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Fast, high-sensitivity, multipesticide analysis of complex mixtures with supersonic gas chromatography–mass spectrometry

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Abstract

We developed a new instrumental approach, termed Supersonic GC–MS, which achieves fast, sensitive, confirmatory and quantitative analysis of a broad range of pesticides in complex agricultural matrices. Our Supersonic GC–MS system is a modification of a bench-top Agilent 6890 GC+5972 MSD with a supersonic molecular beam (SMB) interface and fly-through EI ion source. One of the main advantages of Supersonic GC–MS is an enhanced molecular ion (M^+) in the resulting mass spectra. For example, The M^+ was observed in all 88 pesticides that we studied using the Supersonic GC–MS whereas only 36 of 63 (57%) pesticides that we investigated in standard GC–MS exhibited a M^+. We also found that the degree of matrix interference is exponentially reduced with the fragment mass by about 20-fold per 100 amu increasing mass. The enhancement of the M^+ combined with the reduction in matrix background noise permit rapid full scan analysis of a potentially unlimited number of pesticides, unlike selected ion monitoring or MS–MS in which specific conditions are required in segments for targeted pesticides. Furthermore, unlike the case with chemical ionization, EI-SMB-MS spectra still give accurate identification of compounds using common mass spectral libraries. In practice, we found that libraries favor mass spectra in which the M^+ appears, thus Supersonic GC–MS produced better spectra for compound identification than standard GC–MS. To achieve even lower identification limits, the M^+ plus a second major ion (still using full scan data) gives higher signal-to-chemical noise ratios than the traditional 3-ion approach. The replacement of two low-mass ions with the M^+ (supersonic two-ions method) results in a significant reduction of matrix interference by a factor of up to 90. Another main advantage of Supersonic GC–MS is its exceptional suitability for fast GC–MS with high carrier gas flow-rate. Fast Supersonic GC–MS was able to analyze thermally labile pesticides, such as carbamates, that are difficult or impossible to analyze in standard GC–MS. Large volume injection using a ChromatoProbe was also demonstrated, in the 6 min analysis of pesticides at 20 ng/g in a spice matrix.
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1. Introduction

Pesticide analysis in agricultural products is of vital importance for the safety of our food. However,

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much of the pesticide monitoring currently being performed occurs after the food has already been distributed to the markets, and only a very small fraction of the food supply is monitored. This situation emerges in part due to the high cost and long time required for pesticide analysis in agricultural products. A major challenge and need for pesticide residue chemists is to conduct fast, sensitive, confirmatory and quantitative analysis for a broad range of pesticides in less than 30 min including sample preparation.

Ideally, analysts could use GC–MS in full scan mode (of the MS) to simply identify all the pesticides through the extensive 70 eV electron ionization (EI) libraries available. An implicit assumption in this ideal, though, is that GC–MS is compatible with the analysis of all pesticides.

Three major obstacles stand in the way of performing effective pesticide analysis:

1. Pesticide analysis is a lengthy procedure, especially the sample preparation steps but also the chromatographic analysis itself. The long pesticide analysis time inevitably translates into high cost.
2. While most of the pesticides are amenable to GC and GC–MS analysis, a growing portion of the pesticides are thermally labile and require LC or LC–MS for their analysis. This situation further complicates the analysis and precludes the use of GC–MS alone as the ideal tool for universal multipesticide analysis.
3. The complexity of food matrices and the presence of interferences increase the need for clean-up steps, limit the ruggedness of the instrumental methods, and make low level pesticide identification and quantitation difficult.

In routine pesticide monitoring programs, it is common for laboratories to use a variety of different methods and detectors to cover the broad scope of pesticides and food matrices. Due to matrix interferences, a preliminary GC analysis with selective detectors such as the nitrogen phosphorus detector (NPD), electrolytic conductivity detector (ELCD), electron capture detector (ECD), halogen selective detector (XSD), or flame photometric detector (FPD) [1,2] (more recently pulsed flame photometric detector (PFPD) [3,4]) is often employed to mark suspected pesticides at their elution time for their consequent GC–MS confirmatory analysis. The use of a simultaneous PFPD and MS analysis can reduce the adverse effect of matrix and enable lower MS identification limits if PFPD postrun data analysis software is used [5]. However, PFPD-MS works best for organophosphorus and organosulfur pesticides only.

Some routine monitoring laboratories use GC–MS in the selected ion monitoring (SIM) mode to simultaneously quantify and confirm the identity of a wide variety of GC-amenable pesticides [6], but multiple injections are needed, confirmation in SIM is questionable and extensive clean-up is still needed. The use of MS–MS rather than SIM typically provides lower detection limits, higher degree of confirmation, and reduces mass spectral interferences, even under complex matrix interference conditions [7,8]. However, as in SIM, the limited number of target pesticides possible in a single run prevents MS–MS from becoming the ideal multipesticide analytical tool.

In theory, GC–MS analysis in the full scan mode can quantify and confirm any number of GC-amenable analytes in a single injection, but matrix interferences continue to act as the bottleneck in this type of pesticide analysis. The use of an automated background subtraction and deconvolution search algorithm, such as AMDIS (automated mass spectral deconvolution identification software) [9–11], helps to reduce the effect of background interferences, but detection limits are typically too high.

When full scan MS fails to provide library identification, the confidence level in pesticide identification is sacrificed and traded for lower level detection typically through the isolation of the three most abundant ions at the correct GC elution time. Sphon [12] described and evaluated the use of this method for drug analysis in 1978, and in regulatory circles it was extended and adopted for pesticide analysis. Clearly, if only three major ions are required for pesticide identification instead of the full mass spectrum with its many weak ions, lower identification limits can be achieved in most cases. An important issue is how many and which ions are required for achieving sufficient confidence level in the chemical identification. It was shown by Sphon that the three ions substantially reduce the number of potential interfering compounds from the mass spec-

tral library (assuming that the relatively small set of library compounds constitute the interferants). The three-ion method has been extensively discussed in terms of its suitability [13–15], but it is still in widespread use today. Unfortunately, many pesticides only give one or two strong ions in MS, which makes their analysis using the three-ion approach unrealistic.

It should be mentioned that in 1978, the need for compound identification through its three major ions using SIM was essential, regardless of matrix interference, merely due to instrument sensitivity issues. Thus, the 3-ion method was mainly devised to lower the limit of confirmation at a time when analytical needs could not be adequately met by alternate approaches. Currently, the sensitivity of modern GC–MS instruments is much better, and with full scan sensitivity specification of <1 pg (available from most vendors), a reasonable target of 10 ppb pesticide detection and identification is easily met for clean samples. For the typical 1 μl injection of a sample extract equivalent to 5 g/ml, a 10 ng/g (ppb) pesticide concentration in the sample would mean that 50 pg pesticide is actually introduced into the column. For stable pesticides, this is an easy task for analysis by practically any of the current commercially available GC–MS instruments. In theory, one could inject larger equivalent volumes of sample to further lower the analytical detection limit. However, matrix interferences are almost always the limiting source of noise in GC–MS analysis of pesticide residues in food, and clearly, matrix constitutes the bottleneck that limits the realization of low-level pesticide analysis. In practice, the instrumental sensitivity is often meaningless in the overall detection limits.

Matrix interference may be reduced through more extensive sample clean-up, but such procedures increase the time and cost of the analysis, and clean-up generally requires a sacrifice in the number of pesticides that can be analyzed in the method (due to its inherent selectivity). More efficient chromatographic separation can reduce the number of co-eluting peaks, but the standard 30 m, 0.25 mm I.D. capillary columns provide a good balance in terms of chromatographic resolution and sample capacity and any further increase in the GC separation resolution through the use of a longer column could considerably prolong the chromatography analysis time and adversely affect the analysis of several thermally labile pesticides.

Thus, while new regulations and monitoring needs tend to require more sensitive analysis of an ever-growing number of pesticides in a variety of matrices, the process of pesticide analysis is confronted with difficulties to meet the needed analytical sensitivity combined with the excessive cost and time of analysis. These current limitations severely impede the potential achievement of pesticide analysis prior to the distribution of agricultural products, which, if implemented, would improve food safety.

In the last decade we developed and explored the performance capabilities of a new type of GC–MS, based on the use of a supersonic molecular beam (SMB). SMB was used for interfacing the GC to the MS [16–22] and as a medium for ionization of sample compounds while in the SMB, either by electron ionization [23–25] or by hyperthermal surface ionization (HSI) [24,26–31].

Supersonic molecular beams are characterized by unidirectional motion with controlled hyperthermal kinetic energy (1–20 eV), intramolecular vibrational supercooling, mass focusing similar to that in a jet separator, and the capability to handle very high column flow-rates of up to 240 ml/min [19,24]. While our research has employed a quadrupole mass analyzer [16–22], GC–SMB-MS was also implemented with a time-of-flight mass analyzer [32,33].

Recently, we have incorporated GC–MS with SMB into a new instrument and approach, which we have titled Supersonic GC–MS. The instrument has been described in detail previously [34]. Its design involves minimal modifications of a commercially available Agilent (Wilmington, DE, USA) GC–MS system (6890 GC plus 5972 MSD) to include an SMB interface. In this system the standard EI ion source was replaced with a fly-through EI ion source, mounted in the path of the SMB. A HSI ion source, combined with a 90° ion mirror (for the EI-produced ions), was also fitted inside the quadrupole mass analyzer in lieu of the original EI ion source. The Supersonic GC–MS requires the addition of an air-cooled 60 l/s diffusion pump and 537 l/min rotary pump. All the gas flow-rates, heated zones, sampling and data analysis are performed the same way as

with the original system and are computer-controlled via the original Agilent CHEMSTATION software.

GC–MS with SMB has been demonstrated to improve several GC–MS performance aspects and features including:

1. The M^+ intensity is enhanced in EI with SMB and it is practically always exhibited. A tunable degree of fragmentation is obtained through the control of the electron energy [23–25] and the M^+ may be the only MS peak at low electron energies.
2. Very effective fast and ultra-fast GC–MS is enabled, compatible with any mass analyzer including quadrupole devices [16–21]. Fast splitless injections are achieved, and flow programming is possible with very large high-to-low flow-rate ratios [16]. Any column can be used without restriction on diameter, length, or carrier gas flow-rate for achieving optimal trade-off of GC resolution, speed and sensitivity.
3. Combination with the ChromatoProbe sample introduction device [35] is an option, which enables fast, extract-free sampling of 'dirty' samples. The ChromatoProbe, laboratory-built or commercially available from Varian (Walnut Creek, CA, USA), is based on GC sample introduction using a disposable microvial and intra-GC injector sample thermal desorption. Its effectiveness improves with high column flow-rates as used with the Supersonic GC–MS [19,22].
4. Lowest detection limits can be achieved for a wide range of drugs and aromatics using HSI in GC–SMB-MS [16,34], while the EI sensitivity is similar to that of standard thermal EI [34]. Enhanced SIM sensitivity is exhibited in EI of alkanes due to the large enhancement in M^+ abundance [34].
5. Thermally labile molecules are amenable to fast and ultra-fast GC–MS analysis [16,17] due to the significantly shorter time spent in the injector and column at the higher column flow-rate, lower elution temperatures, and elimination of thermally-induced dissociation in the ion source. Additionally, tailing-free GC–MS was achieved through the use of SMB, background ion filtration, and short column, fast GC–MS [16].

