoveries and LODs of target analytes

Data derived from the recovery studies of samples fortified at the 0.5 μg/l level are presented in Table 2. The mean recoveries for the majority of the analytes (60 compounds) extracted by SPE ranged from 75 to 120% and the respective recoveries for 26 analytes ranged from 50 to 75%. In the latter group, the most lipophilic compounds among the target analytes, such as the organochlorines and the pyrethroids, were included. For these compounds, in spite of their high (>1000 ml) breakthrough volumes, relatively low recovery values were also recorded when water samples were analysed by an on-line SPE–HPLC–DAD system utilising PRP-1 cartridges for sample preconcentration [18].

The mean recoveries for the relatively polar analytes (chloridazon, fenpropimorph, diethylatrazine or G-30, isofenphos, metamitron, molinate and monocrotophos) ranged from 30 to 50%, while the respective recoveries of the most polar analytes, such as demeton-S-methyl sulphone, dimethoate and de-isopropylatrazine (G-28) were 18, 24 and 12%, respectively. The low recovery values for these polar analytes were obviously due to the sample volume exceeding, by far, their respective breakthrough volumes [18].

The precision of the SPE system, expressed by the relative standard deviation (R.S.D.) of the respective mean recovery values, when triplicate water samples fortified at 0.5 μg/l were extracted and analysed, was better than 10% for the majority of the analytes (Table 2). The R.S.D. values for chloridazon, decamethrin, demeton-S-methyl sulphone, fenpropimorph, metamitron and molinate ranged from 14 to 18%. These analytes, with the exception of decamethrin and molinate, were among the most polar compounds included in the target analyte list and their recoveries were in the <50% range. Also, for all of these analytes, except for molinate, their amenability for GC analysis was marginal.

In order to evaluate the performance of the selected SPE system, the recoveries of the analytes of interest when fortified (0.5 μg/l) samples (1-l) extracted by LLE with dichloromethane were also determined. The profile of the mean recovery values (%) was approximately the same in both extraction methods while the extraction precision, expressed by the respective R.S.D. values, was slightly better with SPE than with LLE (Table 2). The mean recovery values of the polar analytes, except for monocrotophos, as was expected, were higher when samples were extracted by LLE than by SPE, while the recovery of molinate was approximately the same

Table 2
Mean retention times (min), quantification masses, mean recovery (%) and respective R.S.D. values, and LODs of the target pesticides

Pesticide	Mean retention time (min)	Quantified mass (m/z)	Mean recovery (%) (R.S.D.) at 0.5 μg/l LLE	Mean recovery (%) (R.S.D.) at 0.5 μg/l SPE	LOD (μg/l)
Alachlor	23.19	160	92 (10)	94 (3)	0.005
Aldrin	25.26	66	37 (4)	59 (5)	0.01
Ametryne	23.45	227	78 (13)	65 (8)	0.01
Atrazine	20.26	200	97 (1)	93 (9)	0.005
Azinphos ethyl	37.45	132	99 (1)	78 (4)	0.01
Azinphos methyl	36.11	132	109 (2.)	94 (2)	0.01
α-BHC	19.26	181	69 (12)	67 (8)	0.005
β-BHC	20.18	183	84 (9)	86 (3)	0.005
γ-BHC (lindane)	20.40	181	74 (8)	82 (7)	0.005
δ-BHC	21.44	181	90 (9)	89 (2)	0.01
Cadusafos	19.08	159	85 (8)	82 (4)	0.01
Captan	27.33	79	105 (16)	87 (3)	0.1
Carbofenothion	32.25	157	75 (14)	51 (6)	0.1
Carbofuran	20.11	164	106 (1)	112 (5)	0.005
Chlorfenvinphos	27.18	267	79 (8)	92 (2)	0.005
Chloridazon	32.48	77	40 (32)	34 (18)	0.5
Chlorobenzilate	31.03	139	108 (3)	94 (3)	0.005
Chloropropylate	31.02	251	87 (9)	90 (2)	0.005
Chlorothalonil	21.14	266	95 (6)	90 (3)	0.05
Chlorpyrifos ethyl	25.19	197	87 (9)	75 (5)	0.05
Chlorpyrifos methyl	23.02	286	84 (4)	79 (4)	0.005
Chlorthiophos	31.28	269	74 (11)	54 (10)	0.01
Coumaphos	39.36	362	106 (5)	86 (9)	0.01
Cyanofos	20.52	109	77 (7)	80 (7)	0.005
o,p-DDE	28.21	246	66 (4)	59 (6)	0.005
p,p-DDE	29.43	246	71 (4)	57 (8)	0.005
DDD	31.2	235	83 (11)	72 (1)	0.01
DDT	32.45	235	103 (5)	72 (5)	0.005
Decamethrin	52.0	181	92 (8)	52 (18)	0.1
Demeton-S-methyl sulfone	24.14	169	102 (21)	18 (15)	0.1
Desmetryn	22.43	213	95 (11)	76 (10)	0.05
Dialifos	37.55	208	89 (12)	88 (3)	0.01
Diazinon	21.06	179	85 (8)	86 (3)	0.005
Dicofol	26.01	139	105 (5)	55 (9)	0.01
Dieldrin	29.50	79	95 (10)	89 (1)	0.01
Dimethoate	20.00	125	88 (4)	24 (7)	0.05
Endosulfan I	28.45	195	76 (12)	99 (2)	0.05
Endosulfan II	31.06	195	97 (5)	92 (3)	0.05
Endosulfan sulfate	32.38	272	112 (6)	89 (5)	0.05
Endrin	30.42	81	84 (9)	94 (1)	0.05
Ethion	31.25	231	89 (1)	76 (6)	0.05
Ethofumesate	24.43	207	93 (1)	85 (5)	0.005
Fenitrothion	24.29	125	96 (10)	88 (5)	0.01
Fenpropimorph	25.41	128	67 (22)	21 (12)	0.01
Fensulfothion	31.08	293	82 (8)	74 (7)	0.1
Fenvalerate I	47.00	125	100 (7)	55 (10)	0.01
Fenvalerate II	48.16	125	98 (1)	56 (10)	0.05
Fluometuron	18.00	72	83 (10)	84 (3)	0.005
Flutriafol	29.04	123	109 (1)	75 (4)	0.05

Table 2. Continued

Pesticide	Mean retention time (min)	Quantified mass (*m/z*)	Mean recovery (%) (R.S.D.) at 0.5 μg/l LLE	Mean recovery (%) (R.S.D.) at 0.5 μg/l SPE	LOD (μg/l)
Formothion	22.21	93	80 (11)	62 (1)	0.01
G-28	18.25	173	21 (9)	12 (4)	0.1
G-30	18.38	172	65 (4)	31 (6)	0.01
Heptachlor	23.41	100	43 (9)	66 (3)	0.01
Heptachlor epoxide	27.10	81	78 (11)	85 (2)	0.05
Isofenphos	27.10	58	77 (13)	44 (10)	0.01
Linuron	24.49	61	94 (11)	95 (5)	0.05
Malathion	24.58	125	113 (5)	99 (2)	0.01
Malaoxon	23.15	127	111 (8)	120 (5)	0.01
Metamitron	30.14	104	59 (3)	20 (14)	0.1
Metalaxyl	23.42	206	102 (7)	98 (2)	0.005
Methacrifos	15.19	125	62 (4)	59 (6)	0.01
Methidathion	28.10	145	100 (2)	85 (3)	0.005
Methoxychlor	34.54	227	126 (4)	82 (7)	0.01
Metolachlor	25.15	162	94 (2)	80 (8)	0.005
Metobromuron	22.26	61	98 (9)	88 (2)	0.01
Mevinphos (*cis/trans*)	13.41–13.46	127	68 (5)	71 (2)	0.005
Molinate	16.18	126	48 (12)	48 (18)	0.005
Monocrotophos	19.07	127	11 (13)	32 (8)	0.05
Monolinuron	20.22	61	86 (10)	78 (3)	0.005
Parathion	25.37	109	94 (9)	91 (6)	0.01
Parathion methyl	23.15	109	89 (10)	97 (3)	0.003
Paraoxon	23.59	109	82 (9)	92 (4)	0.05
Paraoxon methyl	21.32	109	76 (14)	89 (6)	0.05
Pendimethalin	26.49	252	97 (4)	72 (2)	0.005
cis-Permethrin	39.17	183	100 (3)	53 (9)	0.005
trans-Permethrin	39.37	183	105 (7)	67 (4)	0.005
Phenthoate	27.31	274	86 (10)	78 (4)	0.01
Phosalone	36.00	182	89 (10)	93 (3)	0.01
Phosmet	34.29	160	95 (5)	99 (5)	0.005
Pirimifos ethyl	26.21	168	83 (6)	73 (5)	0.005
Pirimifos methyl	24.26	276	74 (11)	78 (5)	0.01
Procymidone	27.39	96	84 (11)	86 (7)	0.005
Prometryne	23.59	184	100 (4)	73 (6)	0.001
Propachlor	17.35	120	80 (1)	73 (4)	0.005
Propanil	22.50	161	101 (5)	83 (8)	0.01
Prothoate	23.02	115	78 (10)	76 (4)	0.01
Pyrazophos	37.22	221	88 (8)	88 (1)	0.05
Quinalphos	27.35	146	89 (4)	79 (2)	0.05
Simazine	20.14	201	72 (11)	87 (1)	0.005
Terbumeton	20.36	210	79 (17)	77 (8)	0.01
Terbuthylazine	20.56	214	93 (4)	75 (8)	0.005
Terbutryne	24.33	226	83 (5)	71 (9)	0.01
Tetrachlorvinphos	28.25	109	93 (7)	114 (3)	0.005
Tetradifon	35.43	75	91 (2)	82 (1)	0.5
Triazophos	31.59	161	89 (7)	93 (4)	0.05
Trifluralin	18.44	306	52 (16)	69 (2)	0.005

in both methods (Table 2). Apparently, the most significant parameter in reduced recovery values of the thiocarbamoyl herbicide, molinate, was its relatively high vapour pressure, which resulted in a substantial amount being lost during concentration of either the dichloromethane or ethyl acetate extract. For the same reason, the recovery of the dinitroaniline herbicide, trifluraline, was in the 50–70% range with both extraction methods (Table 2).

Thus, for the extraction of the target analytes from water samples, a SPE technique was selected, as it was as efficient, more precise, more environmentally friendly, less dangerous to the analyst and much faster than the conventional LLE method with dichloromethane. The merits of SPE over LLE methods have been discussed extensively already [24–28]. However, water extracts derived by LLE were significantly cleaner than the respective extracts derived by SPE. In Fig. 1, total ion chromatograms of solvent blank samples derived from SPE (trace A) and LLE (trace B) are shown. Trace B contains three major peaks identified as phthalate esters (peaks 2 and 7) and a hydrocarbon (peak 8), their presence traced to either sodium sulphate and glass wool or to the glassware used, as dichloromethane, itself concentrated to the same ratio as that found in samples,

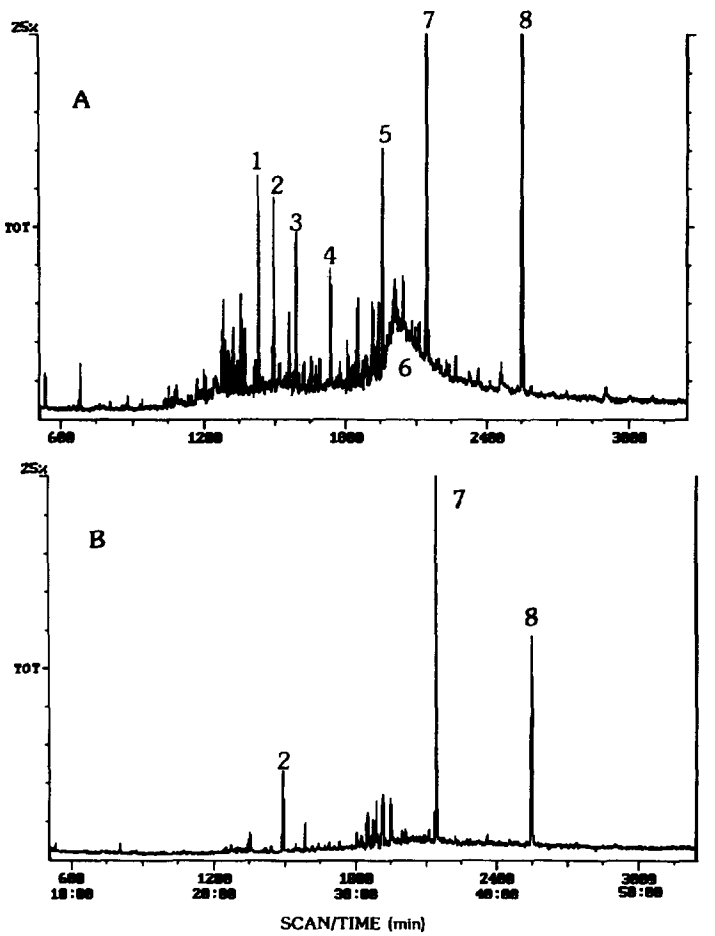

Fig. 1. Sample chromatograms obtained under GC–IT-EI-MS conditions of solvent blank samples, processed by SPE (trace A) and LLE (trace B). For other conditions see Section 2.3. Peaks numbered 2, 3 and 7 are phthalate esters, peak 8 is a hydrocarbon, peak 6 is vinyldimethyl(acetoxymethyl)silane and peaks 1, 4 and 5 are unknowns. *x*-Axis, (upper) scan number and (lower) retention time (min); *y*-axis, total ion intensity.

did not contain such impurities. These same impurities were also present in trace A, apparently due to the same sources, however, the latter trace contained a substantial number of other compounds, mostly long-chain hydrocarbons. In addition, in trace A, a highly tailed vinyldimethyl(acetoxymethyl)silane peak (peak 6) was also present, in the middle of the chromatographic run. Impurities, to a lesser degree, were also released from C_{18} bound membrane disks used in SPE of micropollutants from water samples [28,29]. However, since the cartridges used in the work presented here were reusable for more than ten samples and thus the cost of the analysis per sample was reduced significantly, they were preferred over membrane disks.

The limit of detection (LOD, μg/l) of the proposed method for more than two-thirds (65 compounds) of the analytes of interest was better than 0.01 μg/l, while of these compounds, 53 analytes could be detected when present at much lower levels. For nineteen analytes (chlorpyrifos ethyl, chlorothalonil, desmetryn, dimethoate, endosulfan I and II, endosulfan sulfate, endrin, ethion, fenvalerate II, flutriafol, heptachlor epoxide, linuron, monocrotophos, paraoxon, paraoxon methyl, pyrazophos, quinalphos and triazophos), the LOD was set at 0.05 μg/l. For captan, carbofenothion, decamethrin, demeton-S-methyl sulphone, fensulfothion, deisopropylatrazine (G-28) and metamitron, the LOD was set at 0.1 μg/l and only for chloridazon and tetradifon was the respective LOD level set at 0.5 μg/l. Sample chromatograms with respective mass spectral data of analytes with LODs better than 0.005 and 0.01 μg/l are shown in Fig. 2 and Fig. 3, respectively. At $S/N \geq 3$, the spectral fit of sought analytes with respective library spectral data of better than 850 was obtained.

Certainly, care was taken to avoid interference during the quantitation of poorly resolved analytes or of analytes co-eluted with matrix components. In order to minimise the effect of matrix components on the precision of the quantitation process of pesticides in foods, the preparation of calibration standard solutions in control sample extracts was recommended [15]. However, in the present study such a measure was not necessary since the matrix interference during analysis of the different field water samples was very limited and in the few cases of such interference, the effect was minimised by simply selecting fragment ions that were not present in the spectra of the matrix components, as quantitation masses for these analytes.

The LOD values reported in Table 2 are comparable to, and in some cases are even better than, those previously reported for twenty pesticides extracted from surface waters by SPE, using mixed bed XAD-2/XAD-7 resin columns and analysed by GC–IT-MS, the MS being operated in the CI mode [10]. However, in the above study the LOD levels were set at $S/N > 5$, whereas in the present work the finger-print structural information of the EI spectra allowed the reliable identification of analytes, based on full scan mode (sixteen major ions), at $S/N \geq 3$, or even less. The lower sensitivity attained at the EI mode, due to higher fragmentation of the analytes, was compensated for by the increased confirmatory structural information of the EI mass spectra.

3.4. Ion trap–electron impact ionisation mass spectra of the main chemical groups of pesticides under GC (GC–IT-EI-MS)

The EI-MS fragmentation pattern of the analytes investigated here was the same as that previously reported in numerous publications. However, the relative abundance of the different fragments was slightly (and occasionally significantly) different in the IT-EI-MS spectra compared to respective spectra taken by other than ion trap mass spectrometers [4,5,30–33].

To the best of our knowledge, a comprehensive study of the IT-EI-MS spectra of pesticides has never been reported and therefore the IT-EI-MS spectra taken under GC conditions, due to the lack of reference data for absolute comparisons, had to be carefully examined and fragmentation patterns had to be structurally related to the eluted analytes in order to avoid misidentification due to matrix effects and decomposition of analytes during sample handling and chromatography. To exemplify this, a simple case concerning the IT-EI-MS spectra of the organophosphorus pesticides included in this study will be briefly discussed. The typical EI-MS fragmentation pattern of the organophosphorus pesticides [30–33] was present in the IT-EI-MS spectra of the compounds investigated here, however, there were sig-

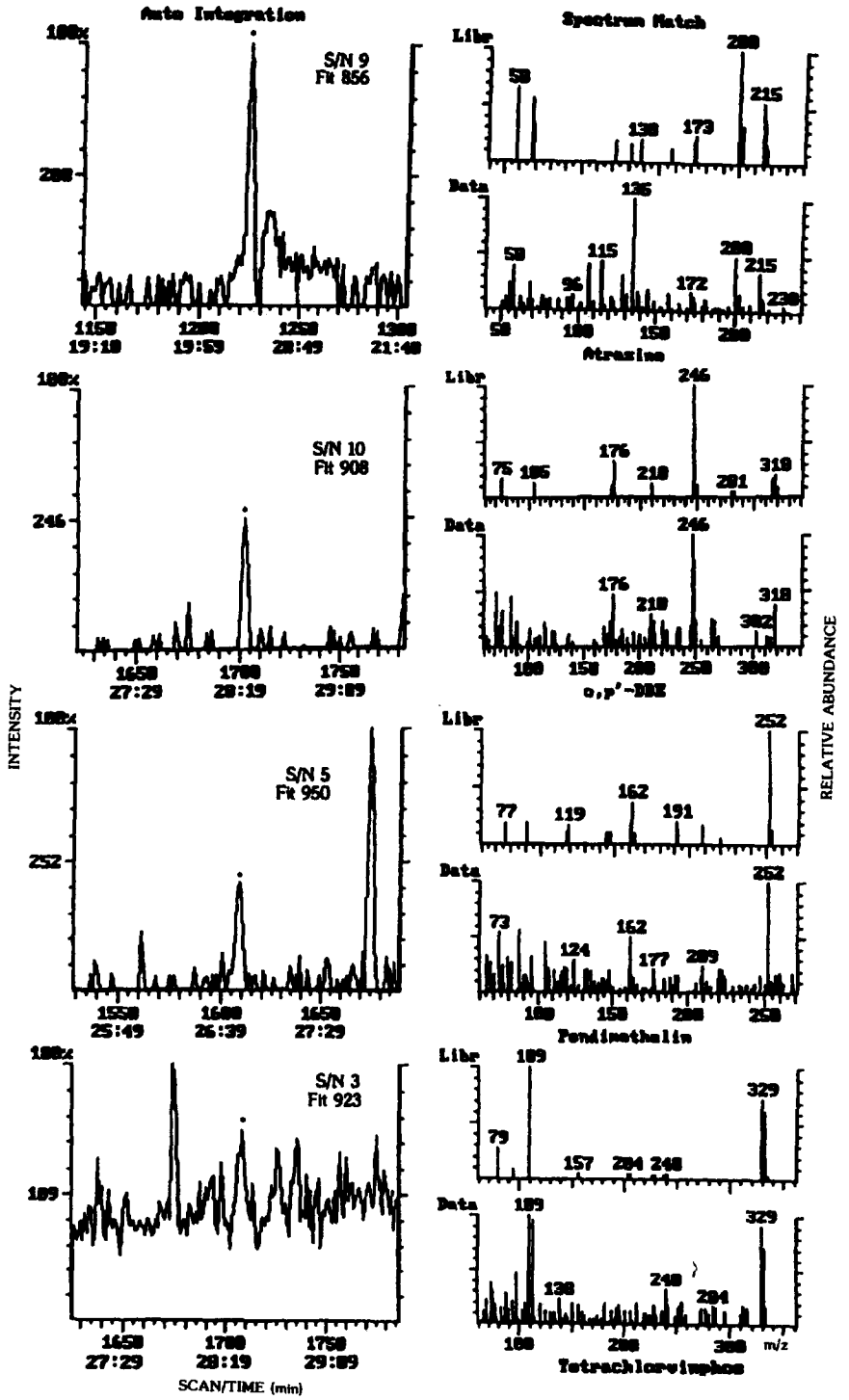

Fig. 2. Sample data from the GC–IT-EI-MS analysis of water samples fortified at the 0.005 μg/l level. For other conditions see Section 2.3. Left-half: x-axis as in Fig. 1 and y-axis, intensity of the quantification ions. Right-half: x-axis, mass (m/z) and y-axis, relative adundance of ions.

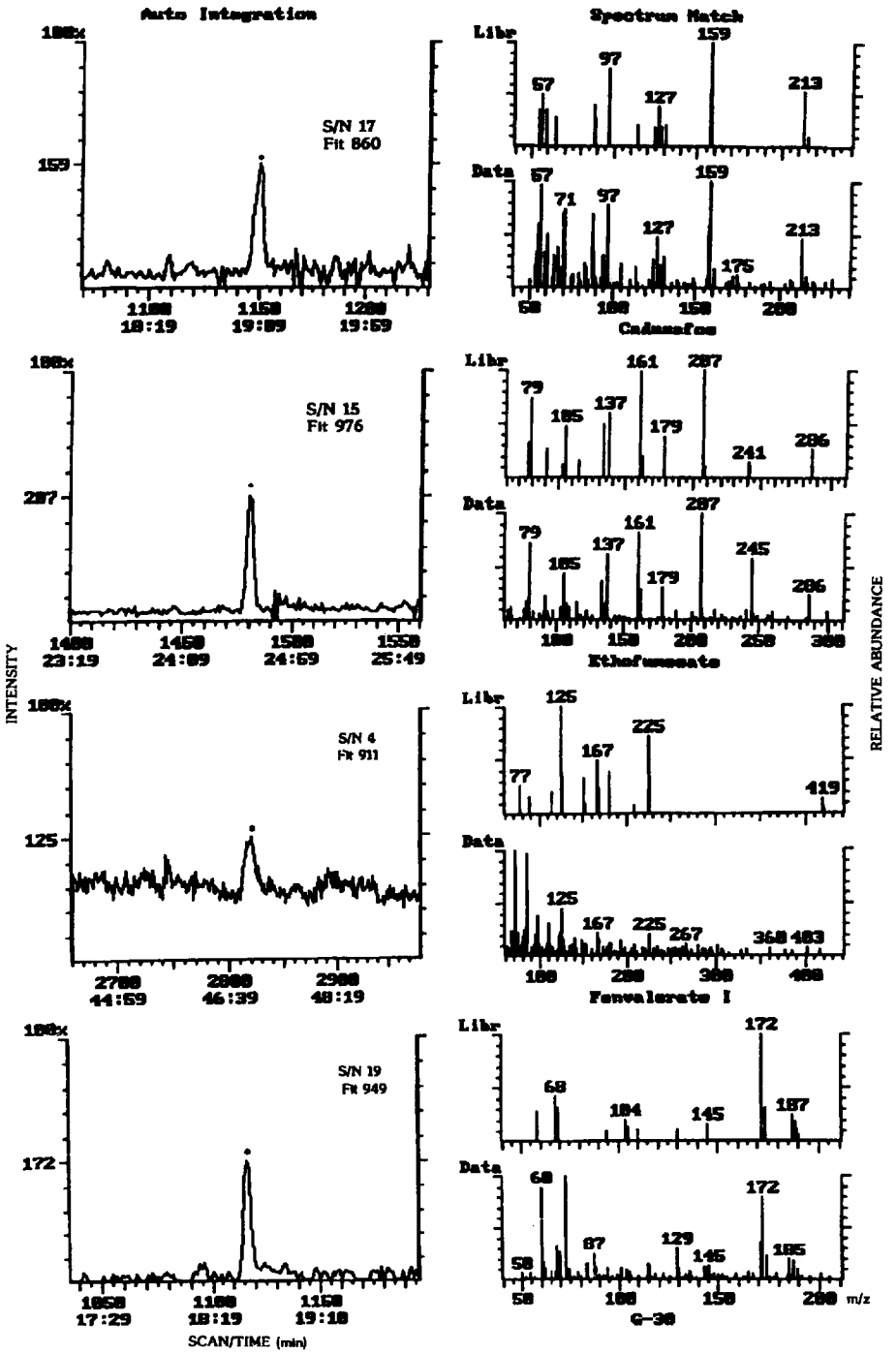

Fig. 3. Sample data from the GC–IT-EI-MS analysis of water samples fortified at the 0.01 μg/l level. For other conditions see Section 2.3. x- and y-axes, as in Fig. 2.

nificant differences in the relative abundance of the so-called typical fragments. For instance, there was a significant difference in the relative abundance of the ions with m/z 125 and 109, reported as being the characteristic and diagnostic base peaks in the EI-MS spectra of O,O-dimethyl phosphorothionates and O,O-dimethyl phosphates, respectively [33]. However, among the O,O-dimethyl phosphorothionates (fourteen compounds) and O,O-dimethyl phosphates (six compounds) investigated under GC–IT-EI-MS, the ions with m/z 125 and 109 were base peaks in the spectra of fenitrothion, malathion and methacrifos and in the spectra of demeton-S-methyl sulfone, paraoxon methyl and tetrachlorvinphos, respectively.

Another aspect concerning the IT-EI-MS spectra of the investigated compounds was the presence of $[M+1]^+$ ions, due to self-CI or self-protonation, in the spectra of a few analytes, such as carbaryl (this is not included in the target analyte list), carbofuran, molinate, terbutryn, terbumeton, and metobromuron. However, it should be mentioned that the relative abundance of $[M+1]^+$ ions in the spectra of the above-mentioned compounds was very low and it did not affect the precision of the quantitation process, since in neither case were $[M+1]^+$ ions used as quantitation masses.

3.5. Pesticide monitoring studies in the Axios River basin, Macedonia, Greece

The proposed system was evaluated with the analysis of field water samples taken from surface and ground aquatic systems of the Axios River basin. In Fig. 4a,b sample data from the analysis of a surface water sample collected from the Axios River at the Greek/FYROM border, are shown. In this sample, molinate, trifluraline, α-BHC and carbofuran were present at 0.03, 0.01, 0.08 and 0.01 μg/l, respectively (Fig. 4a) and β-BHC, γ-BHC (lindane) and propanil were present at 0.02, 0.04 and 0.01 μg/l, respectively (Fig. 4b). The residue levels (mean$\pm$S.D.) of these pesticides at this sampling site of the Axios River during 1994 were 0.05$\pm$0.05 (molinate), 0.01$\pm$0.01 (trifluraline), 0.10$\pm$0.10 (α-BHC), 0.91$\pm$1.07 (carbofuran), 0.03$\pm$0.03 (β-BHC), 0.04$\pm$0.04 (lindane) and 0.29$\pm$0.43 (propanil) μg/l. Sampling was carried out twice per month and in each sample more than three compounds, of the target analytes of this method, were identified.

The same method was used for monitoring pesticides and their conversion products in soil water samples of the Axios River basin. In this case, soil water samples were taken by use of porous suction cups installed in different fields. In a sample taken from a 160-cm soil depth of a corn field, deethylatrazine (G-30), atrazine, carbofuran and lindane at 0.22, 0.23, 0.15 and 0.54 μg/l level, respectively, were determined. The type of pesticides and the respective residue levels present in this type of water sample varied depending upon the crop, the agricultural practices, the soil depth and the time of sampling. For instance, in a soil water sample taken from a depth of 110 cm in a field where cotton was grown, trifluraline, carbofuran, prometryne and isofenphos were determined to be present at levels of 0.03, 0.34, 0.97 and 0.09 μg/l, respectively.

To further validate the GC–IT-MS system, aliquots of field water samples were also analysed by an on-line SPE–HPLC–DAD system [18]. Sample data are presented in Table 3. In this sample, atrazine, propanil and molinate were identified by both systems and the respective concentrations determined were in good agreement with each other. In addition, prometryne and alachlor were determined in the same sample by the GC–IT-MS system at levels lower than the respective LOD levels of the HPLC–DAD system. The complete data derived from pesticide monitoring studies, utilising the proposed method, will be discussed elsewhere.

4. Conclusions

A rapid multiresidue method using GC–IT-MS, the MS operated in the EI mode, and off-line SPE of water samples has been developed for the analysis of a wide chemical range of pesticides and important conversion products in field water samples. For the majority of the analytes, identification in full scan mode with $S/N \geq 3$ was possible, when analytes were present at levels of 0.01 to 0.005 μg/l. However, for about one quarter of the analytes, the respective LOD levels were in the 0.1 to 0.05 μg/l range and only the LOD level of chloridazon and tetradifon

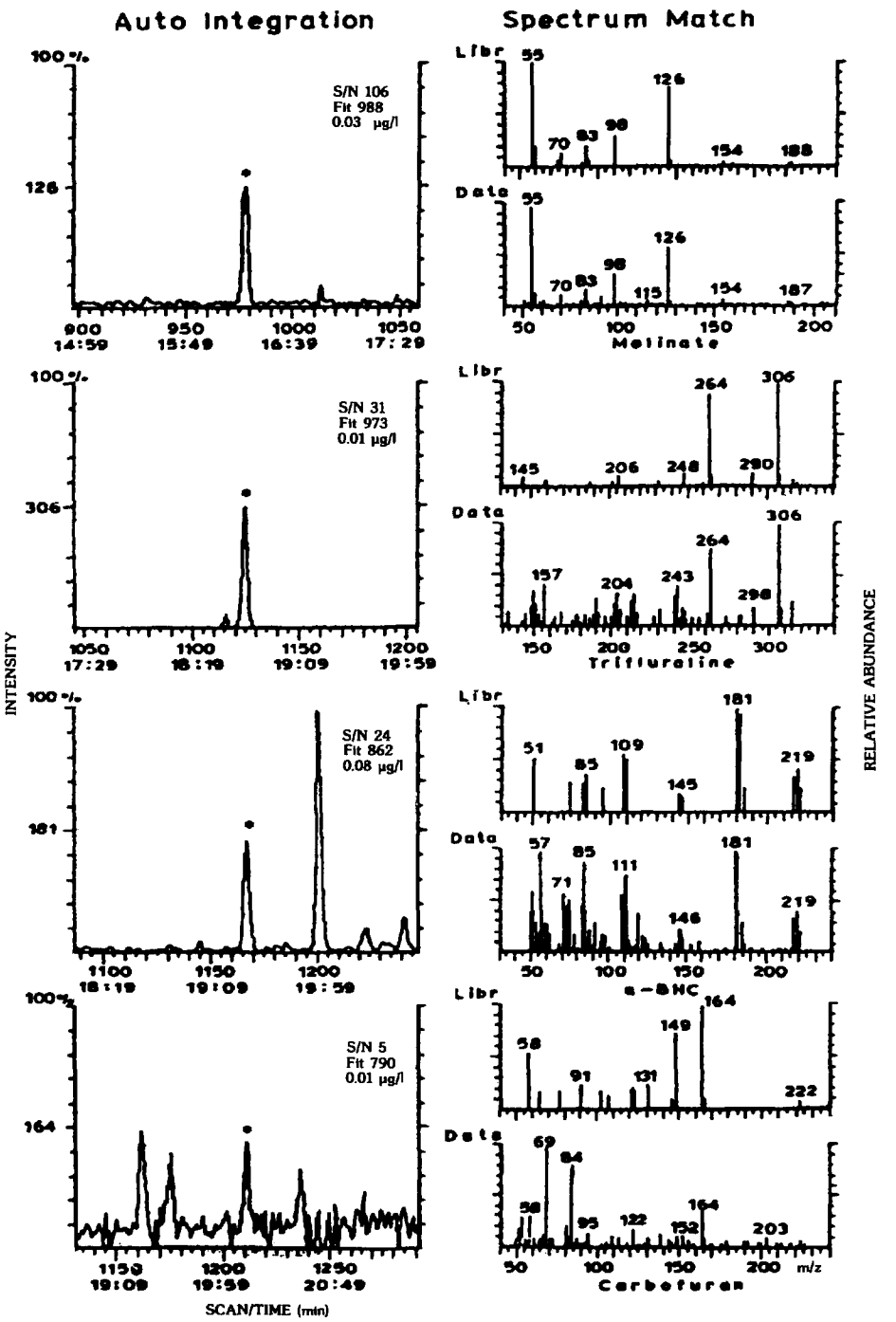

Fig. 4. (a) Sample data from the application of the proposed method for monitoring pesticides in the water of the Axios River at the Greek/FYROM border. The water sample was taken in July, 1994. For the conditions of analysis see Section 2.3; x- and y-axes are the same as in Fig. 2. (b) Sample data from the application of the proposed method for monitoring pesticides in the water of the Axios River at the Greek/FYROM border. This is part of the data derived from the analysis of the sample reported in Fig. 4a. See conditions of analysis in Section 2.3; x- and y-axes are the same as in Fig. 2.

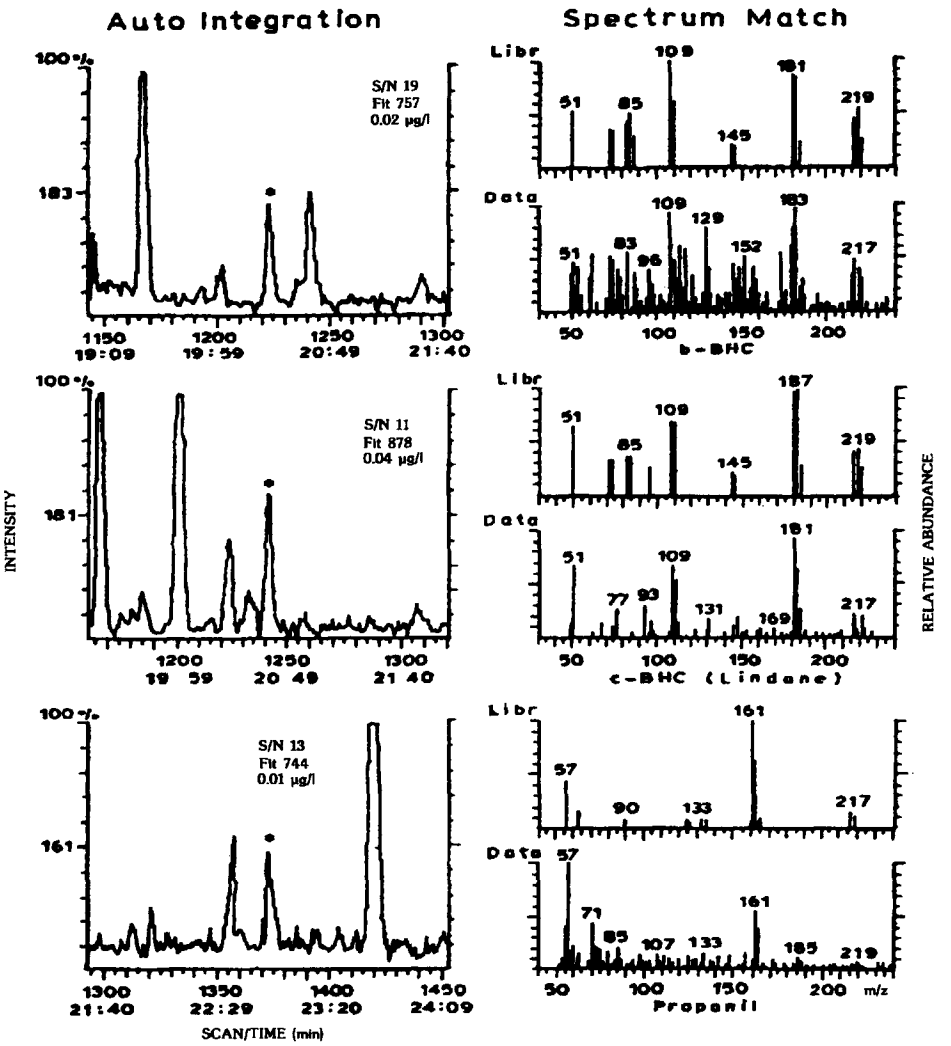

Fig. 4. (*continued*)

Table 3
Comparative pesticide residue[a] data derived from the analysis of a field water sample of the Axios River basin analysed by both on-line SPE–HPLC–DAD and off-line SPE, followed by GC–IT-MS

Pesticide	On-line SPE–HPLC–DAD	Off-line SPE–GC–IT-MS
Atrazine	0.16	0.17
Propanil	0.14	0.12
Molinate	1.08	0.92
Prometryne	ND	0.01
Alachlor	ND	0.02

[a] Residue levels are expressed in $\mu g/l$ and they are not corrected for recoveries.
ND denotes not detectable.

was higher. For some analytes, the low detection limits were primarily due to ineffective extraction of these analytes from water samples, the respective recovery values being <50%, while for only a few others (captan, chlorothalonil, tetradifon) the intrinsic mass fragmentation pattern was the decisive factor for the low detection limits.

Acknowledgments

This work was supported by a grant from European Community, Proj. No. EV5V-CT92-0105 and

the Greek Ministry of Agriculture via the Interreg Program.

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