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Journal of Chromatography A, 868 (2000) 229–247

JOURNAL OF CHROMATOGRAPHY A

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Comparison of gas chromatography–pulsed flame photometric detection–mass spectrometry, automated mass spectral deconvolution and identification system and gas chromatography–tandem mass spectrometry as tools for trace level detection and identification

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Received 8 September 1999; received in revised form 26 October 1999; accepted 28 October 1999

Abstract

The complexity of a matrix is in many cases the major limiting factor in the detection and identification of trace level analytes. In this work, the ability to detect and identify trace level of pesticides in complex matrices was studied and compared in three, relatively new methods: (a) GC–PFPD–MS where simultaneous PFPD (pulsed flame photometric detection) and MS analysis is performed. The PFPD indicates the exact chromatographic time of suspected peaks for their MS identification and provides elemental information; (b) automatic GC–MS data analysis using the AMDIS (''Automated Mass Spectral Deconvolution and Identification System'') software by the National Institute of Standards and Technology; (c) GC–MS–MS analysis. A pesticide mixture (MX-5), containing diazinon, methyl parathion, ethyl parathion, methyl trithion and ethion was spiked, in descending levels from 1 ppm to 10 ppb, into soil and sage (spice) extracts and the detection level and identification quality were evaluated in each experiment. PFPD–MS and AMDIS exhibited similar performance, both superior to standard GC–MS, revealing and identifying compounds that did not exhibit an observable GC peak (either buried under the chromatographic background baseline or co-eluting with other interfering GC peaks). GC–MS–MS featured improved detection limits (lower by a factor of 6–8) compared to AMDIS and PFPD–MS. The GC–PFPD–MS–MS combination was found useful in several cases, where no reconstructed ion chromatogram MS–MS peaks existed, but an MS–MS spectrum could still be extracted at the elution time indicated by PFPD. The level of identification and confirmation with MS–MS was inferior to that of the other two techniques. In comparison with the soil matrix, detection limits obtained with the loaded sage matrix were poorer by similar factors for all the techniques studied (factors of 5.8, >6.5 and 4.0 for AMDIS, PFPD–MS and MS–MS, respectively). Based on the above results, the paper discusses the trade-offs between detectivity and identification level with the compared three techniques as well as other more traditional techniques and approaches.

Keywords: Detection, GC; Pulsed flame photometric detection; Mass spectrometry; Soil; Pesticides

1. Introduction

Multi-residue screening of trace level pesticides in

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01138-3

complex matrices is one of the widespread applications in environmental laboratories. The common practice of such screening is to run the prepared samples on several gas chromatography (GC) systems equipped with selective detectors, and if suspicious peaks appear, perform a GC–MS analysis and try to identify the relevant peaks. Since mass spectrometry (MS) is a non-selective method, very often under complex matrix conditions and trace levels of the analyte, identification can not be accomplished by MS alone.

In such cases, where standard GC–MS fails, several other approaches have been adopted. This included running the MS system in the time-shared selected ion monitoring (SIM) mode or incorporating GC retention times for identification. Another approach was incorporating selective multi-element detection either with several detection methods such as NPD (nitrogen–phosphorus detection)–ECD (electron-capture detection) [1] or ECD–FID (flame ionization detection) [2] combinations or with atomic emission detection (AED) [3].

During the last decade GC–MS–MS methods, mainly daughter ion scan, have also become a routine tool for trace level detection and identification of pesticides. With MS–MS, selectivity is obtained by rejecting matrix interference through the selection of the parent ion, while providing molecular specific information through the daughter ions' spectrum. Combined with the excellent sensitivity of ion-trap MS technology, this method enabled low ppb level informative detection under complex mixture conditions [4–6]. Several advantages of ion trap MS–MS have been found compared with the traditional selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) MS–MS techniques used in sector or quadrupole instruments [7]. MS–MS methods are molecular specific and thus can be applied for predetermined target compounds only.

AMDIS (''Automated Mass Spectral Deconvolution and Identification System'') is a relatively new software by the US National Institute of Standards and Technology (NIST) [8,9]. It rebuilds spectra from a GC–MS chromatogram, using the separate mass chromatograms and combining all the isolated mass peaks having the same retention and shape into a spectrum. AMDIS ignores all the other mass peaks appearing at the same elution time but have a different time profile (shifted, tailing or with no chromatographic peak pattern at all). The rebuilt spectra are then library searched in a target library. This strategy is very promising as an automatic tool for background subtraction and overlapping peaks deconvolution.

A different approach to combine detection and identification is to run a selective detector simultaneously with the MS, both connected in parallel to the end of the analytical column. van Stee et al. showed this in the coupling of AED in parallel with MS [10], Morello et al. used the NPD–MS combination [11], and Amirav and Jing demonstrated this with PFPD (pulsed flame photometric detection)–MS coupling [12]. This approach enables synchronized chromatograms of the specific detector and the MS to be recorded. The specific detector (which must be more sensitive than the full scan MS) indicates the exact elution time of a peak of a suspected compound, and accurate MS background subtraction can thus be obtained and uncover library searchable mass spectral information, even when no MS reconstructed ion chromatogram (RIC) peak exists. Furthermore, the elemental information provided by the specific detector can be incorporated in the MS library search (employing NIST sequential search) and substantially reduce the number of hits in marginal cases where the obtained background subtracted mass spectrum does not provide satisfactory search results [12].

In this work GC–PFPD–MS, AMDIS and GC–PFPD–MS–MS are compared, including several approaches for data processing and interpretation. The comparison relates to detection limits and identification quality, where other factors such as complexity and drawbacks of each method will be discussed as well.

2. Experimental

2.1. GC–PFPD–MS

The system used was the Varian Saturn 2000 GC–MS system (Varian, Walnut Creek CA, USA) equipped with the GC 3800 with a PFPD system. A drawing of the system configuration is shown in Fig. 1.

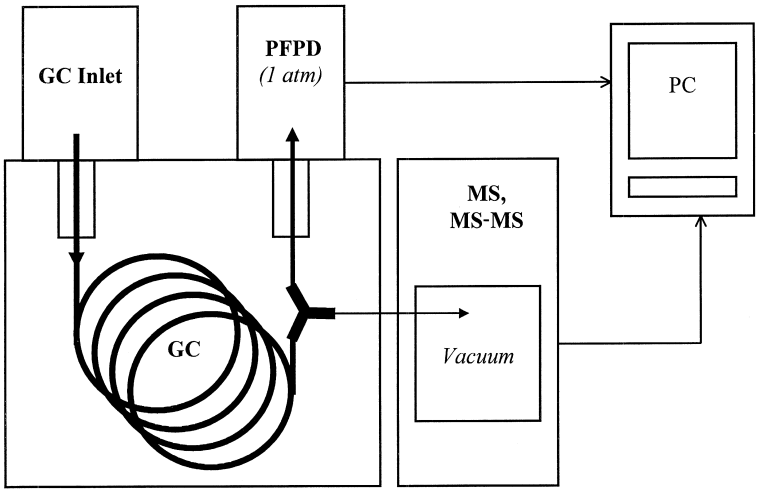

Fig. 1. GC–PFPD–MS system configuration on the Varian 2000 GC–MS system. A Y presstight connector splits the column flow equally into two different transfer lines to the MS and PFPD systems with almost simultaneous elution times (see Experimental section for details). PC=Personal computer.

The PFPD–MS coupling was achieved by using a Y presstight glass connector (Restek, Bellefonte, PA, USA), splitting the gas flow at the end of the analytical column to the MS and the PFPD systems. A 17 cm×100 μm I.D. transfer line from the Y connector to the MS system was calculated to produce a flow of about 1 ml/min, under the fixed pressure gradient of 1 atm between the Y connector and the vacuum in the MS system. Since the total column flow-rate was set to be constant at 2 ml/min, this resulted in an equal split between the MS and PFPD systems. The transfer line to the PFPD system was a short (about 25 cm long), 0.25 mm I.D. capillary, allowing minimum impedance between the Y connector and the PFPD system, which is operated at an atmospheric pressure. A slight, practically constant delay of ~0.02 min between the PFPD signal and the MS signal was corrected using the MS data analysis software. The glass Y connector was found to be superior to metal zero-dead-volume connectors that suffered from solvent memory effects. On the other hand, the Y glass connector can cause leaks and its installation requires some skill and caution.

The PFPD system was operated with a glass transparent filter and a wide gate (4.5 to 24.5 ms) to allow simultaneous screening for S and P containing compounds. The PFPD temperature was 270°C. A PFPD new data analysis software (courtesy of Y. Bao, Varian) acquiring and processing raw PFPD data, enabled one to determine whether a PFPD peak originated from P, S or P+S containing analytes.

The GC analytical column was a DB-5 one (J&W), 15 m×0.25 mm I.D., 0.25 μm film thickness. The carrier gas was helium at a constant flow-rate of 2 ml/min. The GC oven was programmed as follows: 110°C for 0.5 min, ramp at 80°C/min to 170°C, ramp at 25°C/min to 260°C, ramp at 60°C/min to 300°C for 1 min (total 6.5 min). Splitless manual injections of 2-μl volumes were performed.

The MS system was operated in the electron impact ionization (EI) mode both in MS and MS–MS experiments. The emission current was reduced from the typical value 10 μA to 5 μA to minimize protonation effects in the trap and improve MS library suitability. In order to improve the detection limits in very low analyte concentrations (low ppb level), especially in the MS–MS experiments, the voltage of the MS channeltron ion detector was raised from its software-optimized value of 2150 V (at this time) to 2400 V.

2.2. Samples

A MX-5 pesticide mixture (Nanogen) containing the compounds listed in Table 1 was spiked into two

Table 1
MS–MS optimized parameters for the MX-5 pesticide mixture (non-resonance mode)[a]

		Molecular mass	MS–MS parent	Major daughter ions (>10%)	Excitation amplitude (V)	Excitation storage level (V)
1	Diazinon	304	304	276, 179, 162	53	120
2	Methyl parathion	263	263	246, 233, 153, 136	47	100
3	Ethyl parathion	291	291	263, 235, 142, 114	32	100
4	Methyl trithion	314	314	313, 268, 157	26	120
5	Ethion	384	231	203, 175	52	100

[a] The underlined daughter ions have an abundance of more than 50%.

matrices, a soil extract (1 g/ml) and a sage (spice) extract (2 g/ml, diluted 1:3 in acetone). The experiment was performed in two levels of matrix complexity where sage is a very loaded one compared to the soil. The MX-5 mixture was spiked at eight concentration levels, descending in factors of 2 from 1 μg/ml down to 10 ng/ml into both matrices.

2.3. MS–MS optimization

EI-MS–MS experiments were performed in the non-resonance mode, where the excitation time was 20 ms. A five-segment time shared MS–MS method (daughter scan) was developed; the optimum parameters for each compound are given in Table 1.

MS–MS optimization was obtained using continuous sampling of standard solutions of the individual pesticides with the ChromatoProbe sample introduction device (Varian CSB, Walnut Creek, CA, USA). During the method development, Chemical ionization (CI) MS–MS was evaluated as well for some of the compounds exhibiting low or no molecular ions in EI (diazinon, ethion and methyl trithion). CI-MS–MS on the quasi-molecular ion proved to be less sensitive than EI-MS–MS on the molecular ion of diazinon and methyl trithion. With ethion, that demonstrated no molecular ion in EI, the m/z 231 fragment was chosen as the parent ion. All the other parent ions were molecular ions.

2.4. AMDIS processing

GC–MS files that were obtained under GC–PFPD–MS runs were processed with the AMDIS software. The software deconvolution parameters were chosen to be at the maximum software capabilities: ''*Two* adjacent peaks subtraction'', ''*High* resolution'' and ''*Very high* sensitivity''. No retention index was introduced and identification was based on searching a user-made library containing spectra of the five pesticides. In order to make broader library searches, complementary ''elimination'' searches were also performed on the ''Nistdrug'' (778 spectra), ''Nistfda'' (419 spectra) and ''Nisttox'' (1251 spectra) libraries provided with the AMDIS.

3. Results and discussion

Both the soil and sage matrices spiked at eight concentration levels underwent GC–PFPD–MS and MS–MS analyses. Further reprocessing, including AMDIS processing was performed with the data files obtained. The following graphic data is representative of the results, that are all summarized and discussed at the end of this section.

3.1. Soil

In Fig. 2, a typical PFPD–MS chromatogram of the MX-5 mixture spiked at a 50 ppb level in the soil extract matrix, is shown. At this level most of the MS RIC peaks of the five pesticides are buried in the matrix chemical background (bottom trace). On the other hand, the five pesticides were selectively detected by PFPD and resulted in the upper trace. A blow-up of the diazinon peak is shown in Fig. 3. MS background subtraction was performed on both sides of PFPD indicated elution time of the diazinon (flagged), and resulted in the library searchable spectrum. The search result was correct and the library spectrum of diazinon is shown at the bottom. The ''purity'' factor, achieved with the Varian library

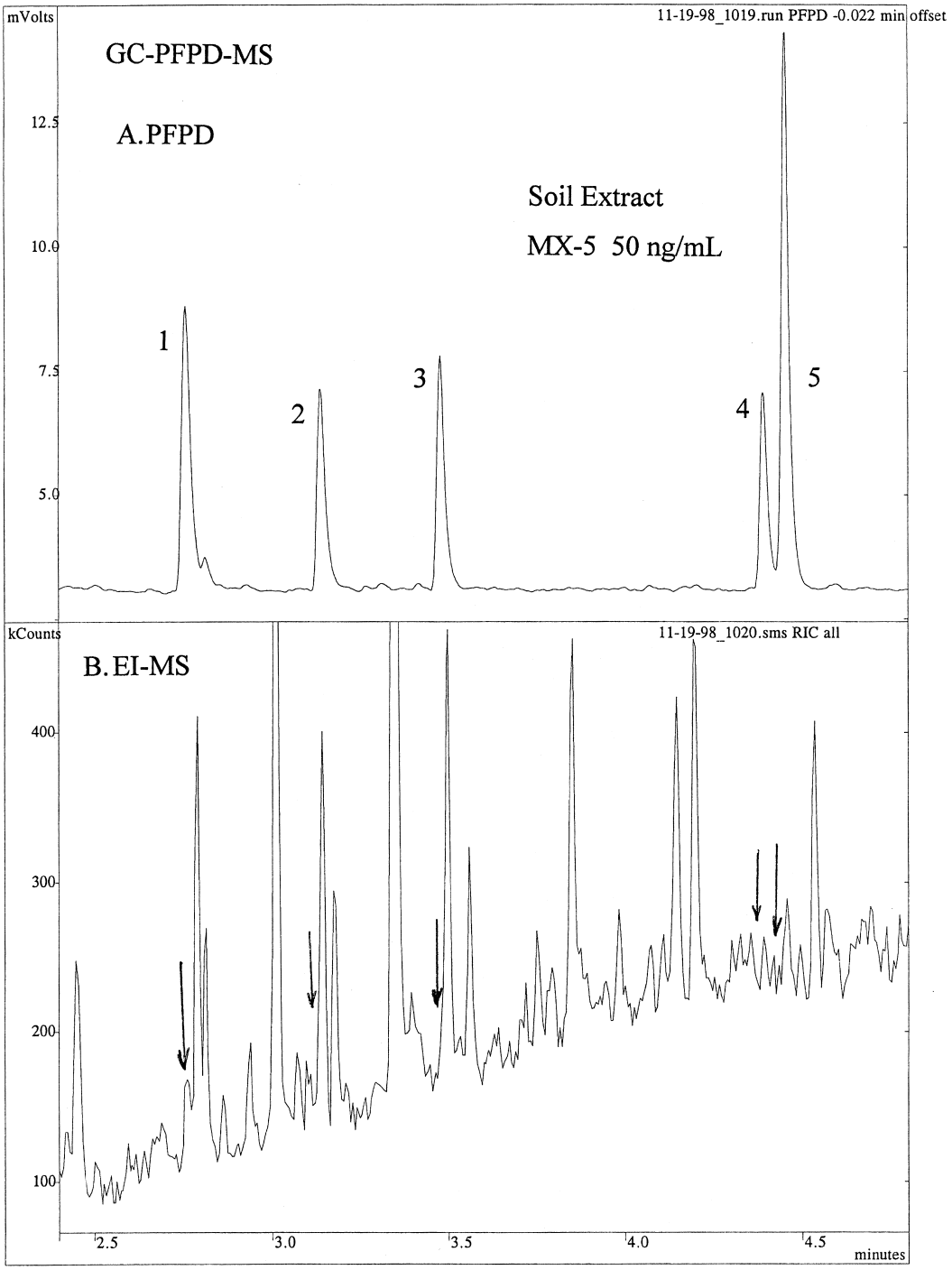

Fig. 2. GC–PFPD–MS of a spiked soil extract. At the spiking level of 50 ng/ml, clear indication of the five pesticides in the PFPD chromatogram is evident. However, in the EI-MS RIC the pesticide peaks are buried in the chemical background. The pesticides are: (1) diazinon, (2) methyl parathion, (3) ethyl parathion, (4) methyl trithion, (5) ethion.

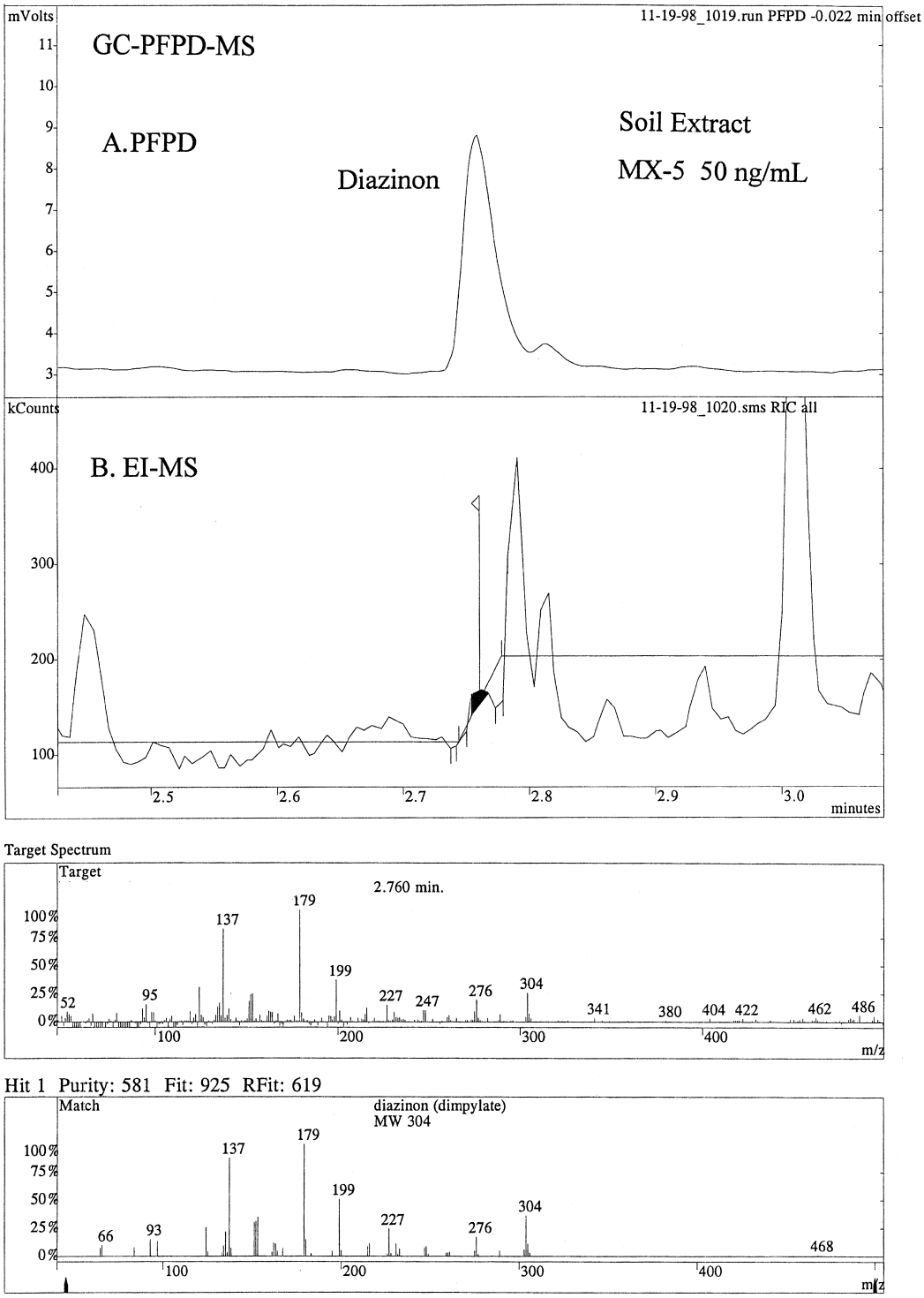

Fig. 3. GC–PFPD–MS detection and identification of diazinon in the soil extract – a blow up of Fig. 2. Careful background subtraction (marked by small vertical lines) around the exact elution time indicated by PFPD, uncovered the library (NIST) searchable spectrum of diazinon.

algorithm under the NIST92 database (~62 000 compounds) was 581, compared with ''user-made'' spectra obtained with a standard under the same experimental conditions. The criterion for a positive NIST search result was achieving a first hit for the correct compound, with a purity search factor above 500 (out of 1000).

The same GC–MS data file was loaded and run on the AMDIS software and the result is presented in Fig. 4. The upper chromatogram shows the re-combination of co-eluting typical mass chromatograms at *m/z* 137, 179 and 304. Other mass peaks that are not fully co-eluting were ignored by the software, and thus the complex mass spectrum (middle trace) could be simplified to the extracted spectrum shown at the bottom. The spectrum was automatically searched for in a dedicated user-made library, and diazinon was identified with a search net fit result of 71. The

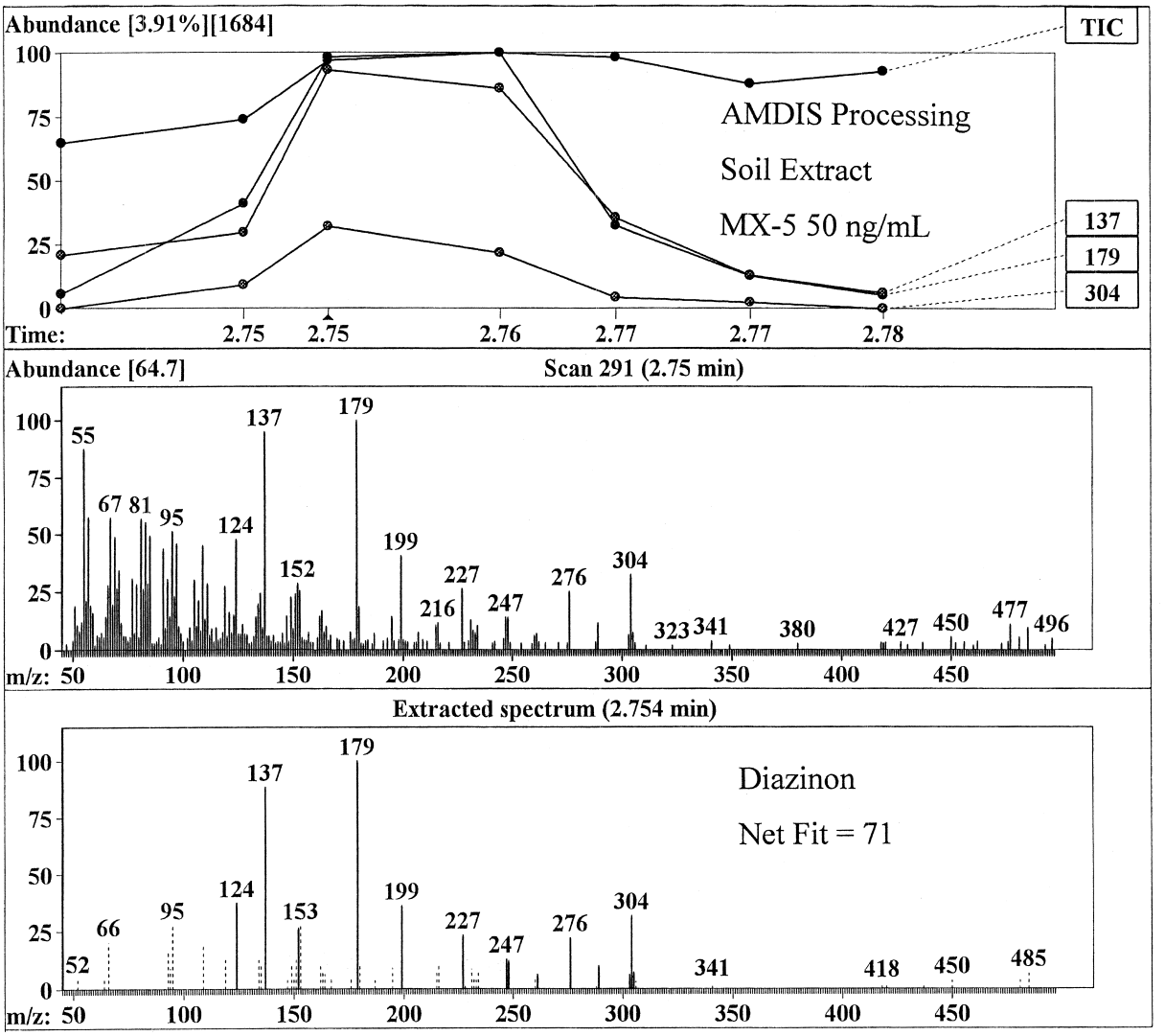

Fig. 4. AMDIS processing. The AMDIS software was run on the GC–MS file of the soil extract shown in Fig. 2. A combination of co-eluting mass chromatograms (such as the *m/z* 137, 179, 304) produced a library searchable diazinon spectrum. The AMDIS dedicated user-made library has been established from spectra acquired on the Varian GC–MS system.

criterion for a positive AMDIS identification was a net fit above 50 (out of 100) for the correct compound, with no fit above 50 for any other compound in other libraries available for search in the AMDIS.

In Fig. 5 the GC–PFPD–MS–MS of a lower level of 10 ng/ml spiked soil extract is shown. Again, the PFPD indicated the elution time of the suspected sulfur and phosphorus containing compounds. Some of the compounds, such as diazinon (1) exhibited peaks in the MS–MS RIC chromatogram, while other compounds did not demonstrate a clear RIC peak. Yet, the MS–MS spectra could be extracted by background subtraction around the PFPD indicated time, as shown in the blow-up in Fig. 6 for methyl parathion (2). In Fig. 6 the chromatogram of the daughter ion at m/z 246 is shown as well. Since MS–MS is always performed with known targets, the daughter ion chromatograms can serve also to allocate the MS–MS chromatographic peak. The MS–MS library spectra were constructed by injecting standards under the same experimental conditions. A library match under MS–MS experiment was considered positive if the purity was above 500 (out of 1000). With the low signal and poor ion statistics obtained under MS–MS analyses such as the one shown in Fig. 6, library compatibility was limited.

3.2. Sage

An example of a GC–PFPD–MS analysis with the sage matrix appears in Fig. 7. The chemical background level is 10–20-times higher than with the soil matrix. Thus the detection limits were affected and the representative example shown is with a high spiking level of 500 ng/ml. PFPD marked the pesticide peaks, as well as several natural sulfur-containing compounds in the matrix. The arrows indicate the exact elution of the five pesticides, which did not exhibit any noticeable RIC MS peak, some of them co-eluting with background dominant peaks [diazinon (1), ethyl parathion (3)]. The blow-up in Fig. 8 demonstrates again the use of PFPD for allocating the exact elution time of the suspected compound, ethion in this case. Background subtraction around this specific point, where no RIC peak existed, resulted in the library-identified spectrum of ethion (purity of 622). The AMDIS processing of the same GC–MS data file resulted in the identification of ethion with a net fit value of 68.

GC–PFPD–MS–MS measurements with the sage extract exhibited an increase in the background level for all the specific parent ions in the separate time segments and thus the detection limits were reduced. In Fig. 9 the GC–PFPD–MS–MS chromatograms are shown for a spiked concentration level of 50 ng/ml. It is evident that at these levels, even the PFPD chromatogram is interfered with some matrix related compounds. The MS–MS RIC features the diazinon and partially the methyl parathion peaks while there are no peaks for the other three pesticides. The blow-up in Fig. 10 demonstrates how the PFPD chromatogram or the daughter ion chromatogram at m/z 175 could be used for the extraction of the hidden ethion MS–MS spectrum.

As is well known, MS–MS spectra are not similar to EI-MS ones. In particular in the case of ethion, where the parent ion was not the molecular ion, there was actually very little resemblance to the EI-MS spectrum. This reduces the credibility of identification based on MS–MS spectra, since there is no wide database for MS–MS spectra available for comparison, mainly due to the variance of spectra between different MS–MS techniques and methods.

3.3. Summary of the results

In Tables 2 (soil) and 3 (sage) the results obtained in the various measurements are summarized. The data consists of the following:

Each column describes a method for detection and identification. Each left sub-column (indicated by ng) indicates the minimum detected level obtained in ng spiked per ml extract, whereas the right sub-columns indicate the identification criteria. The minimum detected level is the minimum level that met the corresponding identification criterion. The first left column contains the AMDIS search results. As mentioned before, the AMDIS criterion was a ''net fit'' result above 50 out of 100, and the ng level (or actually ppm level in the matrix) indicated, is the minimum that met this criterion. The second column is the NIST library search of background subtracted MS spectra at the PFPD indicated time. The criterion was a ''purity'' factor of more than 500 out of 1000 in a search of the background subtracted mass

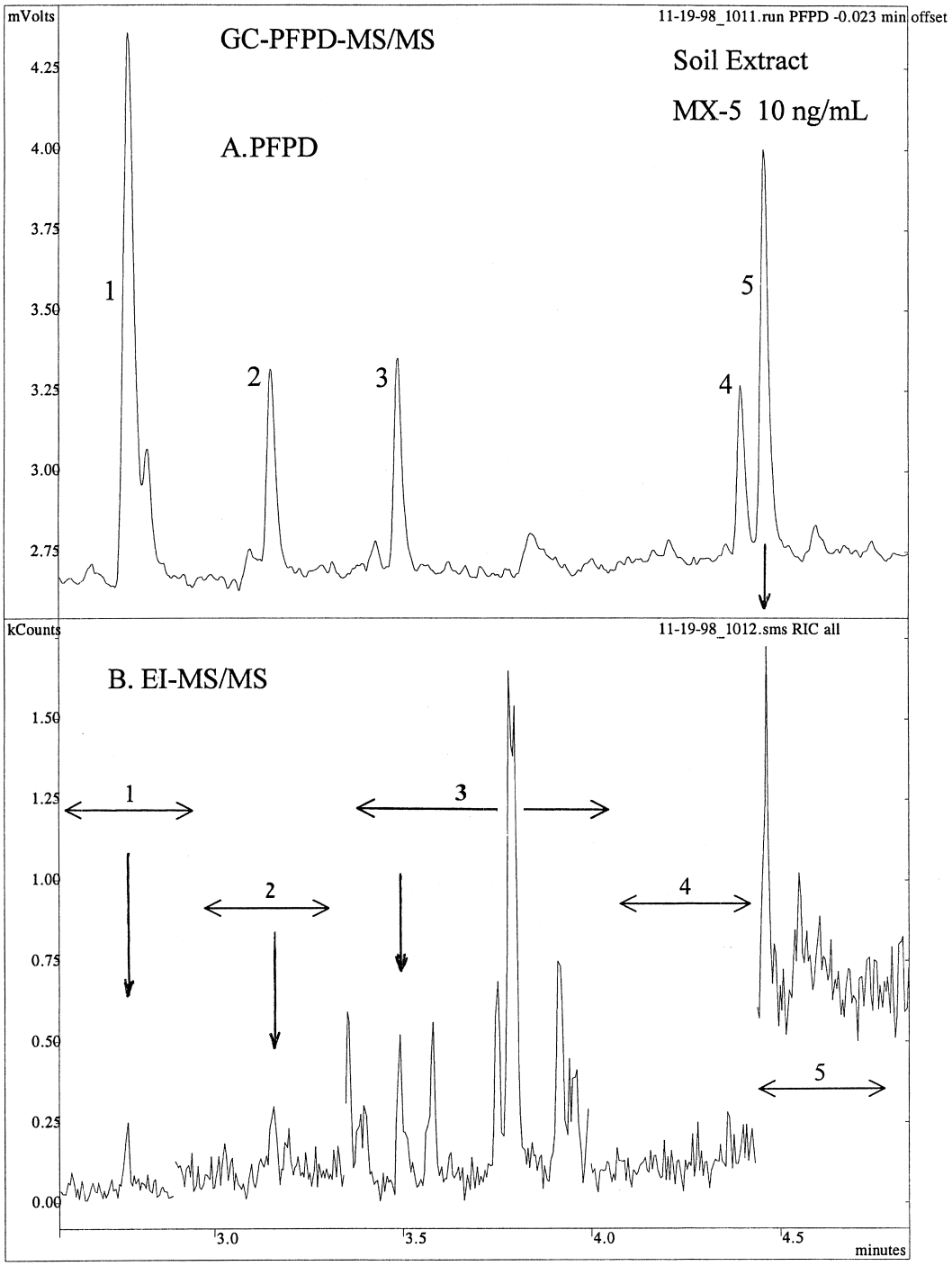

Fig. 5. GC–PFPD–MS–MS of MX-5 in a soil extract. A spiked level of 10 ng/ml MX-5 was detected by PFPD. The time segmented MS–MS RIC features several peaks, among them are peaks at the PFPD indicated elution times for some of the pesticides, where for no clear RIC peak existed for methyl parathion (2) and methyl trithion (4). The arrows, numbered 1 to 5, mark the five consecutive time-segments.

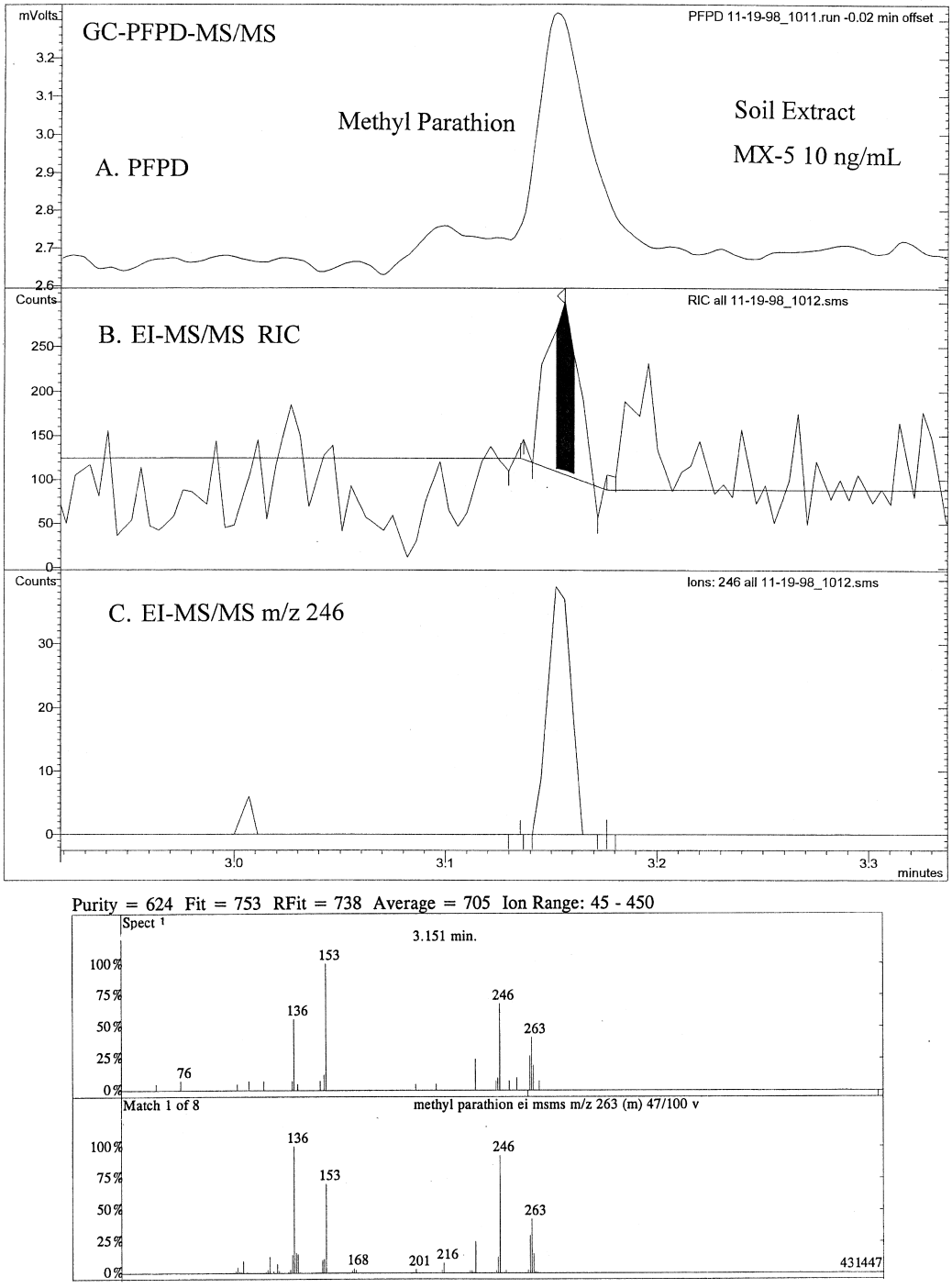

Fig. 6. MS–MS identification of methyl parathion – a blow up of Fig. 5. Background subtraction around the peak indicated by PFPD (or by the daughter ion peak at m/z 246) produced the MS–MS library searchable spectrum. The library spectrum was obtained by running a standard under the same MS–MS conditions.

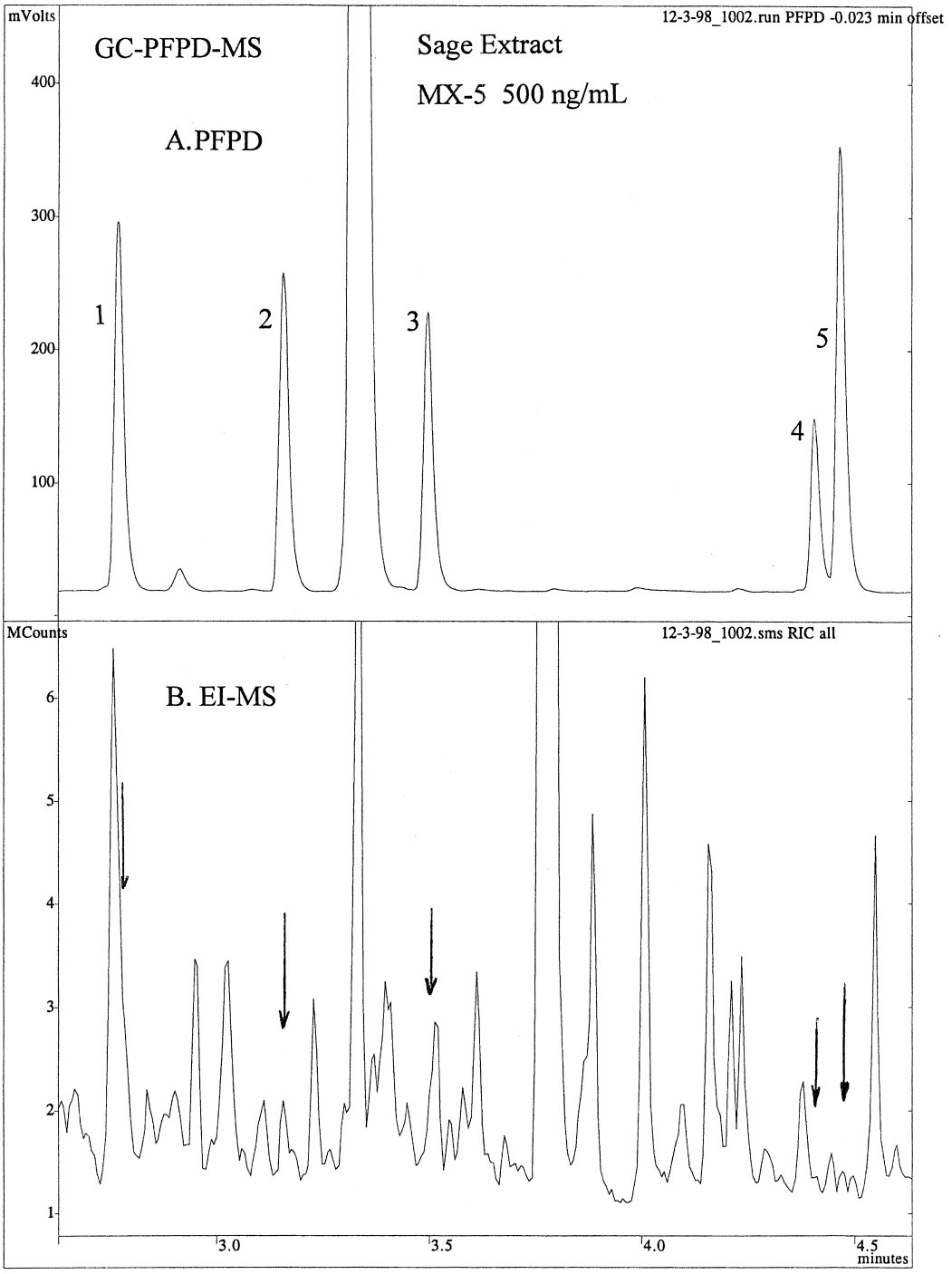

Fig. 7. GC–PFPD–MS of a spiked sage extract. At the spiking level of 500 ng/ml, clear indication of the five pesticides in the PFPD chromatogram is demonstrated. However, in the EI-MS RIC the pesticide peaks are buried in the very intense chemical background (baseline level is more than 10-times higher than with the soil extract). The pesticides are: (1) diazinon, (2) methyl parathion, (3) ethyl parathion, (4) methyl trithion, (5) ethion.

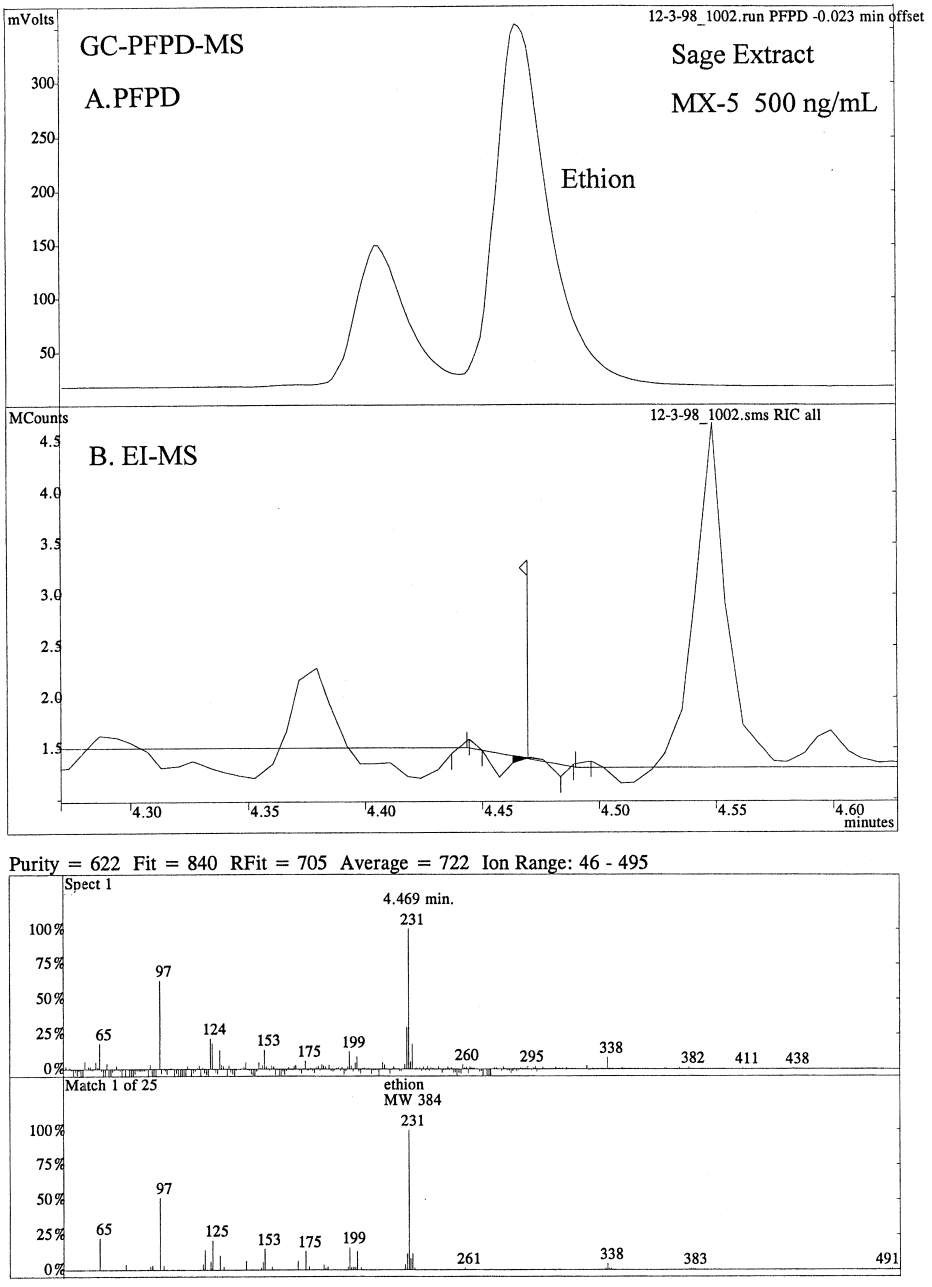

Fig. 8. GC–PFPD–MS detection and identification of ethion in the sage extract – a blow up of Fig. 7. Careful background subtraction (marked by small vertical lines) around the exact elution time indicated by PFPD, revealed the library (NIST) searchable spectrum of ethion, which was completely buried under the matrix chemical noise.

spectrum in the NIST92 database, using the Varian search algorithm. This should be similar to the AMDIS criterion [13]. The third column was based on the same data and library searches, but the criterion was obtaining first hits in the library search, under any fit. Usually a correct first hit would be

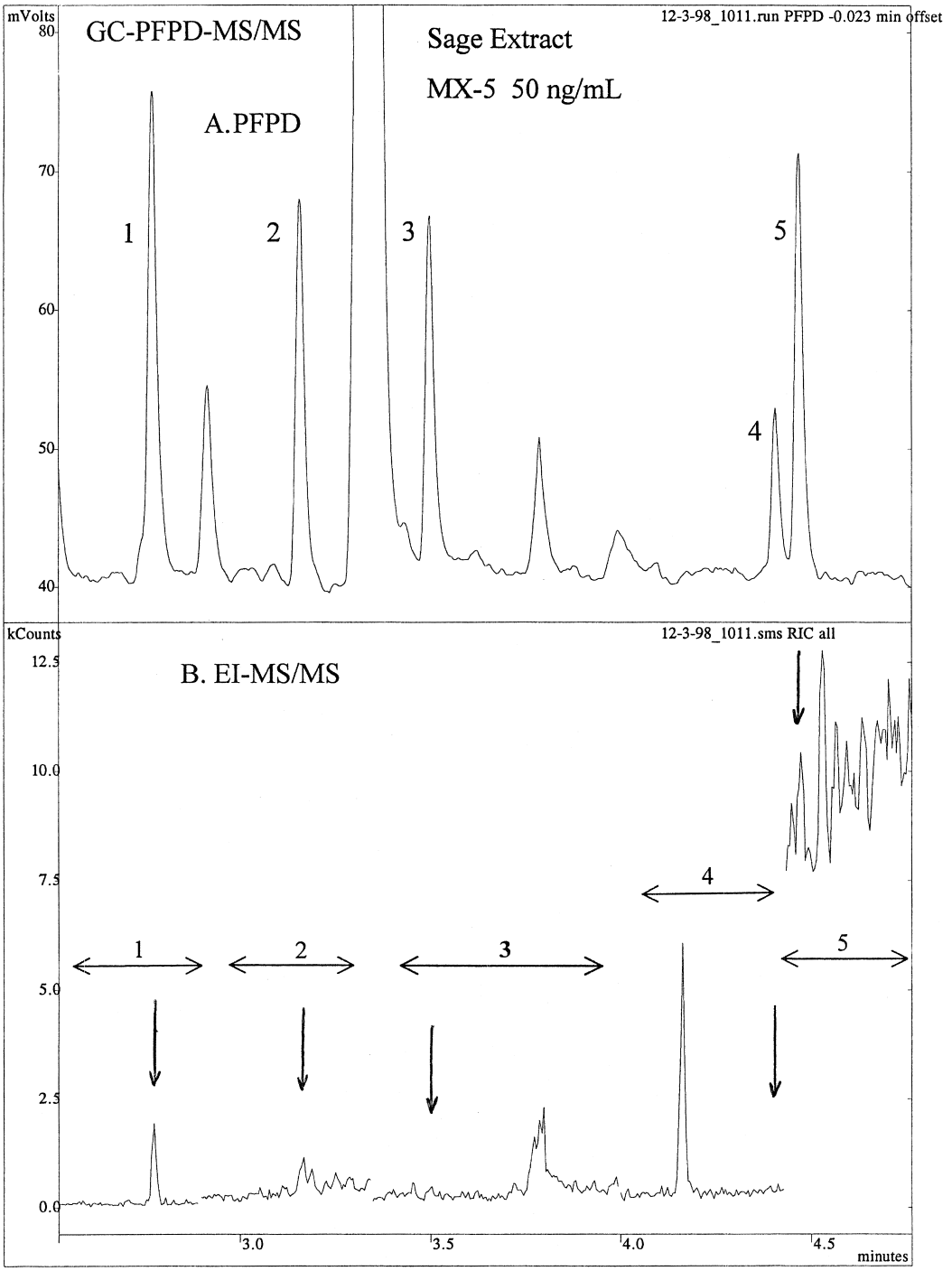

Fig. 9. GC–PFPD–MS–MS of MX-5 in a sage extract. A spiked level of 50 ng/ml MX-5 was detected by PFPD. The time segmented MS–MS RIC features several peaks, among them are peaks at the PFPD indicated elution times for two of the pesticides [no RIC peak for ethyl parathion (3), methyl trithion (4) and ethion (5)]. The arrows, numbered 1 to 5, mark the five consecutive time-segments.

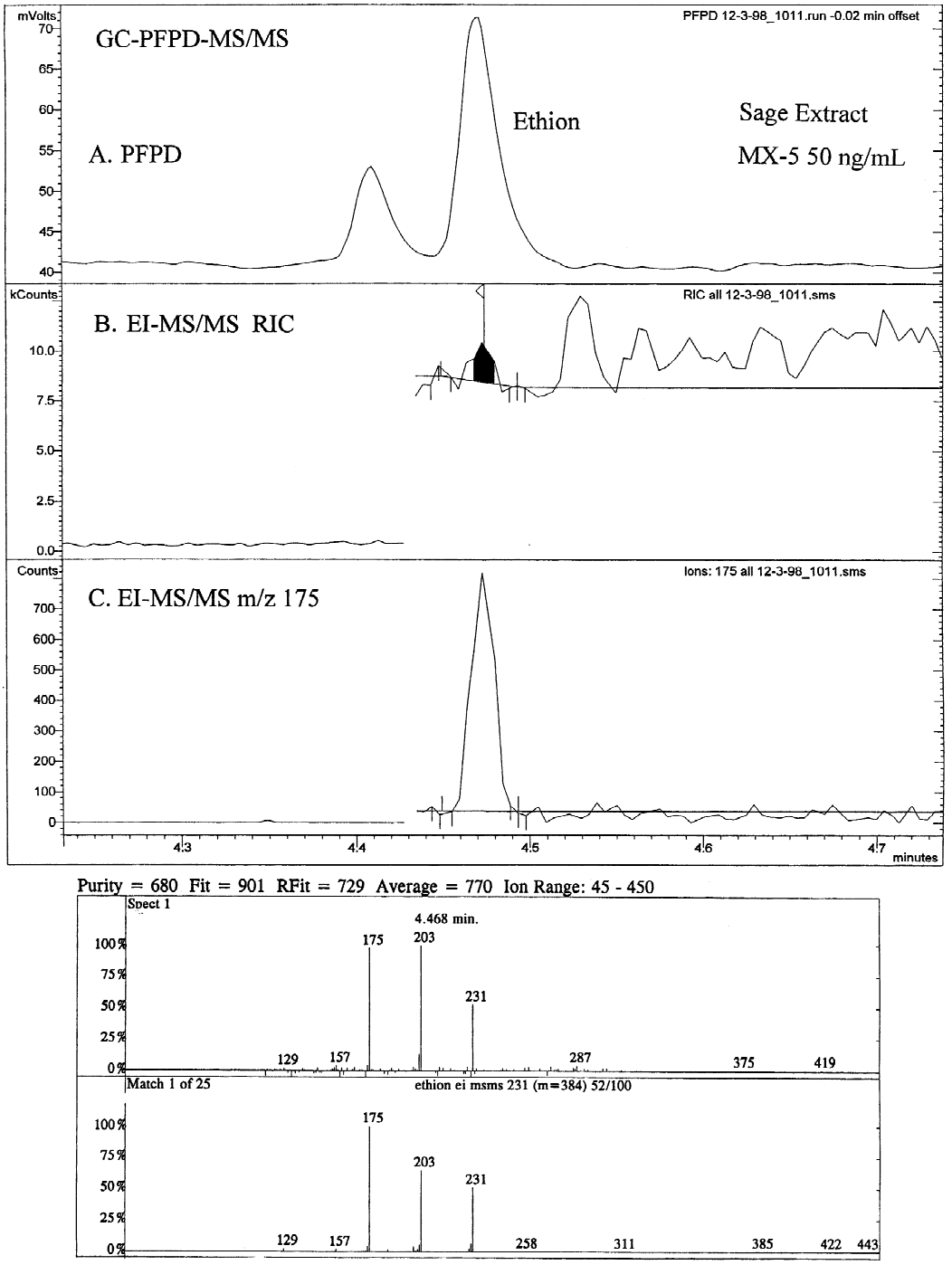

Fig. 10. MS–MS identification of ethion in the sage extract – a blow up of Fig. 9. Background subtraction around the elution time indicated by PFPD (or by the daughter ion peak at m/z 175) produced the MS–MS library searchable spectrum. The library spectrum was obtained by running a standard under the same MS–MS conditions.

Table 2
Summary of the results; soil matrix (the ng amounts are the minimum detected levels in ng spiked per ml extract)

	AMDIS:		PFPD–MS:								PFPD–MS–MS:	
	Net>50 (out of 100)		NIST>500 (out of 1000)		First hit in NIST search		Ions and elements, single NIST match		RSIM peaks, 4 ions or more		NIST match	
	ng	Match	ng	Match	ng	Match	ng	Constraints	ng	Ions	ng	Match
Diazinon	0.05	71	0.02	582	0.01	379	0.01	*m/z* 304>10% *m/z* 179>95% P>0	0.01	*m/z* 304 *m/z* 199 *m/z* 179 *m/z* 137	0.01	839
Methyl parathion	0.2	54	0.2	563*	0.1	315	0.1	*m/z* 263>10% *m/z* 246>5% *m/z* 109>20% P>0 S>0	0.05	*m/z* 263 *m/z* 246 *m/z* 125 *m/z* 109 *m/z* 79	0.01	624
Ethyl parathion	0.1	53	0.2	549	0.02	290	0.02	*m/z* 291>20% *m/z* 97>60% P>0	0.05	*m/z* 291 *m/z* 235 *m/z* 186 *m/z* 139	0.05	772
Methyl trithion	0.2	64	0.2	538	0.05	302	0.02	*m/z* 314>15% *m/z* 157>50% P>0	0.05	*m/z* 314 *m/z* 157 *m/z* 125 *m/z* 63	0.02** 0.2	566 769
Ethion	0.05	64	0.05	510	0.02	352	0.01	*m/z* 231>95% P>0 S>0	0.05	*m/z* 338 *m/z* 231 *m/z* 199 *m/z* 153 *m/z* 97	0.01	827
Average	0.12		0.13		0.04		0.03		0.04		0.02** 0.055	

*Background subtraction from a neighbor hydrocarbon peak.
**No GC peak in RIC.

obtained with purity factors above 200. This criterion significantly reduces the level of confirmation but improves the detection limits. The forth column describes identification based on combined elemental information (PFPD) and mass spectral one (major ions). The PFPD data analysis software served to distinguish between S, P and P+S containing compounds. Library searches were performed under constraints drawn from the above information and the criterion was achieving a single correct hit, in this case out of the NIST98 database (110 000 compounds). Further incorporation of the PFPD elemental ratio information is possible for achieving even lower detection and identification levels [12]. The fifth column uses a criterion of presence of detectable four ion peaks of the analyte, and was constructed for comparison. The results are based on reconstructed mass chromatograms of the specific ions out of the full scan runs (real SIM is not possible with ion trap analyzers). The sixth and last column is of the GC–PFPD–MS–MS analyses. The criterion was a library match to the user-made MS–MS spectrum. The results marked with ** are of cases where no MS RIC peaks existed and the elution time indication of the PFPD or of a daughter ion chromatogram was necessary to allow exposure of the analytes spectrum.

The differences in detection limits from compound to compound emerge mostly from different matrix interferences for each analyte. In the MS–MS ex-

Table 3
Summary of the results; sage matrix (the ng amounts are the minimum detected levels in ng spiked per ml extract)

	AMDIS: Net>50 (out of 100)		PFPD–MS: NIST>500 (out of 1000)		First hit in NIST search		Ions and elements, single NIST match		RSIM peaks, 4 ions or more		PFPD–MS–MS: NIST match	
	ng	Match	ng	Match	ng	Match	ng	Constraints	ng	Ions	ng	Match
Diazinon	0.5	57	1	666	0.25	370	0.25	*m/z* 304>7% *m/z* 276>15% P>0	0.25	*m/z* 304 *m/z* 276 *m/z* 227 *m/z* 199 *m/z* 179 *m/z* 137	0.02	833
Methyl parathion	1	59	>1*	385	0.5 0.25	285 218	0.5	*m/z* 263>50% *m/z* 125>50% *m/z* 109>50% P>0	0.5	*m/z* 263 *m/z* 246 *m/z* 229 *m/z* 125	0.02** 0.05	512 625
Ethyl parathion	1	63	1	523	0.25	298	0.5	*m/z* 291>10% *m/z* 109>70% P>0	0.5	*m/z* 291 *m/z* 263 *m/z* 155 *m/z* 109 *m/z* 97	0.25	674
Methyl trithion	0.5	52	1	517	0.5	245	0.25	*m/z* 157>50% *m/z* 125>50% P>0	1	*m/z* 314 *m/z* 157 *m/z* 125 *m/z* 63	0.1** 0.25	684 715
Ethion	0.5	68	0.25	510	0.1	231	0.05	*m/z* 231>95% P>0 S>0	0.5	*m/z* 338 *m/z* 231 *m/z* 97 *m/z* 65	0.02** 0.1	531 852
Average	0.70		>0.85		0.30		0.31		0.55		0.08** 0.13	

*Purity of 385 was achieved with 1 ng, no higher concentrations were injected.
**No GC peak in RIC.

periments, the differences may have also emerged from different MS–MS efficiencies and different abundance of the parent ions. In particular, methyl trithion exhibited poorer MS–MS sensitivity compared with the other compounds. The average minimum detected level obtained for each method was calculated and appears at the bottom of each column of Tables 2 and 3.

4. Conclusions

The following conclusions arise from the above results:

(1) GC–PFPD–MS coupling provided enhancement in the detection and library confirmation level compared to standard GC–MS. It is estimated that on average, signals that are about one third of the background baseline level can still be extracted and identified by library searches, and meet the criterion of ''purity'' >500. The signal, in that case, is measured by ion count of the background-subtracted spectrum compared to the background average baseline ion count near the analyte elution time. The knowledge of the elemental presence of P or S (and potentially several other elements) can support or reject a library search result. The PFPD–MS combination is mostly powerful for unknown or unexpected compounds, where the PFPD indication becomes critical.

(2) AMDIS provided detection capabilities similar or even slightly better than PFPD–MS. A limitation of the AMDIS is the reduced size of the databases. The results presented here were based on the criteria of net fit >50 for the correct compound while no other hit existed among the ''NISTdrugs'', ''NIS-Ttox'' and ''NISTFDA'' available libraries. This enabled an elimination of about 2000 compounds. The confirmation level would thus be inferior to a standard library search as long as a complete NIST library search is not accomplished. It is noted however, that the AMDIS has an option to ''manually'' carry out a full NIST search for an extracted spectrum in order to improve the level of identification and make sure that there is no similar or better fit for the selected spectrum in the NIST full database. AMDIS is naturally valid only for a limited group of compounds whose spectra exist in its database, and this may be a drawback in screening. The major advantage of the AMDIS is the relatively *fast* (few minutes to few seconds, depending on the computer and the data) and *fully automatic* processing, almost without any operator decision making required.

(3) NIST library search using constraints of dominant ions and elemental information (obtained by PFPD) provided single fits, at detection levels lower by a factor of 2–3 compared to the PFPD–MS full NIST library searches. These detection levels are similar to those obtained under ''first hit–any fit'' searches. The confirmation level obtained by both methods may be arguable. Although the use of an eliminatory constrained library search reduced the number of available options to one, yet, there was no indication (such as a match factor) to better indicate that this is the correct result and not another compound that does not exist in the library at all. Therefore it is concluded that this method may be poorer than a standard full library search with a high match. Incorporation of the elemental information coming from a selective detector into the identification algorithm of the library search or AMDIS may provide the ultimate identification level.

(4) With GC–MS–MS a substantial improvement in the detection limits was observed (an average factor of 8 compared to PFPD–MS). The PFPD marking proved to be useful, especially where MS–MS RIC peaks were absent (4 out of 10 cases). It is noted, however, that the compound peak could also be allocated by its known daughter ion chromatograms as demonstrated in Figs. 5 and 10. MS–MS spectra could be extracted as long as daughter ion chromatographic peaks were present.

The confirmation level with MS–MS is relatively low due to the following: (a) MS–MS spectra are different than EI spectra and can not be easily searched for in standard EI libraries, in particular if the parent ion is not the molecular ion. (b) No MS–MS spectra database is available, and it is difficult to establish since MS–MS spectra depend on the instrument and the experimental conditions. The absence of a MS–MS database reduces the confirmation level obtained, since no elimination of spectra of other compounds is possible. In particular, very important is the elimination of compounds of the same family having the same functional groups and fragment ions as the analyte. (c) Even if there were a MS–MS library, MS–MS spectra usually feature only few major mass fragments (3–4) and thus are not ideal for a library search.

Other major disadvantages of MS–MS are the need for compound specific method development and the ''blindness'' to any other compound beside the programmed ones. Due to these, MS–MS is basically suitable for target compound analysis rather than for general screening. It is mentioned, however, that GC–MS–MS methods for a few dozen ''target'' compounds can still be established. In principle, the use of alternate scan MS–MS, where several MS–MS methods are applied alternately, all in the same time segment, has the potential to increase the number of analyzed compounds number significantly and provide a tool for partial screening. Under this type of a ''many target'' GC–MS–MS analysis, the PFPD time indication can become very useful and easier to use than the normal product ion mass chromatographic indication.

(5) In comparison to the soil matrix, the detection limits obtained for the loaded sage matrix were poorer by similar factors for all the techniques studied (factors of 5.8, >6.5 and 4 for AMDIS, PFPD–MS and PFPD–MS–MS respectively). This implies that GC–MS–MS, in this experiment, was affected by the complexity of the matrix similarly to standard GC–MS.

(6) The technique of monitoring four ions provided detection limits better by a factor of 2–3 compared with PFPD–MS, but with a lower level of

confirmation. The synchronous PFPD signal can significantly support identification based on this method. As far as detection levels are concerned, it is noted that these results were obtained under full scan MS since no true SIM experiments can be performed with ion trap MS. It seems that time-shared SIM experiments with quadrupole or sector instruments may produce better detection limits, which may be comparable to those obtained by ion trap MS–MS. Since MS–MS produces spectra usually containing also 3–4 major ions, one can assume that the confirmation level obtained with both time-shared four ion SIM and MS–MS may be comparable as well. However, there is a higher confidence that the ions in the MS–MS spectrum originated from the analyte (through the parent ion) and not from any background interference.

(7) Fig. 11 is a two-dimensional plot summarizing the results in terms of trade-offs between detection limits and confirmation level based on the above results and conclusions. The range of improvement in the detection limits is estimated to be more than a factor of 24 (more than a factor of 3 improvement from standard GC–MS to PFPD–MS, and about a factor of 8 further improvement with GC–PFPD–MS–MS). The range of reduction in the confirmation level is more difficult to estimate and is very much molecular and sample dependent. The parameters that govern the quality of the identification are: (a) the library search match level (if a library search is involved). (b) The size of the MS spectra database (to allow the comparison with and elimination of other compounds). (c) The richness of the database with spectra of compounds of the same family of the analyzed one, having a similar fragmentation pattern in many cases.

Although it is evident that the price of improved detection limits is usually a lower confirmation level, it can be compensated for by the use of the GC retention index. It is well practiced and known that this GC added dimension of information, together with any of the above mentioned methods would provide unambiguous confirmations (albeit not always legally accepted) in many cases.

Acknowledgements

The advice and support of Gary Mallard of NIST concerning the installation, operation and optimization of the AMDIS software is well appreciated. The advice and support of Yihan Bao of Varian CSB providing the PFPD data analysis software is gratefully acknowledged. The company for Life Science

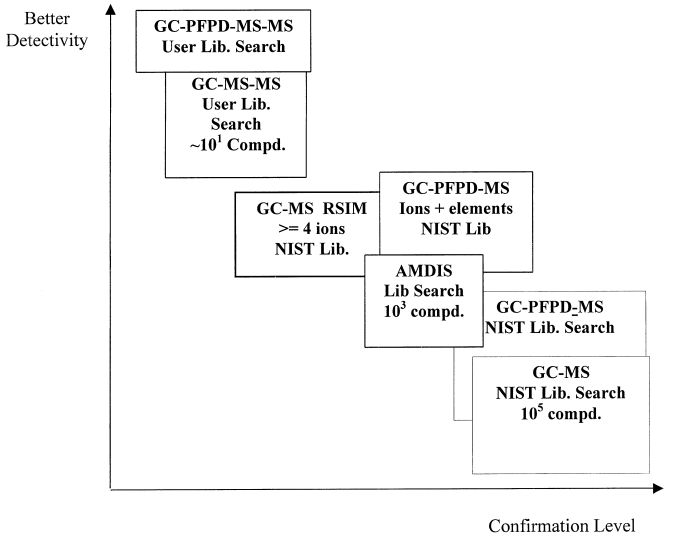

Fig. 11. The suggested trade-off between better detectivity (lower minimum detected level) and confirmation level, based on the results and conclusions.

Research in Israel (LSRI) is acknowledged for access to the Varian Saturn 2000 GC–MS instrument.

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