In this paper, the use of Supersonic GC–MS for multipesticide analysis is described, and the implications of its unique features are explored.

2. Experimental

The Supersonic GC–MS system is described in detail elsewhere [34], and thus, it will be only briefly presented here beyond what is described above. An Agilent 6890 GC was used with an Optic 2 temperature programmable injector (Atas, Veldhoven, The Netherlands). A laboratory-made ChromatoProbe could be coupled with this injector. This ChromatoProbe is similar to our previous ChromatoProbe, which is available from Varian, except that it is slightly longer due to the longer liner in the Optic injector.

A 6 m×0.20 mm I.D., 0.33 μm film thickness DB-5ms column (Agilent, Folsom, CA, USA) was used in most of the Supersonic GC–MS experiments, except a few experiments that were conducted with a 15 m×0.53 mm I.D., 1 μm film thickness DB-1 column. The Supersonic GC–MS transfer line, a 20 cm×0.53 mm I.D. deactivated Silcosteel tube provided by Restek (Bellefonte, PA, USA), was operated at 250 °C with 130 ml/min He flow-rate. The nozzle was kept at the same temperature as the transfer line. After the supersonic expansion, the supersonic free jet was skimmed, differentially pumped, and passed into a fly-through EI ion source (laboratory-made) inside the vacuum chamber of the original 5972 MS instrument located perpendicular to the quadrupole MS. A 10-mA ionizing electron emission current was used with 70 eV electron energy. The original 5972 MS was used without its standard EI ion source, which was replaced by our laboratory-made ion mirror and ion optics [34]. The transfer line temperature and all flow-rates were controlled by the CHEMSTATION software, and data analysis was similarly performed with the original CHEMSTATION software and either NIST'98 or Wiley mass spectral libraries.

The experiments with standard GC–MS systems were performed on the following systems: (a) Agilent 6890 GC+5972 MSD before it was converted into a Supersonic GC–MS (transfer line temperature was 280 °C with the resulting estimated 180 °C ion source temperature); (b) Varian Saturn 2000 ion trap GC–MS with 30 m×0.25 mm I.D., 0.25 μm DB-5ms and 1 ml/min He column flow-rate. The ion trap temperature was 180 °C; (c) Agilent 5890 GC+5972 MSD with 30 m×0.25 mm I.D., 0.25 μm Rtx-5 ms (Restek) and 1 ml/min He column flow-rate. The

transfer line temperature was 260 °C with the resulting estimated 167 °C ion source temperature; (d) Agilent 5890 GC+GCD MS with 30 m×0.25 mm I.D., 0.25 μm DB-5ms and 1 ml/min He column flow-rate. The transfer line temperature was 280 °C with the resulting estimated 155 °C ion source temperature.

All 88 pesticides that were studied in this project were obtained from Chemservice (West Chester, PA, USA), Dr. Ehrenstorfer (Augsburg, Germany), Accustandard (New Haven, CT, USA), or the EPA National Pesticide Repository (Fort Meade, MD, USA). Stock solutions of approximately 2000 ng/μl were prepared of each pesticide in toluene, ethyl acetate, or acetone, and mixtures were prepared at typical initial concentration of 7 ppm in methanol. Some degradation occurred for methomyl and carbaryl in solution, and their concentrations reduced with time. Analytical parameters and conditions specific to each experiment are given in the text.

3. Results and discussion

3.1. Mass dependence of matrix interference

It is well known that matrix interference is substantially higher at lower masses in GC–MS analysis of agricultural matrices. However, to our knowledge, this aspect has not been reported quantitatively in the case of a real matrix in order to determine how much matrix interference occurs with respect to higher and lower masses. Thus, we explored this issue with a standard GC–MS (6890+5972) in the analysis of several pesticides in oregano. We chose oregano because spices and herbs are known to possess a relatively high degree of matrix interference and would provide a reliable result representative of nearly any complex matrix.

Fig. 1 shows the mass dependence of the matrix interference in oregano extract. The upper trace gives the NIST library mass spectrum of the pesticide diazinon with letters (A)–(F) above six selected major ions. The lower mass chromatograms (A)–(F) are the postrun reconstructed selected ion monitoring (RSIM) chromatograms, obtained from the full scan analysis of diazinon in oregano. Diazinon was spiked at 200 ng/g in the oregano extract (1 g/ml) and gave a retention time of 12.3 min in the chromatograms. Trace A, which is the RSIM chromatogram of the M^+ ($m/z=304$), essentially has no matrix interference. In trace B ($m/z=199$), the pesticide peak is still clean around its elution time but some matrix interference is now observed at higher elution times. As the fragment masses get smaller, the relative matrix interference shows marked increases and appears throughout the chromatogram. At $m/z=137$, the detection of diazinon is severely hampered, and by $m/z=93$, it is practically useless for the identification of the pesticide.

Fig. 2 gives another example of the same trend in the case of ethion (200 ng/g in oregano). The same observations are demonstrated displaying a strong increase of the matrix interference with respect to decreasing fragment mass.

Fig. 3 plots the results from Figs. 1 and 2 in a quantitative way. For each mass chromatogram, the areas of all the matrix peaks were integrated over an elution time range of 3 min (±1.5 min) around the pesticide elution time. These were summed and normalized (by division) to the library mass spectral relative ion abundances. In Fig. 3, the log of the normalized matrix interference (N_M) is plotted against the fragment mass, and a clear exponential reduction in the extent of matrix interference versus mass is observed for both ethion and diazinon in oregano. Very similar slopes were obtained demonstrating that matrix interference was reduced 28–30 times per each 100 amu increase in mass. Thus, matrix interference was exponentially reduced with mass and the reduction factor was about 29 times per 100 amu. However, more extensive work would have to be done to prove this as a general rule as well as to provide a general quantitative matrix interference reduction factor. Thus, in order to make a more conservative estimate, we shall assume a lower factor of 20-fold decrease per 100 amu as a reasonable number for our further calculations.

In order to rationalize this finding we assume that EI of average matrix compounds gives an exponential increase in the abundance of low mass fragments. Accordingly, even if the M^+ is observed, the mass spectral region near it is relatively ‘empty’ while at low masses almost every mass has some peak intensity. Probably the distribution function of the average matrix mass spectrum is closer to a Maxwell Boltzmann, which shows an exponential reduction at high masses as well as an increase at the

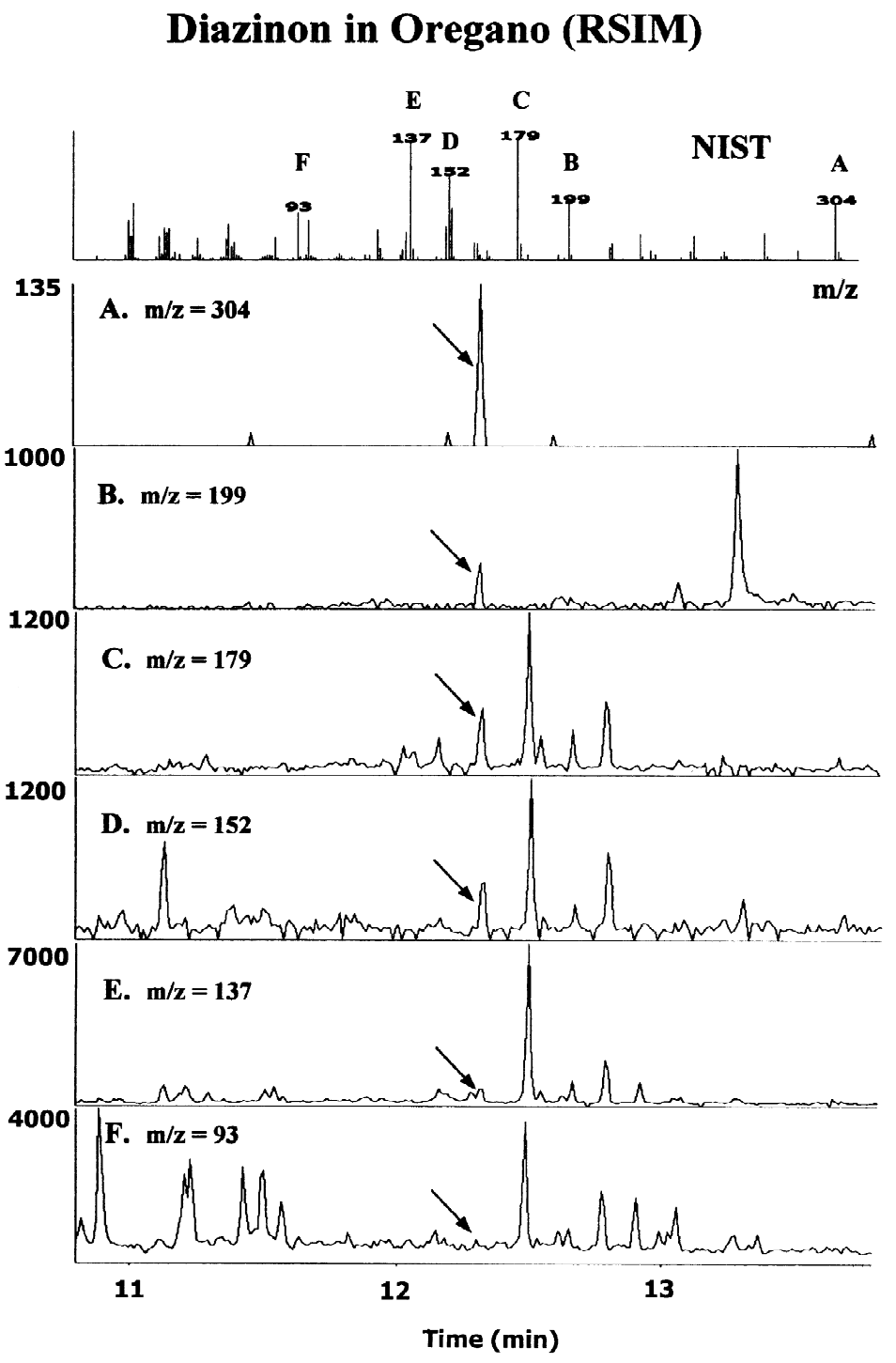

Fig. 1. Analysis of 200 ng/g diazinon in oregano extract with a standard GC–MS. The upper trace is the NIST library mass spectrum of diazinon with its major fragments indicated in letters (A)–(F). The lower 6 traces are corresponding mass chromatograms (A)–(F) obtained through postrun data analysis at the indicated m/z. The arrows show the elution time of the pesticide.

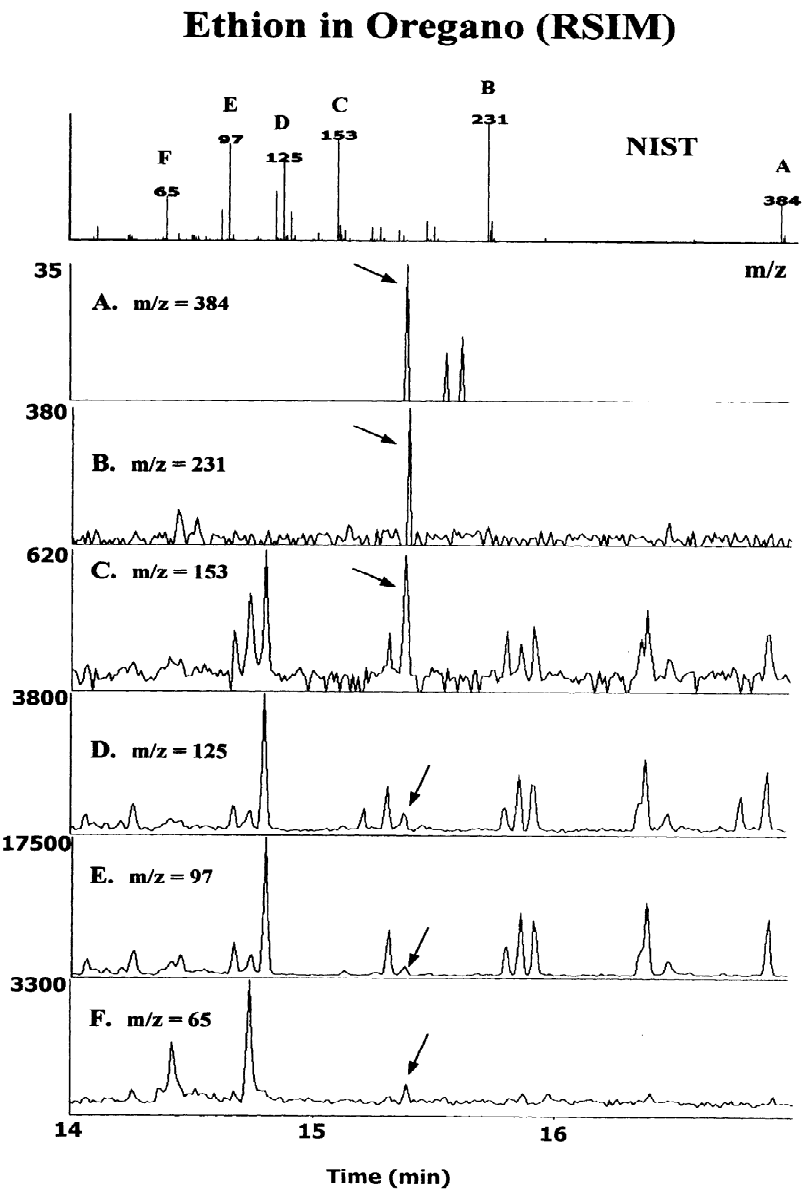

Fig. 2. Analysis of 200 ng/g ethion in oregano extract with a standard GC–MS. The upper trace is the NIST library mass spectrum of ethion with its major fragments indicated in letters (A)–(F). The lower 6 traces are corresponding mass chromatograms (A)–(F) obtained through post run data analysis at the indicated m/z. The arrows show the elution time of the pesticide.

very low masses as is evident from the lowest fragment mass chromatogram $m/z=65$ in Fig. 2. Statistically, fewer fragment permutations are available from organic compounds at $m/z=65$, for which ethion had a fairly unique fragment. McLafferty et al. [36] showed that fewer compounds give $m/z=65$ as a strong ion, and that $m/z=41$, 43, 57, 77, 91 and 105 are very common ions in the MS of organic molecules. The results shown in Figs. 1–3 are too few to conclude a general behavior and thus we confirmed them with a few other cases. In fact, a statistical analysis of the NIST library itself shows

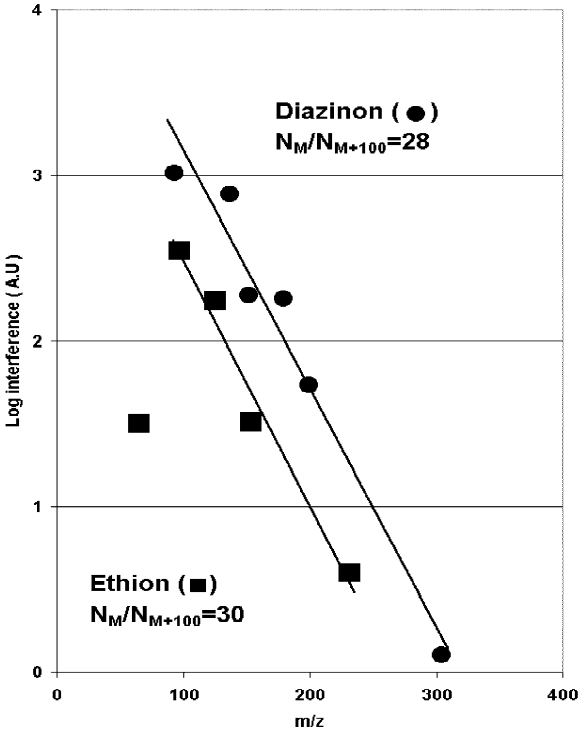

Fig. 3. Plot showing the relationship between normalized matrix interference (N_M) versus mass. The log of the integrated matrix peaks in Figs. 1 and 2 is plotted versus the analyzed masses (A)–(F) for both ethion and diazinon in oregano. Clear exponential decay of the normalized matrix interference with mass is observed with an average slope of 29 per 100 amu.

that the sum of all the library compounds shows an exponential probability reduction with mass and similarly every summation of matrix mass spectra (averaging over an extended elution time range) that we performed gave us also an exponential reduction of the matrix interference with mass. However, with broader averaging time duration the slope was found to be smaller due to lower effect of GC separation. In fact, the readers of this article can easily open their existing files, average the mass spectra around a given pesticide elution time and analyze the fragment height distribution function if it behaves in the same way as ours.

This observation of mass dependence of matrix interference has important implications in planning strategies to reduce matrix interference as will be described.

3.2. Fast pesticide analysis with the supersonic GC–MS

Fig. 4 displays a demonstration of fast analysis using Supersonic GC–MS for 13 representative pesticides. Several aspects of this fast analysis with Supersonic GC–MS are different from other types of fast GC–MS, which include:

(a) The column length was only 6 m instead of the standard 30 m.
(b) The column I.D. was 0.2 mm (0.33 μm film

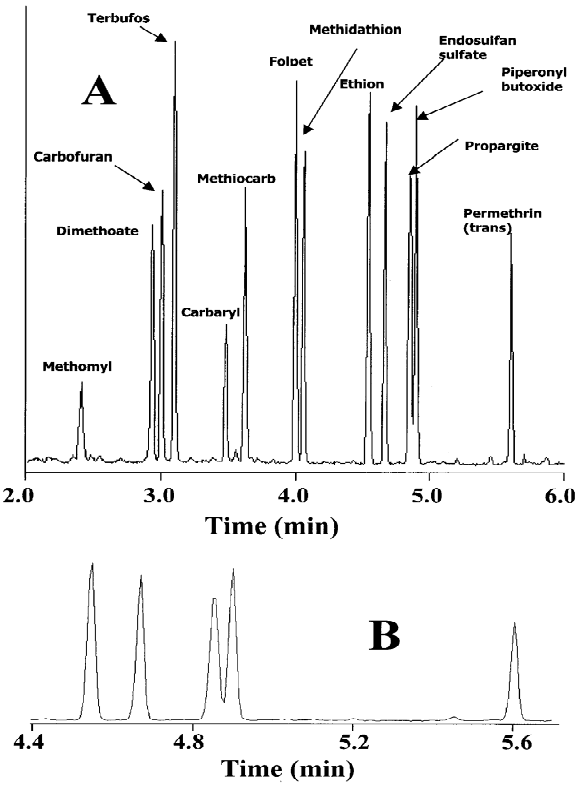

Fig. 4. Fast GC–MS analysis of the indicated 13 pesticides obtained with the Supersonic GC–MS. (B) This is a zoom of the upper trace (A) in order to demonstrate the symmetric tailing-free peak shapes. A 6 m×0.2 mm I.D., capillary column with a 0.33 μm DB-5ms film was used with a 10 ml/min He flow-rate. A 1-μl sample volume was injected with an initial concentration of seven ppm. Methomyl and carbaryl slowly degraded in the methanol solution and their concentration is assumed to be 3 ppm. The optic injector initial temperature was 100 °C and programmed to 260 °C at a rate of 4 °C/s. The GC oven started at 80 °C for 1 min following by temperature programming rate of 35 °C/min up to 310 °C.

thickness), which was slightly narrower than the standard 0.25 mm I.D. (the use of 0.32 or 0.53 mm I.D. columns could also have been used to increase sample capacity and column life).

(c) The He flow-rate was 10 ml/min as measured at 60 °C, and thus the linear velocity in the 0.2 mm I.D. column was about 12 times greater than in a standard 0.25 mm I.D. column.

(d) The speed enhancement factor (SEF), which is the multiplication of column length reduction times carrier gas linear velocity increase (sometimes also defined as void time reduction) [17], was 60. This SEF is higher than most other fast GC–MS schemes. The SEF is a direct measure of the capability to analyze thermally labile molecules [17,37].

As demonstrated in Fig. 4, the least volatile pesticide, *trans*-permethrin, eluted at 5.6 min, and the total chromatographic time, including the time needed to clean the column of late-eluting matrix components, was only 8 min. In comparison, typical pesticide analyses last 30–50 min. Furthermore, typical GC analyses of pesticides do not include thermally labile analytes such as carbamates, but the Supersonic GC–MS was able to provide high quality peaks of the thermally labile folpet, and carbamates, methomyl, carbofuran, carbaryl, and methiocarb.

3.2.1. Peak tailing

Fig. 4B (bottom trace) focuses on the last 5 of the 13 pesticides in order to show the excellent peak shapes achieved for the least volatile pesticides. Sharp and highly symmetric peaks were obtained, without any peak tailing. All the pesticide peak widths are in the range of 1.2–1.5 s and are thus easily amenable for analysis with the scan rate of standard quadrupole mass analyzers. We used 3.2 Hz scan rate, which led to 4–5 scans per peak (FWHM) and clearly nice (and reproducible) peak shapes were obtained.

In GC–MS the EI ion source temperature is maintained at a relatively low temperature in comparison with other GC detectors, and its temperature is practically always lower than the upper GC column temperature. This relatively low ion source temperature is chosen in order to obtain high quality mass spectra, with observable molecular ion and good matching factors to the library mass spectra. As a result, in traditional GC–MS systems, the late eluting peaks tend to tail due to thermal interactions with the metal surfaces of the EI source. These time extended adsorption–desorption cycles inside the ion source results in tailing peaks. Increasing the ion source temperature can reduce this tailing for relatively nonvolatile pesticides, but then the source is too hot for the other pesticides, and the relative abundance of the M^+ is reduced for all pesticides due to increased thermal energy in the pesticide molecular ion.

The reason for the tailing-free symmetric peak shapes obtained with the Supersonic GC–MS is the use of a molecular beam and a fly through EI ion source combined with vacuum background ion filtration [23,24]. This vacuum background filtration is based on the differences in kinetic energies of ions that were produced from thermal nondirectional vacuum background molecules and pesticides in the SMB. The field in our EI ion cage is close to zero and thus background ions are practically not extracted. Even if some of them are extracted, they are defocused with the ion optic lens voltage that is optimized for ions formed from SMB compounds that fly through the ion source and whose ion energy is higher. Any pesticide that will scatter from a surface, thermally equilibrate with the ion source temperature and will be ionized after that event will be filtered out and not detected. Thus, peak tailing due to lengthy cycles of pesticides adsorption–desorption inside the ion source is completely avoided regardless the pesticide volatility.

3.2.2. Column bleed

Additionally, note how the baseline of the total ion chromatogram in Fig. 4 is flat from the start to finish, even near the elution time of *trans*-permethrin. A closer examination of the obtained mass spectra showed that no mass spectral peaks of $m/z = 207$ or 281 from column bleed were observed. Accordingly, column bleed was completely eliminated. The major reason for elimination of column bleed is that the use of a short column and high column flow-rate considerably reduced the elution temperature of all pesticides. For example, permethrin eluted at 242 °C rather than the 290 °C elution temperature that occurred in standard GC–MS at the GC conditions we utilized. The effect of increased column flow-rate

and shorter column length on lowering sample elution temperatures will be further described in detail elsewhere [37].

3.2.3. Thermally labile pesticides

The lower pesticide elution temperatures and higher injector splitless flow-rate had a considerable effect on increasing the recovery of thermally labile compounds, such as methomyl, in the GC step. To better evaluate the relative gain in our ability to analyze thermally labile pesticides, the same pesticide mixture used in the analysis showed in Fig. 4 was also analyzed with standard GC–MS instruments [mass selective detector (MSD) and gas chromatography detector (GCD)] in two other laboratories that frequently conduct pesticide residue analysis.

Table 1 summarizes the results for the 13 pesticides. We used terbufos as a stable reference compound and normalized the peak heights of all pesticides to its height in each chromatogram. Clearly, ethion, *trans*-permethrin, piperonyl butoxide and endosulfan sulfate proved to be stable (along with terbufos), whereas propargite, methidathion and dimethoate showed some relative reduced yield in the standard GC–MS analysis. In the cases of methomyl, carbofuran, carbaryl, methiocarb and folpet, a clear reduction occurred in their analytical yield relative to the Supersonic GC–MS due to thermal degradation at the injector and/or in the column. The yield of carbofuran was about 20%, while only about 10% each of carbaryl, methiocarb and folpet were detected, and methomyl could not be observed at all in the standard GC–MS systems. It should be emphasized that 90% pesticide degradation also implies an order of magnitude increase in the relative magnitude of matrix interference. We consider this feature of greatly improved analysis of thermally labile pesticides in Supersonic GC–MS as very important.

3.3. Library identification with 'cold EI' mass spectra

The most valuable reason for using Supersonic GC–MS is its feature of enhanced M^+, which is a very useful tool for reducing matrix interference and achieving lower detection and identification limits. This enhancement of the molecular ion emerges from the vibrational cooling of molecules in the SMB during the supersonic expansion, and concomitant greater stability of the molecular and other larger ions.

Currently, pesticide identification is frequently accomplished by comparing experimental mass spectra with extensive 70 eV standard EI libraries available (or self made libraries). The first question that needs to be addressed is how compatible are 'cold EI' mass spectra, namely EI mass spectra obtained in

Table 1
Relative pesticide degradation in GC–MS analysis

No.	Pesticide	SMB	5972 (USDA ARS)		GCD (PRL-Israel)		Average gain
1	Methomyl	38	0	[∞]	0	[∞]	∞
2	Dimethoate	93	13	[7.1]	53	[1.8]	4.5
3	Carbofuran	93	40	[2.4]	14	[6.8]	4.6
4	Terbufos	100	100	[1.0]	100	[1.0]	1.0
5	Carbaryl	40	9	[4.5]	1	[36.3]	20.4
6	Methiocarb	89	16	[5.4]	7	[13.4]	9.4
7	Folpet	54	16	[3.4]	3	[16.1]	9.8
8	Methidathion	84	18	[4.6]	57	[1.5]	3.1
9	Ethion	100	100	[1.0]	100	[1.0]	1.0
10	Endosulfan sulfate	71	42	[1.7]	35	[2.0]	1.9
11	Propargite	91	41	[2.3]	41	[2.2]	2.3
12	Piperonyl butoxide	95	73	[1.3]	93	[1.0]	1.2
13	*trans*-Permethrin	43	37	[1.2]	37	[1.2]	1.2

SMB means pesticide peak height relative to that of terbufos, 5972 means results obtained with the Agilent 5972 MSD at the USDA (USA) and GCD means results obtained with the Agilent GCD at the Israel Plant Protection and Inspection Services. The numbers in brackets are the degradation factor, which is the relative pesticide peak height obtained with the Supersonic GC–MS divided by the relative peak height obtained with the MSD or GCD. The last column shows the average gain considering both MSD and GCD.

Supersonic GC–MS, with current libraries. The simple answer is that the cold EI spectra in Supersonic GC–MS are quite compatible with existing MS libraries (and certainly compatible with laboratory-made libraries).

Small molecules, and those which exhibit a dominant molecular ion in conventional 70 eV EI, show cold EI mass spectra which are practically identical to those of thermal (traditional) EI, as in the NIST library. Examples of this 'no effect' category are small aromatic compounds including octafluoronaphthalene (OFN) and hexachlorobenzene (HCB). In this categorization, a 'small' molecule means a molecule with less than about 15 atoms and without many low-frequency vibrations (low vibrational heat capacity). Medium size compounds, such as most of the pesticides, give a moderate M^+ enhancement and thus exhibit quite good library search capability with high matching factors. Only large molecules such as large aliphatic compounds with over 50 atoms may show substantial change in the appearance of the mass spectrum and result in enhancement of both the molecular ion and other high mass fragments.

Fig. 5 shows different degrees of M^+ enhancement in typical cold EI mass spectra of propargite, HCB, and methiocarb compared with the standard 70 eV EI NIST library mass spectra. Propargite ($C_{19}H_{26}O_4S$) is a relatively large pesticide with 50 atoms and a 'floppy' structure. Thus, as shown in the upper two traces in Fig. 5, its cold EI mass spectrum is characterized by an enhanced M^+ as well as enhanced high mass ions at $m/z=201$ and 173. Conversely, HCB with only 12 atoms and a highly rigid structure shows a cold EI mass spectrum that is practically identical to the library spectrum. However, the majority of pesticides are medium-size compounds, and the vibrational cooling in SMB results only in moderate enhancement of the M^+ as shown in Fig. 5 for methiocarb.

The reason for this typical behavior is that upon cooling, the molecular ion M^+ as the highest mass ion can only be increased upon vibrational cooling. In contrast, other fragment ions can both gain population upon cooling from lower mass fragments and lose population to higher mass fragments (or to the M^+) and thus are in a relative steady state and are practically unchanged. Accordingly, only the M^+ is practically enhanced while on the other hand only very low-mass fragment ions are depleted as shown in Fig. 5 for methiocarb

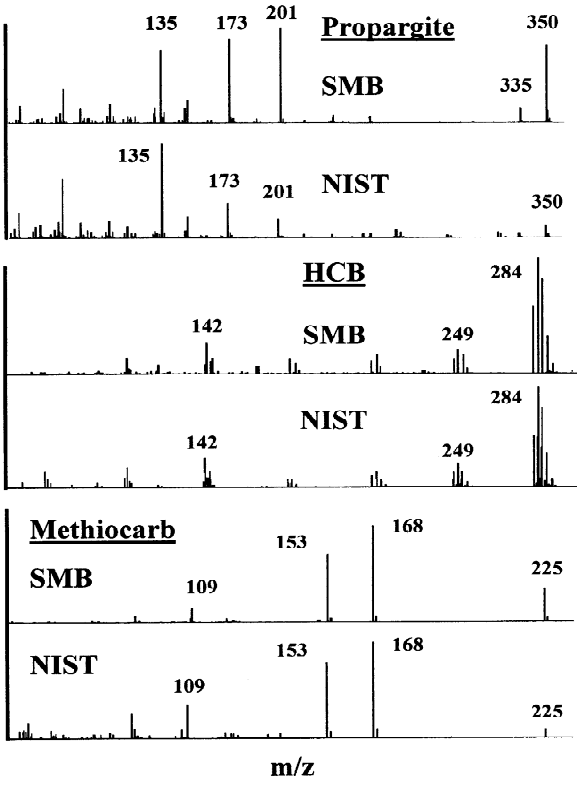

Fig. 5. Comparisons of mass spectra obtained with the Supersonic GC–MS with those taken from the NIST'98 library for different pesticides. Propargite is an example of a pesticide with a relatively large enhancement of the M^+, hexachlorobenzene (HCB) is an example of a pesticide with minimal enhancement, and methiocarb is an example of the majority of pesticides with a moderate enhancement.

The Supersonic GC–MS is not the only instrument that gives somewhat different spectra than the traditional 70 eV mass spectra in commercial databases. Ion trap, time-of-flight, and even quadrupole instruments at different MS ion source temperatures, tuning parameters, and other settings produce different mass spectra for the same molecule. In fact, the library spectra are usually generated from high concentrations of the pure chemical of interest at 'ideal' conditions designed to produce a M^+. Real analyses are often conducted in complex mixtures at

higher source temperatures, low analyte concentration, and nonoptimal GC–MS conditions.

Fig. 6 gives typical cold EI mass spectra of four pesticides in Supersonic GC–MS in comparison with both their 70 eV EI NIST library mass spectra and mass spectra obtained using an ion trap GC–MS (Saturn 2000). We studied the mass spectra of 88 pesticides and found these examples to be typical and highly representative of our findings. Several conclusions were drawn from these results, including: (a) In all mass spectra obtained with the Supersonic GC–MS, the M^+ is noticeably enhanced whereas the relative abundances of the low-mass fragments are reduced; (b) The NIST library mass spectra of these pesticides are characterized by higher relative abundance of the M^+ than in the case of those from the conventional GC–MS (ion trap or quadrupole). Thus, while all four pesticides, methiocarb, terbufos, ethion and methidathion, gave a cold EI M^+ of around 30–50% relative abundance, the relative abundances of the molecular ions were lower in the NIST library. In the case of the ion trap MS results, the M^+ was absent or very low, especially for methidathion, terbufos and methiocarb (the $m/z=226$ for methiocarb was due to self-chemical ionization at the relatively high concentration injected and is not the M^+).

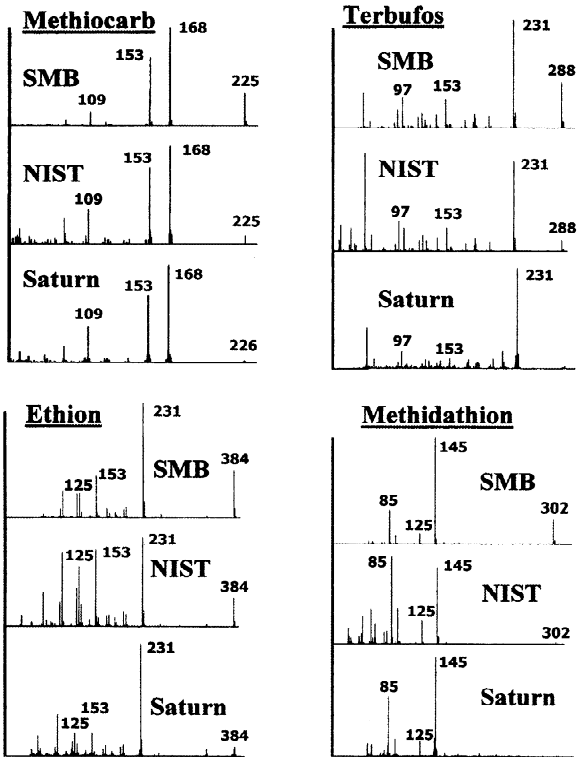

Fig. 6. Comparisons of the mass spectra of methiocarb, terbufos, ethion, and methidathion obtained with the Supersonic GC–MS, Saturn 2000 GC–MS and those from the NIST'98 library. These examples are considered as typical and representative of most pesticides. Note that while the mass spectra obtained with the Supersonic GC–MS are characterized by enhanced M^+ in comparison with both the library mass spectra and those obtained with the standard GC–MS. The reduced M^+ of the Saturn 2000 GC–MS spectra versus those in NIST'98 are probably due to higher ion source temperature.

In general, the NIST library mass spectra almost always show equal or higher relative abundance of the M^+ than found in experimental GC–MS data. The most likely reason is that a higher ion source temperature is typically used for pesticide analysis by GC–MS than was used to generate the library mass spectra. Ion source temperature effects are well known and documented [38–40], and actually the enhanced M^+ with the Supersonic GC–MS is an extension of the same effect to very low (cold) ion source temperatures. The ion source temperature is commonly maintained at 180–220 °C in pesticide analysis to reduce the effect of peak tailing of late-eluting pesticides in the ion source. The need for higher temperature for certain pesticides sacrifices the better spectra obtained at lower temperatures of the majority of pesticides. This constraint was not a factor in the case of those who generated the mass spectra for the libraries.

The cold EI mass spectra of eight other pesticides are shown in Fig. 7. The chromatogram that includes these pesticides is shown in Fig. 4. Clearly, the mass spectra of all these pesticides show an enhanced M^+.

Table 2 shows a comparison of the NIST'98 library identification results for the 13 pesticides from Fig. 4 using the Supersonic GC–MS and standard 5972 MS. All of these pesticides were easily identified as the highest probability compound with matching factors and reverse matching factors of 800–900 in the Supersonic GC–MS results. The matching factors were somewhat higher in the case of 5972 MS results, however, the Supersonic GC–MS better excluded compounds in the library. The probability of correct identification of the pesticide as the first choice was always very high with the

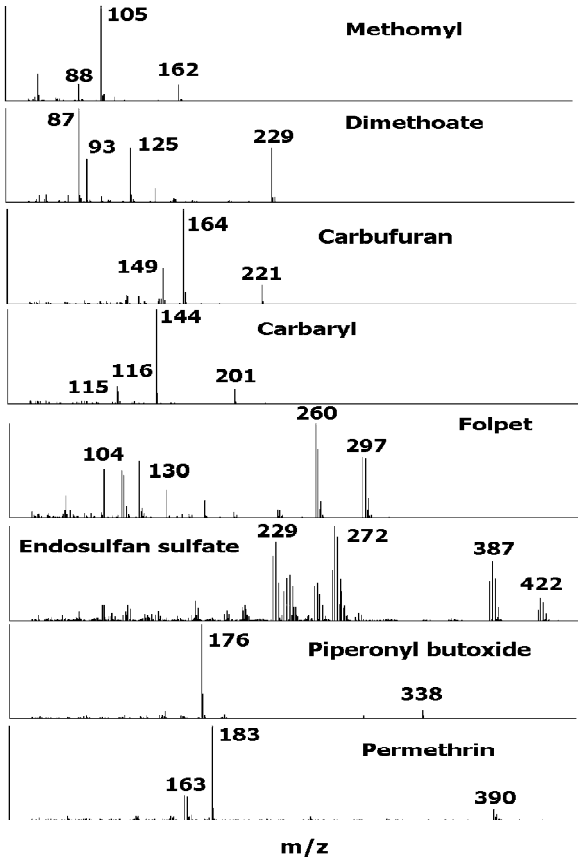

Fig. 7. Cold EI mass spectra of the indicated eight pesticides in Supersonic GC–MS.

Supersonic GC–MS. In fact, 12 of the 13 pesticides gave a greater ratio of the matching probabilities of the first choice and the second choice in Supersonic GC–MS than in standard GC–MS (often considerably greater ratio). Thus, even though the enhanced M^+ somewhat reduced the matching factors, it more importantly provided greater selectivity in the identification. This effect can be described as 'lower fit, but better hit.'

In most instances, pesticide analysts generate their own libraries from reference standards analyzed on their own GC–MS instrument under their own conditions. The issue of 'fit' vs. the commercial libraries is not an issue with any instrument in that targeted set of analytes. However, the advantage of full scan MS library searching is that an identification of any compound in the library, and exclusion of all compounds in the library, may be made even if the analyst does not have the reference standard.

3.4. Isotopomer ratio analysis for further confirmation

Pesticide identification often relies on library searching for identification. However, in certain cases the library match for a particular pesticide is close to that of another library compound due to the ion source temperature effect, column bleed, matrix interference or co-elution with another analyte. In other cases, the pesticide in question may not give the highest probability match, or it gives an unacceptable matching factor due to poor ion abundance statistics or other reasons. In these cases, and even for clean matrices, a second, independent type of pesticide identification is desirable.

An additional feature of the Supersonic GC–MS is that since the M^+ is practically always observed, one can analyze the associated isotopomer group of the M^+ or other fragments. Due to the nature of ionization in an SMB, self-CI does not occur and vacuum background is eliminated. Thus, the 'true' isotopomer abundance distribution is obtained. The isotopomer ratios provide a long-established way to elucidate empirical formulas from the known elemental isotope abundances [41–43] and serve to provide additional independent evidence for identification in MS. However, this is often not possible in traditional GC–MS analysis due to weakness or absence of the molecular ion, self-CI and background noise in the low mass-resolution instruments.

Fig. 8 demonstrates how the isotopomer ratios can be useful for independent evidence to help identify chemical species in Supersonic GC–MS. In the figure, the isotopomer patterns were from actual mass spectra taken 'on the fly' in the chromatogram shown in Fig. 4. The indicated pesticides are compared with the calculated isotope abundance patterns using the NIST library isotope calculator, and a close agreement between the experimental and calculated results was observed. Certainly, a second run with a narrower mass spectral range could further improve the results by reducing mass spectral skewing and statistical noise, however, the observed agreement suggests that an additional approach for compound

Table 2
Pesticide identification with the NIST'98 library using cold EI and its comparison with standard MS (Agilent 5972 MSD) results. Data is processed from the chromatogram shown in Fig. 4. Note the significantly lower probability for the second library compound obtained with the Supersonic GC–MS (SMB)

No.	Pesticide	Hit no.	Match factor		Reverse match factor		Probability			
			MSD	SMB	MSD	SMB	MSD		SMB	
							1st	2nd	1st	2nd
1	Methomyl	1	ND	854	ND	866	ND	ND	96.7	0.96
2	Dimethoate	1	871	824	903	826	97.5	2.3	98.8	0.98
3	Carbofuran	1	920	845	930	848	78.7	14.5	92.6	2.3
4	Terbufos	1	930	872	933	874	98.9	0.98	98.8	0.98
5	Carbaryl	1	900	892	909	900	56.2	13.5	75.9	8.4
6	Methiocarb	1	907	896	913	916	91.8	3.8	98.3	0.98
7	Folpet	1	909	844	912	846	98.2	0.98	97.8	0.97
8	Methidathion	1	908	876	910	877	95.4	0.95	97.6	0.97
9	Ethion	1	890	916	891	916	97.7	1.3	98.9	0.98
10	Endosulfan sulfate	1	873	859	878	869	95.8	1.8	96.9	0.96
11	Propargite	1	798	677	878	685	81.8	1.1	93.0	0.93
12	Piperonyl butoxide	1	908	864	911	866	97.1	0.97	96.3	0.96
13	*trans*-Permethrin	1	894	844	898	845	73.6	22.0	79.3	15.8

identification can be explored in the future, especially with the Supersonic GC–MS.

Currently, we do not have the proper software to convert experimental results into a range of empirical formulas with matching factors as in high-resolution sector instruments but the algorithm for this software has been developed [41–43] and can be implemented. Moreover, if we assume or suspect that a given group of peaks is from a particular compound of interest, we can restrict the search to about 300–500 library compounds based on the determined molecular mass and achieve a much easier confirmation

In Fig. 9 we show some raw data to further illuminate this subject of pesticide identification through isotope abundance analysis. The figure provides the cold EI mass spectra of terbufos, methidathion, folpet, ethion and carbofuran obtained in a Supersonic GC–MS analysis of these pesticides spiked at 70 ng/g in coriander extract. The substantial coriander matrix interference is clearly observed as 'grass' at almost every mass in the low mass spectral range. However, the region near the M^+ is relatively clean, which permits an independent isotope abundance analysis that can be performed in a subsequent run through fast, limited mass spectral scan range around the M^+. It should be emphasized that this type of confirmation identification requires further research and must be tested with appropriate software.

3.5. Cold EI mass spectra of pesticides

Table 3 summarizes the results for 88 pesticides that we studied using Supersonic GC–MS. The table contains the following details for each of the 88 pesticides: (A) Pesticide number (a few pesticide have two columns such as 3A and 3B for two different sets of data as described below); (B) Pesticide name; (C) Compound class; (D) Pesticide molecular mass; (E) The three major ions from the NIST library to estimate possibility for interferences in the manner of Sphon [12] (The number at the line below the three ion masses shows how many compounds in the NIST'98 library could interfere with the identification of the pesticide using the NIST sequential search mode with >40% of the pesticide fragment abundance for all the three ions); (F) The M^+ plus the most prominent additional ion. Below each of these two ion masses is a number that shows how many compounds in the NIST'98 library could interfere with the identification of the pesticide as in (E) above, but with the indicated two ions only and using >40% of the molecular and second ion

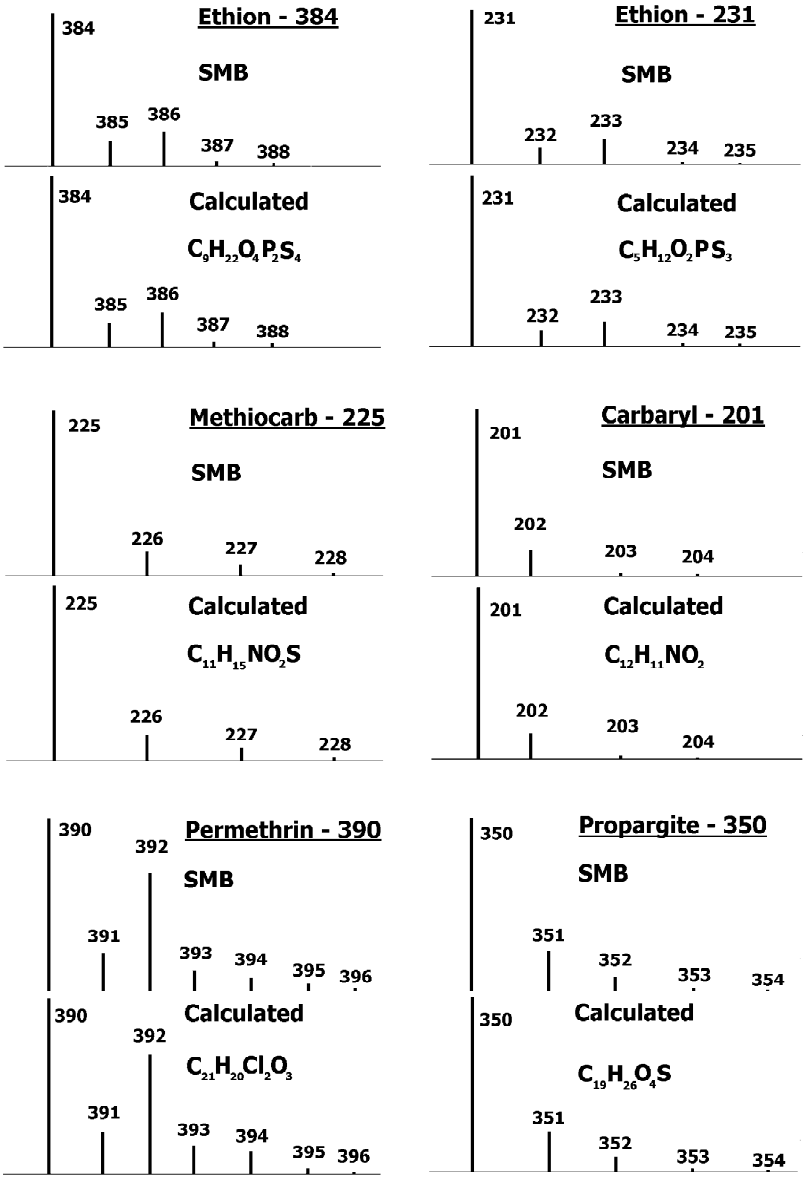

Fig. 8. Comparison of the indicated mass spectra for pesticides achieved with the Supersonic GC–MS and those calculated by the NIST'98 isotope calculator at the M^+ group of isotopomers. For each pesticide (ethion, methiocarb, carbaril, permethrin, and propargite), the upper trace is the experimental results while the lower trace is the calculated trace using the indicated pesticide empirical formula. This procedure was used twice for ethion, once for the M^+ and once for its high mass fragment, $m/z = 231$.

intensities as measured with the Supersonic GC–MS. In a few rare cases when the M^+ was weak, the data were also analyzed with a high mass fragment ion and a second most prominent high mass ion such as for deltamethrin (3) trifluralin (37) and malathion (52); (G) The relative abundance of the pesticide M^+ as measured with the Supersonic GC–MS; (H) The relative abundance of the pesticide M^+ as taken from the NIST'98 library; (I) The relative abundance of the pesticide M^+ as measured with the Varian Saturn 2000 GC–MS (180 °C ion trap temperature); (J) The relative abundance of the pesticide M^+ as

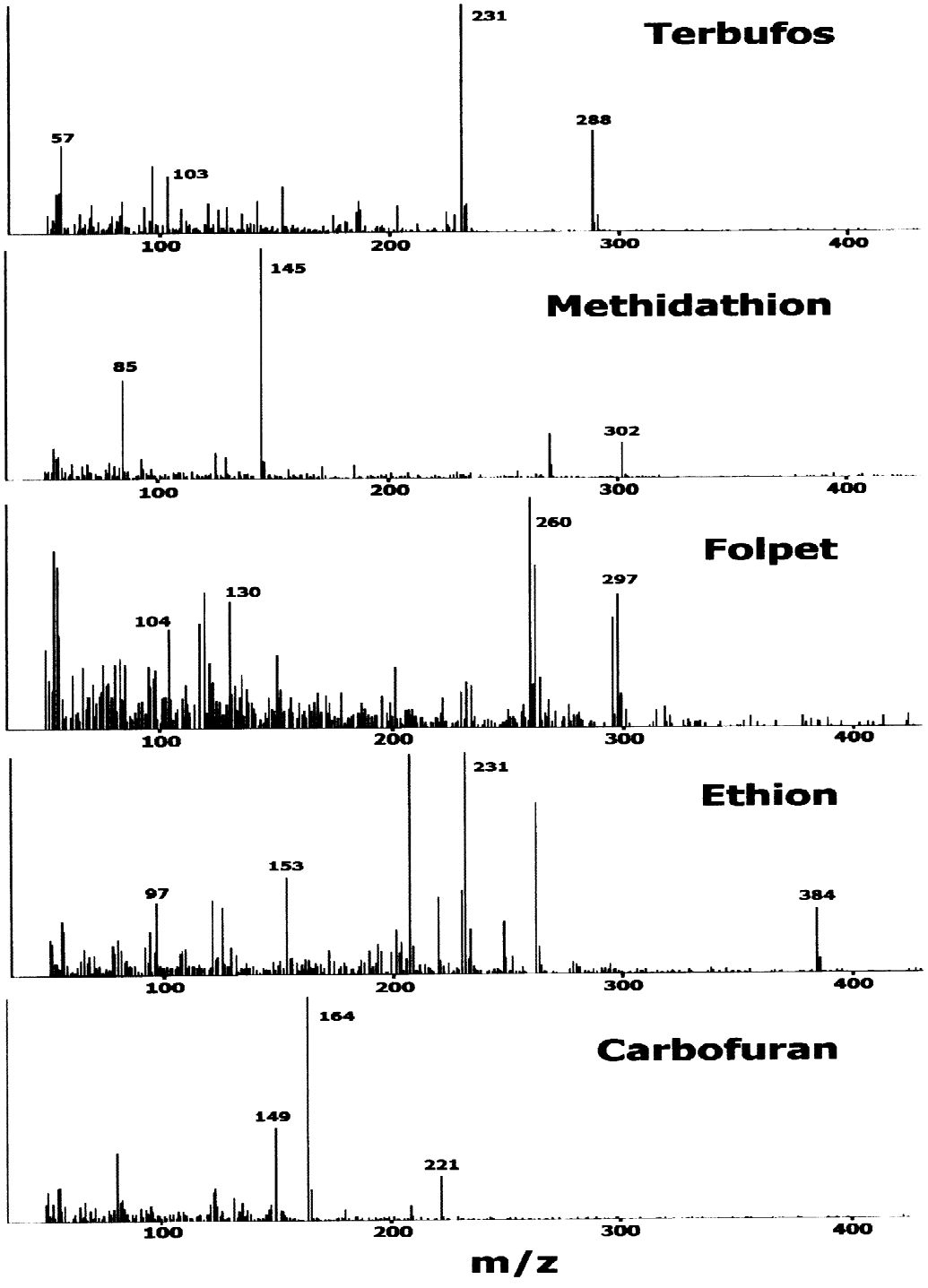

Fig. 9. Raw data of the indicated pesticides spiked at 70 ng/g in coriander obtained with the Supersonic GC–MS. Note the background congestion at the low mass spectral range.

Table 3
Summary of pesticide mass spectral data obtained with the Supersonic GC–MS and standard GC–MS (Saturn 2000, 5972 MSD and GCD); see the discussion in the text (Section 3.5) for details about the table

A	B Name	C Type	D M.W.	E 3 Ions	F 2 SMB ions	G M^+ in SMB	H M^+ in NIST	I M^+ in saturn	J M+ in GCD	K M+ in 5972	L SMB/NIST	M SMB/SAT	N Interference reduction	O Sensitivity loss
1	Cyfluthrin	Pyr	433	163+226+206 1	433+226 0	12.1	3.3	0.0			3.7	12.1	3.4	2.9
2	Cypermethrin	Pyr	415	163+181+91 6	415+163 2	9.2	1.7	1.3			5.4	7.1	22.4	4.2
3A	Deltamethrin	Pyr	503	253+181+208 1	422+253 0	25.2	5.0				5.0		10.0	1.5
3B	Deltamethrin	Pyr	503	253+181+208 1	503+253 1	10.0	5.0				2.0		10.0	3.7
4	Esfenvalerate fenvalerate	Pyr	419	125+167+225 1	419+225 0	37.6	54.0	13.0			0.7	2.9	25.2	2.1
5	Permetrin (T+C)	Pyr	390	183+163+77 1	390+183 5	11.0	1.4	0.5	0.0	0.3	7.9	22.0	134.5	2.2
6	Piperonyl butoxide	Pyr	338	176+149+119 30	338+176 7	9.5	3.3	0.0	0.6	1.8	2.9	7.2	56.3	1.4
7	Butylate	Carb	217	57+156+174 2	217+174 14	74.0	15.0	4.0			4.9	18.5	14.3	0.5
8	Carbaryl	Carb	201	144+115+116 57	201+144 40	14.0	7.5	1.0	0.0	4.7	1.9	14.0	9.0	1.9
9	Carbofuran	Carb	221	164+149+122 17	221+164 14	28.0	6.2	4.0	5.2	6.0	4.5	7.0	17.0	0.7
10	Methiocarb	Carb	225	168+153+109 0	225+168 12	33.4	8.2	0.0	5.0	8.2	4.1	33.4	16.5	1.1
11	Methomyl	Carb	162	105+58+88 1	162+105 466	16.0	2.5	ND	ND	ND	6.4		4.8	5.1
12	Propoxur	Carb	209	110+152+81 61	209+110 17	11.5	0.0	0.0			11.5	11.5	21.1	1.0
13	Vernolate	Carb	203	128+86+146 22	203+128 3	44.0	5.2	0.0			8.5	44.0	8.4	0.2
14	Alachlor	N	269	160+188+237 2	269+188 0	41.0	6.4	11.3			6.4	3.6	5.6	0.4
15	Atrazine	N	215	200+215+173 1	215+200 9	92.0	62.0	66.0			1.5	1.4	15.2	0.4
16	Chlorothalonil	N	264	266+229+109 5	266+229 7	100	100	100			1.0	1.0	40.0	0.9
17	Chlorpropham	N	213	127+213+171 4	213+171 16	63.4	32.2				2.0		10.0	0.5
18	Cyanazine	N	240	225+198+68 0	240+225 25	100	46.0	50.0			2.2	2.0	176.0	0.6
19	Dicloran	N	206	124+176+206 1	206+176 28	100	86.7	94.7			1.2	1.1	1.6	2.6
20	Diphenylamine	N	169	169+168+167 40	169+168 100	100	100	100			1.0	1.0	2.1	0.5
21	Diuron	N	232	72+232+187 10	232+72 2	68.0	20.0				3.4		1.0	0.1
22	Folpet	N	295	260+104+130 1	297+260 0	47.0	24.6	5.3	ND	3.6	1.9	8.9	176.0	1.0
23	Imazalil	N	296	215+173+81 105	296+215 11	23.0	2.0				11.5		461.5	0.5

Table 3. Continued

A	B Name	C Type	D M.W.	E 3 Ions	F 2 SMB ions	G M^+ in SMB	H M^+ in NIST	I M^+ in saturn	J M+ in GCD	K M+ in 5972	L SMB/NIST	M SMB/SAT	N Interference reduction	O Sensitivity loss
24	Iprodione	N	329	56+70+187 9	329+314 13	26.4	0.0	2.7	26.4	9.8	26.4	9.8	2273.0	0.8
25	Linuron	N	248	61+248+160 3	248+160 3	100	9.8				10.2		4.4	0.3
26	Metolachlor	N	283	162+238+146 1	283+238 13	10.1	0.0	1.0			10.1	10.1	78.0	1.5
27	Metribuzin	N	214	198+144+103 2	214+198 10	29.3	3.8	0.0			7.7	29.3	115.0	0.5
28	Myclobutanil	N	288	82+179+150 0	288+179 30	26.8	14.0				1.9		24.6	1.7
29	Pendimethalin	N	281	252+162+192 1	281+252 19	11.5	11.5	0.0			1.0	11.5	75.3	1.2
30	Prometryn	N	241	58+184+241 0	241+184 5	100	65.6				1.6		34.9	0.7
31	Propachlor	N	211	120+77+176 12	211+176 3	41.4	8.2				5.0		31.4	0.8
32	Propanil	N	217	57+161+63 1	217+161 39	36.4	14.0				2.6		22.5	0.4
33	Propyzamide	N	255	173+145+255 4	255+173 4	77.9	23.8				3.3		7.4	0.4
34	Simazine	N	201	201+186+173 2	201+186 25	100	79.0				1.3		1.8	0.9
35	Tetrahydro-phthalimide	N	151	79+151+80 19	151+123 404	100	47.0				2.1		7.8	3.7
36	Thiabendazole	N	201	201+174+129 3	201+174 8	100	100	100			1.0	1.0	26.0	0.2
37A	Trifluralin	N	335	306+264+248 2	306+264 12	100	9.8				10.2		0.7	0.1
37B	Trifluralin	N	335	306+264+248 2	335+306 9	10.0	9.8			7.6	1.0		0.7	1.0
38	Vinclozolin	N	285	285+212+198 0	285+212 3	100	100				1.0		1.8	0.8
39	Acephate	P	183	136+94+183 11	183+136 30	19.0	7.0				2.7		5.5	0.4
40	Azinphos-methyl	P	317	160+132+77 22	317+160 10	7.7	0.0	0.0			7.7	7.7	14.1	11.0
41	Carbophenothion	P	342	157+342+97 0	342+157 2	58.0	76.0				0.8		10.4	1.0
42A	Chlorfenvinphos	P	358	81+109+267 12	358 +323 4	9.1	0.0				9.1		1675.8	0.9
42B	Chlorfenvinphos	P	358	81+109+267 12	358+267 12	9.1	0.0				9.1		391.3	3.1
43	Chlorpyrifos-methyl	P	323	286+125+109 0	323±286 1	23.9	2.5				9.6		892.0	1.0
44	Diazinon	P	304	179+137+152 1	304+179 1	71.0	47.0	73.0		35.3	1.5	1.0	5.0	1.0
45	Dimethoate	P	229	87+125+93 2	229+125 2	86.0	9.0	8.0	2.5	5.3	9.6	10.8	2.3	0.5

Table 3. Continued

A	B Name	C Type	D M.W.	E 3 Ions	F 2 SMB ions	G M^+ in SMB	H M^+ in NIST	I M^+ in saturn	J M+ in GCD	K M+ in 5972	L SMB/NIST	M SMB/SAT	N Interference reduction	O Sensitivity loss
46	Disulfoton	P	274	88+97+60 1	274+186 1	82.1	4.9	0.0			16.8	82.1	31.0	3.0
47	Disulfoton sulfone	P	306	213+153+97 0	306 +213 6	12.0	0.5				24.0		45.3	5.9
48	Ethion	P	384	97+231+153 0	384+231 0	26.0	33.5	8.2	1.0	5.0	0.8	3.2	66.7	3.2
49	Ethoprophos	P	242	158+97+139 1	242+158 6	53.0	33.0	5.0			1.6	10.6	8.6	1.1
50	Fenamiphos	P	303	303 +154+288 0	303 +288 10	100	100				1.0		44.5	0.7
51	Fenthion	P	278	278+125+109 2	278+169 9	100	100				1.0		4.2	1.4
52A	Malathion	P	330	125+173+93 2	256+173 24	21.0	7.5	1.0			2.8	7.5	12.0	4.4
52B	Malathion	P	330	125+173+93 2	330+173 13	3.0	0.0	0.0		0.0	3.0	3.0	7.0	31.3
53	Methidathion	P	302	85+145+93 8	302+145 10	20.0	1.7	0.0	0.7	1.5	11.8	20.0	15.7	1.9
54	Mevinphos	P	224	127+109+192 3	224+192 40	11.0	1.6	1.8			6.9	6.1	18.5	2.7
55	Omethoate	P	213	156+110+79 3	213+156 8	20.0	2.5				8.0		47.0	1.4
56	Parathion	P	291	97+109+291 4	291+155 18	100	45.0	100			2.2	1.0	2.0	1.3
57	Parathion methyl	P	263	109+125+263 3	263 +125	100	86.0	100		60.8	1.2	1.0	1.0	1.3
58	Phorate	P	260	75+121+260 2	260+121 7	100	17.2				5.8		2.9	0.2
59	Phosalone	P	367	182+121+97 4	367+182 5	31.0	11.0	33.3			2.8	0.9	29.4	1.4
60A	Phosmet	P	317	160+104+133 63	317+160 10	11.1	0.0	0.0			11.1	11.1	36.0	1.4
60B	Phosmet	P	317	160+61+76 8	317+160 10	11.1	0.0	0.0			11.1	11.1	109.0	1.5
61	Terbufos	P	288	231+57+103 2	288+231 9	41.8	5.7	0.0	2.3	3.1	7.3	41.8	184.0	0.4
62	Captafol	Cl	347	79+313+183 16	349+183 14	24.0	6.0				4.0		7.8	0.5
63	Chlordanes	Cl	406	373+237+272 0	408+373 0	17.8	0.0	0.0			17.8	17.8	47.0	2.8
64	Dacthal	Cl	330	301+142+332 0	332+301 4	38.7	32.5	17.3			1.2	2.2	681.0	0.8
65	DDD	Cl	320	235+165+199 12	320+235 5	7.7	3.3	1.3			2.3	5.9	17.7	3.1
66	DDE	Cl	318	246+318+176 2	318+246 2	100	46.7	100			2.1	1.0	31.8	0.3
67	DDT	Cl	354	235+165+199 13	354+235 4	8.2	4.1	0.0			2.0	8.2	16.5	2.6

Table 3. Continued

A	B Name	C Type	D M.W.	E 3 Ions	F 2 SMB ions	G M^+ in SMB	H M^+ in NIST	I M^+ in saturn	J M+ in GCD	K M+ in 5972	L SMB/NIST	M SMB/SAT	N Interference reduction	O Sensitivity loss
68	Dicofol	Cl	368	139+251+111 4	368+251 13	3.8	0.0	0.0			3.8	3.8	104.0	8.0
69	Dieldrin	Cl	378	79+263+277 0	380+277 3	27.0	16.4	15.3			1.6	1.8	176.0	1.1
70	Endosulfan I+II	Cl	404	195+207+241 1	404+339 1	20.0	0.0	0.0			20.0	20.0	29.0	3.6
71	Endosulfan sulfate	Cl	420	272+229+387 0	420+387 1	26.4	22.5	29.3	5.0	7.7	1.2	0.9	107.6	2.6
72	Endrin	Cl	378	263+279+81 0	380+345 2	58.0	0.0	0.0			58.0	58.0	4100.0	0.5
73	Heptachlor	Cl	370	272+337+100 0	374+272 2	10.0	9.4	5.3			1.1	1.9	173.0	1.9
74	Heptachlor epoxide	Cl	386	353+237+81 1	386+353 4	16.3	10.7	0.0			1.5	16.3	3350.0	1.9
75	Hexachlorobenzene	Cl	284	284+249+142 5	284+249 13	100	100	100			1.0	1.0	19.0	0.9
76	Lindane BHCs	Cl	288	181+219+109 5	290+219 3	43.0	6.8	0.0			6.3	43.0	45.5	1.4
77	Methoxychlor	Cl	344	227+152+274 3	344+227 9	4.3	3.3	0.0		1.0	1.3	4.3	120.0	1.1
78	Methoxychlor olefin	Cl	308	238+308+223 0	308+238 3	100	69.2	100			1.4	1.0	2.3	0.7
79	Mirex	Cl	540	272+237+119 7	540+272 0	8.2	0.0	0.0			8.2	8.2	385.2	3.0
80	Nonachlors	Cl	444	409+411+237 4	444+409 3	7.5	2.5	0.0			3.0	7.5	303.3	1.6
81	Pentachloroanisole	Cl	278	265+280+237 2	280+265 15	100	75.0	86.0			1.3	1.2	68.0	0.8
82	Pentachlorobenzene	Cl	248	250+215+108 3	250+215 21	100	100	100			1.0	1.0	22.7	1.1
83	Quintozene	Cl	295	237+249+265 0	295+237 3	100	59.8	100			1.7	1.0	0.9	0.7
84	Tecnazene	Cl	261	203+215+261 1	261+203 4	95.2	72.1	100			1.3	1.0	1.0	0.8
85	Tetrachloro-isophthalnitril	Cl	264	266+229+124 7	266+229 17	100	100	100			1.0	1.0	28.7	0.8
86	Tetrachlorvinphos	Cl	364	329+109+79 5	366+329 2	10.1	0.0	0.0			10.1	10.1	2780.0	2.0
87	*o*-Phenylphenol	Other	170	170+141+115 51	170+141 34	100	100	100			1.0	1.0	4.7	0.4
88	Propargite	Other	350	135+173+81 23	350+201 0	88.4	13.9	52.0	3.0	9.7	6 4	1.7	61.7	0.4

measured with the GCD with ~155 °C ion source temperature; (K) The relative abundance of the pesticide M^+ as measured with the Agilent 5972 MSD with 167 °C ion source temperature; (L) The relative gain in the abundance of the M^+ achieved with the Supersonic GC–MS in comparison with that M^+ abundance in the NIST‘98 library; (M) The relative gain in the abundance of the M^+ achieved with the Supersonic GC–MS in comparison with that measured with the Varian Saturn 2000 GC–MS; (N) Matrix interference reduction factor (MIRF) achieved through the analysis of the pesticide with the Supersonic GC–MS with the two ions indicated in column F in comparison with the 3-ion method

using the ions indicated in column E (further explanations are given below and in Section 3.6); and (O) instrumental sensitivity loss factor through the use of the M^+ with the Supersonic GC–MS instead of the third strongest ion in the NIST'98 library spectrum.

The sensitivity loss factor is simply obtained by the division of the relative abundance of the M^+ in column (G) over the relative abundance of the weakest ion among the three ions in column (E). MIRF was calculated based on data taken from Fig. 3 in which a 29-fold increase in matrix background was calculated for every 100 amu fragment mass decrease. To be more conservative in the calculations, we used a value of 20 per 100 amu fragment mass increase.

We are introducing a concept that we call the Supersonic GC–MS 2-ion method (STIM) for analyte identification as an approach in which the M^+ is always included in the identification, and an additional intense verifying ion (often the base peak or high-mass neighbor) is used as the 2nd verifying ion. In STIM, the M^+ generally replaces the two lowest mass fragment ions in the 3-ion method. In most cases with the 3-ion method, the lowest mass fragment (among the three) is the one which is most affected by interferences. This effect is demonstrated in the calculation in Table 3 for many of the pesticides.

Thus, if for example we consider methiocarb (see Fig. 5 and pesticide no. 10 in Table 3 for a comparison of spectra and data), analysis by STIM would use the M^+ ($m/z=225$) and high mass fragment ($m/z=168$). In the 3-ion method with traditional GC–MS data, the three largest fragments from the NIST'98 spectrum are $m/z=168$ (base peak), $m/z=153$ (93% relative abundance) and 109 (35%). Thus, the ability to identify methiocarb would be limited by matrix interference at the $m/z=109$ mass chromatogram. In a complex matrix, assuming 20 times lower matrix interference every 100 amu, a mass difference of 59 amu between $m/z=168$ and $m/z=109$ implies lower matrix interference at the $m/z=168$ by a factor of 5.85. The $m/z=168$ peak has a 2.82 times greater relative abundance in the NIST library spectrum than the $m/z=109$ peak. Thus, MIRF in this case is $5.85\times 2.82=16.5$. In addition, the $m/z=109$ for methiocarb in the NIST library is 1.1 times higher than the $m/z=225$ M^+ in Supersonic GC–MS, and this factor of 1.1 is the sensitivity loss factor for methiocarb.

Several conclusions emerge from the data presented in Table 3 about the cold EI mass spectra of pesticides and its implications for pesticide analysis as follows.

3.5.1. Presence of the molecular ion

For all 88 pesticides that exhibited a M^+ in Supersonic GC–MS, the weakest M^+ was for malathion (no. 52 in Table 3) with relative abundance of 3%. Even for this pesticide, however, the M^+ could be replaced with the high mass fragment $m/z=256$, which showed increased relative abundance of 21%. In contrast, only 75 out of the 88 pesticides (85.2%) showed a M^+ in the NIST library, and in the Saturn 2000 GC–MS experiment, only 36 among the 63 pesticides (57%) investigated showed a M^+ with relative abundance $>1\%$. No difference was observed in the average abundance of the M^+ obtained with the Saturn 2000 GC–MS and Agilent 5972 MSD and GCD. While some random variations occurred, the average results were very similar with both the ion trap and quadrupole based GC–MS systems. Thus, clearly the M^+ is far less frequent under real analysis conditions than in the NIST library.

3.5.2. Intensity of the molecular ion

The average relative abundance of the M^+ among all 88 investigated pesticides was 50% with the Supersonic GC–MS and 29% in the NIST library. For the 63 pesticides analyzed with Saturn 2000 GC–MS, the average relative abundance of the M^+ was 30%. However, if those pesticides with 100% relative M^+ abundance are removed from the statistics (since the SMB gain cannot be calculated in those cases), the average M^+ abundance becomes 40% with the SMB, 16% in the NIST, and 12% with the Saturn 2000 GC–MS, which suggests about a 3-fold increase in average M^+ abundance in SMB-MS. From the 63 pesticides that were also analyzed by Saturn 2000, MSD, or GCD, 27 pesticides showed no M^+. The M^+ was always observed with the Supersonic GC–MS and had an average relative abundance of 20% in these most difficult but important situations.

3.5.3. Molecular ion gain with Supersonic GC–MS

The average gain in the relative abundance of the M^+ using the Supersonic GC–MS in comparison with the NIST library mass spectra was a factor of 5.6. This factor is the average sum of all the ratios of the M^+ abundance obtained with the Supersonic GC–MS divided by the NIST abundances (average value of the gain factors which is the average of data in column L in Table 3). This factor was obtained by assuming 1% relative abundance for the M^+ if it was absent since a division by zero cannot be attempted. Similarly, the average gain in comparison with Saturn 2000 GC–MS mass spectra is a factor of 10.8 (this factor was also obtained by assuming 1% relative abundance for the M^+ if it was absent). Thus, we claim that the M^+ relative abundance is increased by about a factor of 10 with the Supersonic GC–MS, and that the weaker the molecular ion under thermal EI conditions the higher is this gain factor.

3.5.4. Confidence level in the pesticide identification

The use of STIM in comparison with the standard 3-ion method in conventional GC–MS results in an average 2.4-fold increase in the number of potential interfering NIST library compounds. The use of the base peak in traditional GC–MS pesticide spectra, on average, results in 1440 other compounds that give relative abundance $>40\%$ in NIST'98 (which is a measure of the degree of possible matrix interferences). This number is only approximately 1.3% of the approximately 108 000 compounds in NIST'98. The addition of the 2nd most intense ion reduces the average number of potential interfering compounds to 90.4, and the addition of the 3rd ion further reduces it to 8.4. Thus, each addition of an ion reduces the potential interference with a gradually reduced effect. The use of STIM results in an average of 20.1 as the number of potential interfering compounds (thus the factor of 2.4 difference). However, considering the uniqueness of the M^+ for identification and the considerably increased orthogonality of the GC and MS separations using the M^+, STIM is claimed to provide greater confidence level in the pesticide identification. From the psychological point of view the M^+ is unique (some say singular) in the provision of the greatest confidence level in the identification. Without it, many people feel that the identification cannot be trusted. Such reservations have good justification since homologous compounds or degradation products of the suspected pesticide could have similar mass spectra at the low mass spectral range. Large aliphatic compounds for example are famous for having similar mass spectra for many different compounds. The GC separation adds important information because most co-eluting compounds can have the same low mass fragments, but their probability to have the same M^+ is far smaller (orthogonal GC and MS separation). Furthermore, the 3-ion method in Supersonic GC–MS can still be used to increase the confidence level in the identification if the analyst wishes. The Supersonic 3-ion method improves (reduces) the number of NIST library interfering compounds by a factor of 1.3 in comparison with the standard three ions method while still reducing the matrix interference by a factor of 12. In any case, true evaluations of different approaches to confirmation should be pursued in real applications to determine actual rates of false positives and negatives.

3.5.5. Instrumental sensitivity variation

The average relative height of the M^+ obtained with the Supersonic GC–MS was similar to that of the third ion in the 3-ion method (it was weaker by a factor of 1.05 in our experiments). Thus, because the flux sensitivity of the Supersonic GC–MS is similar to that of standard GC–MS [34], the instrumental sensitivity (difficulty) is equivalent with both methods. Since matrix interference is often the limiting source of noise in pesticide analysis, instrumental sensitivity is not so important. Certainly for thermally labile compounds the Supersonic GC–MS is more sensitive than standard GC–MS systems.

3.5.6. Matrix interference reduction factor (MIRF)

The average reduced degree of matrix interference over all 88 investigated pesticides was calculated as a factor of 225. This is the average of the 88 MIRFs as listed in column (N) in Table 3. If both the four highest and lowest numbers are removed, the average MIRF becomes 90. These numbers were calculated assuming 20 times reduced matrix interference every 100 amu increased mass, and normalizing them to the relative height of the lowest mass peaks

in both methods. This factor was found to be especially high with difficult pesticides, such as iprodione, folpet and metribuzin where Supersonic GC–MS provides clear advantages. Thus, STIM, through the enhanced M^+, provides the most significant gains where it is needed with the more difficult pesticides, and this feature is further strengthened by the increased analysis capabilities of thermally labile pesticides.

3.6. Supersonic 2-ion method (STIM)—a proposed new method for achieving lower matrix interference and identification levels

Our goal is to conduct faster GC–MS analysis, of a broader range of pesticides, with reduced matrix interference and thus lower detection and identification limits, even for 'difficult' pesticides, and with increased confidence level in the pesticide identification. It is realized that there is an internal conflict between making the analysis faster and reducing the degree of matrix interference. We demonstrated (Fig. 3) that matrix interference is exponentially reduced versus increasing monitored mass. Thus, the central ingredient of our proposed new method is the use of the Supersonic GC–MS in order to enhance the abundance of the molecular ion and other high mass fragments. