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Automated recognition of target compounds at low levels in environmental samples by means of capillary gas chromatography–mass spectrometry with dedicated mass spectral libraries and the macro program AUTARG

II. Application to pesticides in groundwater samples

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ABSTRACT

The use of the macro program AUTARG in the daily routine analysis of pesticides in groundwater was investigated. AUTARG Level 1 proved to be a valuable and reliable tool for the automated evaluation of GC–MS data. It is able to replace time-consuming manual evaluation by providing similar reliable results. AUTARG Level 2 is a powerful addition to Level 1, especially in trace level analysis, when looking for specific compounds using dedicated control files. It has been proved that the use of ion traces by Level 2 makes possible the detection of target compounds hidden in the chromatographic background. In our investigations, using an older GC–MS system, it has been shown that the limits of AUTARG are determined by the detection limits. Today, new GC–MS systems promise much lower detection limits. Using AUTARG for automated evaluation of scan chromatograms to analyse water samples according to the tolerances for drinking water of the European Community should present us with no problems.

INTRODUCTION

In Part I [1] of this paper we described the macro program AUTARG, which is designed to reduce the workload of analysts by automating the evaluation of full scan GC–MS chromatograms.

In this part, we wish to demonstrate the merits of this program in daily routine water analysis. The program was applied to a series of groundwater samples, which were screened for the most relevant pesticides. The study was performed with groundwater samples from the Berlin area, which usually contain a considerable amount of interfering humic substances. Therefore, recoveries were analysed for each individual water sample. The three classes of pesticides used for the recoveries were: (1) chlorinated hydrocarbons, (2) triazines and similar nitrogen-containing pesticides and (3) phenoxycarboxylic acids and other acidic herbicides.

In this paper we present a few examples from these field studies, in order to demonstrate the

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potential and limits of automated chromatogram evaluation using AUTARG Level 1 and AUTARG Level 2.

EXPERIMENTAL

Material

All pesticide standards were of analytical purity, purchased from Promochem, Wesel, Germany, or Pestanal quality from Riedel de Haen, Seelze, Germany. Sample vials, screw caps and septa were purchased from Zinsser, Frankfurt, Germany. Inserts of 200 μl for the sample vials were obtained from CS-Chromatographie Service, Langerwehe, Germany. Stock solutions of all compounds were prepared in toluene or methanol. Standards and samples were finally dissolved in toluene. All solvents were Pestanal products from Riedel de Haen. Pentafluorobenzylbromide was obtained from Aldrich, Steinheim, Germany. Triethylamine was purchased from Merck, Darmstadt, Germany. Solid-phase extraction (SPE) cartridges, 6 ml (polypropylene), and RP-18 material were obtained from Baker, Frankfurt, Germany. Adjustable transferpettors (1–10 μl and 10–100 μl) were from Brand, Wertheim, Germany.

Sample preparation

Chlorinated hydrocarbons and triazines (recoveries). The water samples (1 l) were spiked with the mixture of pesticides to achieve a concentration of 100 ng/l of each substance, and then extracted by liquid–liquid partition with 50 ml of dichloromethane. The neutral extract was evaporated and separated into two fractions by chromatography on small silica gel columns. The extracts were finally dissolved in 100 μl of toluene.

Phenoxycarboxylic acids (recoveries). A water sample of 1 l was spiked with a mixture of pesticides to achieve a concentration of 100 ng/l of each substance. The internal standard 2,4-dichlorobenzoic acid was added at twice the concentration level. The sample was then acidified to pH <2 with HCl. Each SPE cartridge was filled with 2 g of RP-18 adsorbent. Conditioning was performed successively with 5 ml of dichloromethane, 5 ml of methanol and finally 5 ml of distilled, deionized water. The solvents were drawn through the cartridges by means of a gentle vacuum and the cartridge was not permitted to run dry after addition of the water. The water sample spiked with the herbicides was then percolated through the cartridge at a flow-rate of *ca.* 8 ml/min. After drying the cartridge for 2–3 h under a gentle stream of nitrogen, the herbicides were eluted with 3 ml of dichloromethane and 5 ml of methanol. The eluate was dried under a gentle stream of nitrogen.

Derivatization was performed at 90°C using 200 μl of pentafluorobenzylbromide (2% in toluene) and 2 μl of triethylamine as catalyst. The derivatized sample was then dried under nitrogen and finally dissolved in 100 μl of toluene.

GC–MS parameters

All MS measurements were performed with an HP 5970 mass-selective detector combined with an HP 5890 gas chromatograph fitted with a 25 m × 0.2 mm I.D. × 0.33 μm HP-5 capillary column. The oven temperature was maintained at 100°C for 1 min following injection, then programmed at 30°C/min to 150°C, which was held for 1 min, then at 3°C/min to 205°C followed by 10°C/min to 260°C, which was held for 23 min. The injector and transfer line temperatures were 210 and 250°C, respectively, and 2-μl quantities of sample were injected by means of an HP 7673 autosampler using hot splitless injection with the split closed for 0.9 min.

Scan parameters: scanned mass range, 50–510; scan rate, 0.93 scans/s; solvent delay, 6 min.

Hardware and software requirements

These were as described in Part I [1].

RESULTS AND DISCUSSION

Since the tolerance for drinking water was fixed by the European Community Commission (EEC) and since it has been established by the individual European member states, it has become general practice to keep these tolerances in mind when developing methods for residue analysis in either groundwater or surface water. Therefore in our study, standard mixtures of

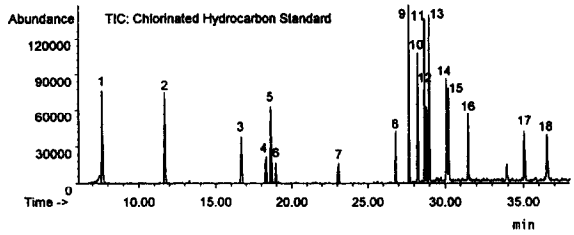

Fig. 1. TIC of the chlorinated hydrocarbon mixture containing eighteen pesticides (each at 1 ng/μl) as listed in Table I.

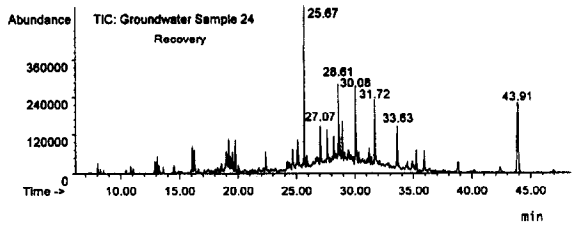

Fig. 2. Recovery of groundwater sample (No. 24) spiked with 100 ng/l of the chlorinated hydrocarbon mix.

pesticides considered relevant in water contamination were applied at a concentration level of 100 ng/l for recovery studies. Because of the detection sensitivity of a GC–MS system applying cyclic scanning, this is a demanding goal, as can be seen from the chromatograms of the standard pesticide mixtures. According to our lengthy experience of pesticide analysis, the recognition of 2 ng of pesticide injected, by means of library search, is a satisfactory result in routine analysis. This cannot, however, be expected to be achieved with all the 400 pesticides documented in the HPPEST Library [2].

Chlorinated pesticides

In Fig. 1, the total-ion current chromatogram (TIC) of a standard mixture of eighteen pesticides (1 ng/μl) is shown. Each standard was recognized, as shown in Table I.

Fig. 2 shows the chromatogram of a groundwater sample spiked with this standard mixture at a concentration of 100 ng/l. An analyst

TABLE I

RESULTS OF THE ANALYSES OF THE CHLORINATED HYDROCARBON STANDARD MIXTURE AND THE RECOVERY OF SAMPLE 24 BY MEANS OF AUTARG LEVELS 1 AND 2

Numbers in columns Level 1 and Level 2 indicate the match quality of the pesticide found by automated library search. HCH = Hexachlorocyclohexane.

Pesticide	Peak No.	Expected t_R (min)	Chlorinated hydrocarbon standard			Recovery (sample 24)			
			Level 1	Level 2	t_R (min)	Level 1	t_R (min)	Level 2	t_R (min)
Dichlobenil	1	7.65	91	91	7.65	Not id.	–	Not id.	–
Pentachlorobenzene	2	11.70	91	91	11.70	Not id.	–	Not id.	–
α-HCH	3	16.69	86	86	16.68	Not id.	–	56	16.62
β-HCH	4	18.30	34	34	18.30	Not id.	–	50	18.25
Lindane	5	18.65	45	45	18.64	78	18.56	72	18.56
Quintozene	6	18.97	72	72	18.98	Not id.	–	Not id.	–
Heptachlor	7	23.09	83	83	23.09	Not id.	–	Not id.	–
Heptachlorepoxid-*trans*	8	26.83	58	58	26.83	Not id.	–	47	26.75
o,p-DDE	9	27.70	99	96	27.70	96	27.64	96	27.64
Chlorfenson	10	28.26	94	94	28.26	91	28.21	91	28.21
p,p-DDE	11	28.71	99	99	28.71	Not id.	–	99	28.66
Dieldrin	12	28.83	53	89	28.83	47	28.76	47	28.77
o,p-DDD	13	29.00	96	96	29.00	50	28.94	96	28.94
p,p-DDD	14	30.11	64	64	30.11	Not id.	–	58	30.05
o,p-DDT	15	30.25	91	91	30.25	Not id.	–	90	30.19
p,p'-DDT	16	31.50	90	90	31.50	Not id.	–	Not id.	–
Tetradifon	17	35.08	94	90	35.08	Not id.	–	32	34.98
Mirex	18	36.54	78	83	36.54	Not id.	–	Not id.	–

performing manual evaluation usually starts by checking the largest peaks, which in Fig. 2 are labelled by their retention times. Comparing the abundance of these peaks with those of the standard mixture makes it clear that the pesticides are to be found among the smaller peaks. Using automated integration, a total of 64 peaks were integrated, which would make manual evaluation a time-consuming task. AUTARG Level 1 has shortened this procedure to a few minutes, recognizing five out of eighteen pesticides, even though these are among the smaller peaks, as documented in Table II. When not checking recovery samples, there is the risk of overlooking these small pesticide peaks because the analyst does not expect any particular pesticide in the sample.

AUTARG Level 2 using a control-file with its dedicated search for chlorinated hydrocarbons succeeded in recognizing twelve pesticides. This example illustrates the merits of Level 2: firstly, the recognition of peaks covered by matrix compounds and, secondly, the recognition of peaks due to the much better signal-to-noise ratio found with single-ion chromatograms. It is obvious that advantage can be taken of this fact when searching for defined pesticides. The results of the evaluation of the spiked groundwater sample No. 24 obtained by automated library searching using first AUTARG Level 1 and then the AUTARG Level 2 program is also compiled in Table I.

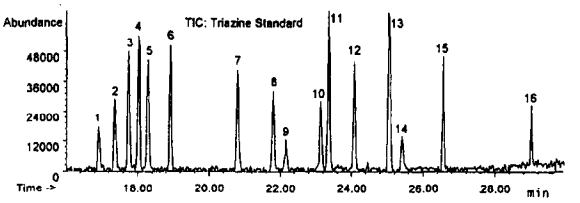

Fig. 3. TIC of the triazine standard mixture (each at 1 ng/μl).

Triazines

The performance of AUTARG was next tested using a standard mixture of sixteen triazines. Fig. 3 shows the TIC of the triazine standard mixture containing 2 ng of each pesticide injected. Fifteen out of the sixteen pesticides were identified with the "Autointegration" option of the integration software. However, the small peak labelled 9 was not recognized in the inte-

TABLE II

RESULTS OF THE ANALYSES OF THE TRIAZINE STANDARD MIXTURE AND THE RECOVERY OF GROUNDWATER SAMPLE 23 BY MEANS OF AUTARG LEVELS 1 AND 2

Pesticide	Peak No.	Expected t_R (min)	Triazine standard mixture			Recovery (sample 23)			
			Level 1	Level 2	t_R (min)	Level 1	t_R (min)	Level 2	t_R (min)
Simeton	1	16.93	78	78	16.93	Not id.	–	Not id.	–
Atraton	2	17.37	91	91	17.38	Not id.	–	58	17.36
Prometon	3	17.76	91	97	17.76	68	17.73	91	17.76
Atrazine	4	18.04	95	95	18.04	38	18.00	53	18.01
Propazine	5	18.30	90	90	18.30	94	18.30	94	18.3
Terbuthylazine	6	18.93	94	94	18.93	94	18.90	97	18.9
Sebuthylazine	7	20.81	90	90	20.81	59	20.79	53	20.8
Desmetryn	8	21.81	78	78	21.82	Not id.	–	Not id.	–
Metribuzine	9	22.17	Not id.	40	22.17	Not id.	–	Not id.	–
Ametryn	10	23.14	72	64	23.15	46	23.13	76	23.12
Prometryn	11	23.37	95	95	23.37	90	23.35	90	23.35
Terbutryn	12	24.09	40	40	24.09	7	24.06	53	24.08
Metolachlor	13	25.06	78	78	25.06	50	25.04	59	25.03
Triadimefon	14	25.43	Not id.	Not id.	–	32	25.38	32	25.38
Metazachlor	15	26.57	90	83	26.57	Not id.	–	Not id.	–
Methoprotryne	16	29.03	72	64	29.03	Not id.	–	Not id.	–

gration procedure, because it was excluded by the automatic threshold setting. While this peak was correctly identified with AUTARG Level 2 as metribuzine, neither AUTARG Level 1 nor Level 2 was able to find triadimefon under peak 14. When investigating the mass spectrum manually, however, several ions characteristic of triadimefon can be revealed, namely m/z 208, 181 and 57. In this particular case, the problem is not with the AUTARG program but with the search software, which for some reason comes to the conclusion that the spectrum recorded does not suffice for a positive library search. Even multiple manual attempts did not reverse this unwanted result. The triadimefon case appears even more confused when examining the recovery sample produced with the same standard mixture added to groundwater containing many matrix compounds. In the gas chromatogram of this particular sample many matrix compounds elute at similar retention times to triadimefon but are well separated, so that AUTARG Level 1 and Level 2 both identified the small peak at 25.38 min as triadimefon.

This case is reported as a representative example of our daily routine work to illustrate the well-known problem in environmental trace analysis with GC–MS and cyclic scanning that trace compounds may be overlooked by unfavourable parameter setting. On the other hand, data processing and storage capacity makes it necessary to filter out unnecessary information from the acquisition signals.

The chromatogram of the triazine recovery sample (Fig. 4) is again dominated by peaks of matrix compounds (peaks labelled), even though AUTARG Level 1 is able to recognize ten out of sixteen pesticides. Once again the specific analysis of Level 2 gives a better result, recognizing eleven pesticides with mostly a better quality of library search than that of Level 1 (see Table II).

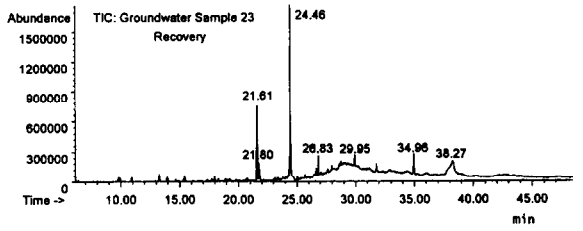

Fig. 4. TIC of groundwater sample No. 23, spiked with 100 ng/l of the triazine standard mixture.

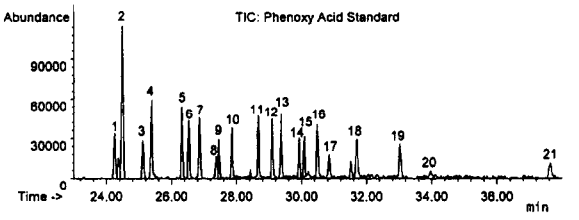

Fig. 5. TIC of the phenoxycarboxylic acid standard mixture (1 ng/μl each, except for the internal standard, which was 2 ng/μl).

Phenoxycarboxylic acids and other acidic herbicides

This group of pesticides has to be derivatized prior to GC analysis. With pentafluorobenzylic esters generated from the standard mixture, the chromatogram shown in Fig. 5 was produced. 2,4-Dichlorobenzoic acid (peak No. 2) was used as internal standard with a concentration twice as high as the other compounds. Fig. 6 shows a chromatogram for the ion trace m/z 181, which is typical of all pentafluorobenzylic esters, and also flurenol-butyl. Usually the ion at m/z 181 is base peak in the mass spectra of these compounds; flamprop is the only exception, with the ion at m/z 105 as base peak.

Analysing this group of pesticides using this type of GC–MS system, the nearness to the detection limit is plain to see from the chromatograms shown in Figs. 5–7. As can be deduced from Table III, 4 out of 21 compounds could not be identified by either AUTARG Level 1 or Level 2. The reason for this has already been

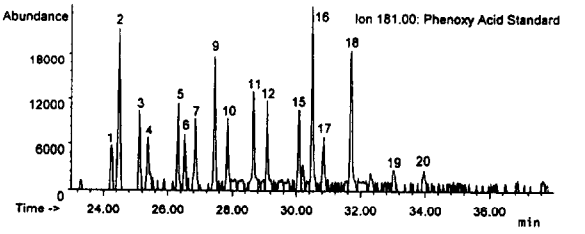

Fig. 6. RIC for m/z 181 of the phenoxycarboxylic acid standard, characteristic of all pentafluorobenzylic esters and flurenol-butyl.

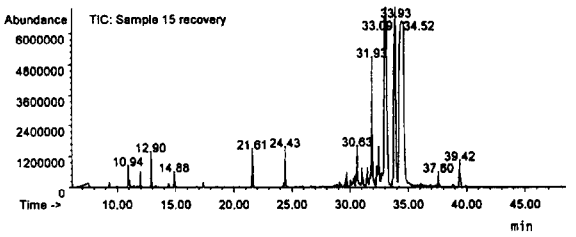

Fig. 7. TIC of the groundwater sample No. 15, spiked with 100 ng/l of the phenoxycarboxylic acid standard mixture and 200 ng/l internal standard.

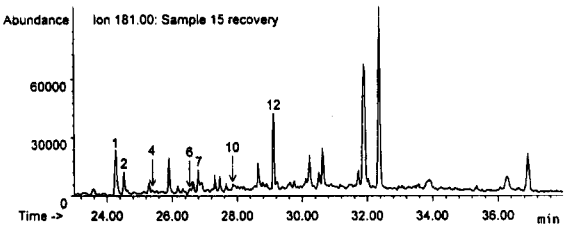

Fig. 8. RIC of m/z 181 of the recovery of groundwater sample No. 15.

referred to in the Triazines section. The compound peaks appear clearly in the chromatogram of the TIC (Fig. 5), but the mass spectra of these four compounds are not sufficient for a successful recognition in the mass spectral library. These spectra consist of 3–5 characteristic masses, but the information is obviously not sufficient for the library search to give positive results.

In Figs. 7 and 8, the TIC and the corresponding reconstructed ion chromatogram (RIC) for m/z 181 of the recovery experiment with groundwater sample No. 15 are shown. With AUTARG Level 1, not one single pesticide was

TABLE III

RESULTS OF THE ANALYSES OF THE PHENOXYCARBOXYLIC ACID STANDARD MIXTURE AND THE RECOVERY OF GROUNDWATER SAMPLE 15 BY MEANS OF AUTARG LEVELS 1 AND 2

2,4-DB = 4-(2,4-dichlorophenoxy) butyric acid; MCPA = 4-chloro-*o*-tolyloxyacetic acid; MCPB = 4-(4-chloro-*o*-tolyloxy)butyric acid.

Pesticide	Peak No.	Expected t_R (min)	Phenoxycarboxylic acid standard			Recovery sample 15			
			Level 1	Level 2	t_R (min)	Level 1	t_R (min)	Level 2	t_R (min)
2-(4)-Chlorophenoxy-2-methylpropionic acid	1	24.26	83	83	24.26	Not id.	–	98	24.28
2,4-Dichlorobenzoic acid	2	24.49	99	98	24.50	Not id.	–	96	24.53
Clopyralid	3	25.13	83	83	25.13	Not id.	–	Not id.	–
Mecoprop	4	25.39	97	97	25.40	Not id.	–	83	25.41
Dicamba	5	26.33	95	95	26.32	Not id.	–	Not id.	–
MCPA	6	26.54	94	94	26.54	Not id.	–	42	26.56
Dichlorprop	7	26.87	91	91	26.86	Not id.	–	58	26.87
Chlorflurenol	8	27.37	90	90	27.39	Not id.	–	Not id.	–
Flurenol-butyl	9	27.46	83	83	27.47	Not id.	–	Not id.	–
2,4-D	10	27.86	94	95	27.87	Not id.	–	50	27.88
Triclopyr	11	28.67	95	95	28.67	Not id.	–	Not id.	–
Fenoprop	12	29.10	91	91	29.11	Not id.	–	40	29.12
Fluazifop-*p*-butyl	13	29.38	94	94	29.38	Not id.	–	Not id.	–
Flammprop-isopropyl	14	29.94	Not id.	Not id.	–	Not id.	–	Not id.	–
2,4,5-T	15	30.09	83	91	30.09	Not id.	–	Not id.	–
MCPB	16	30.50	74	74	30.50	Not id.	–	Not id.	–
Fluroxypyr	17	30.86	72	83	30.87	Not id.	–	Not id.	–
2,4-DB	18	31.71	Not id.	Not id.	–	Not id.	–	Not id.	–
Fluazifop	19	33.04	59	59	33.03	Not id.	–	Not id.	–
Picloram	20	33.98	Not id.	Not id.	–	Not id.	–	Not id.	–
Flamprop	21	37.68	Not id.	Not id.	–	Not id.	–	Not id.	–

recognized, as can be deduced from Table III. Although the abundance of the pesticides is low and some peaks are covered by matrix compounds, with Level 2 seven out of 21 compounds were recognized. The limitation of positive identification by AUTARG Level 2 is again determined by the inadequacy of the mass spectral data passing the noise filter.

Fig. 8 demonstrates how AUTARG Level 2 works. In the RIC of ion trace m/z 181 the pentafluorobenzylates of the acidic pesticides appeared mostly as relatively small peaks but their integration was still possible. The compounds that were found by AUTARG Level 2 are labelled. The 2-(4)-chlorophenoxy-2-methylpropionic acid (peak No. 1) and the internal standard (peak No. 2) were hidden in the TIC by a phthalate peak at 24.43 min. In the reconstructed ion trace, the overlaying of this phthalate peak is not visible. Fenoprop, the large peak No. 12, is covered by a matrix peak in the TIC. The peaks representing three other pesticides (4, 6 and 10) seem to disappear in the noise. Since, as described in Part I, Level 2 works with two ion traces for each compound, the second trace may be even more characteristic, but is generally lower in abundance than mass 181. This example demonstrates the advantages of Level 2 in comparison with Level 1.

Finally, the results of analysing the compounds of three different pesticide classes by means of AUTARG Levels 1 and 2 are summarized in Table IV. The results do not only support our estimation of the value of these tools in daily routine but also demonstrate the differing detection limits observed with the various classes of chemical structures using GC–MS. The experimental approach applied in this study to demonstrate the merits of the macro program AUTARG is also useful for reviewing the detection sensitivity of any target compound group using full-scan mode, identifying those that absolutely require selected-ion monitoring (SIM) for achieving the detection sensitivity needed.

The results obtained using AUTARG for pesticide residue analysis also give a brief overview of the percentage of those pesticides that can be found in water samples above the tolerance level established by the EEC for drinking water when applying cyclic scan mode with our GC–MS system, which represents the first generation of mass selective detectors. All other pesticides or target substances not meeting these criteria have to be analysed separately by means of a SIM programme using defined time windows. This means not only additional analysis time depending on the number of target compounds, but also loss of information with a possible reduction in reliability.

As a consequence, for use in a field study now under way, in addition to the methods described here, a SIM method has been developed for phenoxyalkanoic acids [3] achieving much lower detection limits with the same instrumentation. Furthermore, sample preparation by solid-phase extraction has been improved, with excellent recovery rates for 30 acidic herbicides being reported [4]. This method used for routine analysis offers detection limits for these compounds in drinking water of 1–10 ng/l.

TABLE IV

RESULTS OF THE ANALYSES OF THREE PESTICIDE CLASSES AT A CONCENTRATION LEVEL OF 100 ng/l USING AUTARG LEVEL 1 AND AUTARG LEVEL 2

Classification	Number	Standard/recovery	AUTARG Level 1		AUTARG Level 2	
			No. identified	Identified (%)	No. identified	Identified (%)
Chlorinated hydrocarbons	18	Standard	18	100	18	100
		Recovery (sample 24)	5	28	12	67
Triazines	16	Standard	14	88	15	94
		Recovery (sample 23)	10	63	11	69
Phenoxycarboxylic acids	21	Standard	17	81	17	81
		Recovery (sample 15)	0	0	7	33

CONCLUSIONS

AUTARG Level 1 is a valuable and reliable tool for the automated evaluation of GC–MS data. It replaces the time-consuming manual evaluation by providing similar reliable results. It can be performed immediately after data acquisition using the time elapsed before the next injection.

AUTARG Level 2 is a powerful addition to Level 1, especially in trace level analysis, when looking for specific compounds by using dedicated control files. It has been proved that the use of ion traces by Level 2 makes possible the detection of target compounds hidden in the chromatographic background. This is because the signal-to-noise ratio is much better than that of a TIC and, secondly, because the ion traces suppress overlaying matrix compounds, so that hidden target compound peaks can be found.

In our investigations using an older GC–MS system it has been shown that the limits of AUTARG are determined by the detection limits. Today, new GC–MS systems promise much lower detection limits. Using AUTARG for automated evaluation of scan chromatograms to analyse water samples according to the tolerances for drinking water of the European Community should present the analyst with no problems.

REFERENCES

1 H.-J. Stan and F. Schwarzer, *J. Chromatogr. A*, 653 (1993) 45.
2 H.-J. Stan and J. Lipinski, *HP Pesticide Library*, Hewlett-Packard Company, Palo Alto, USA (1989).
3 Th. Heberer, S. Butz and H.-J. Stan, submitted for publication.
4 S. Butz, Th. Heberer and H.-J. Stan, submitted for publication.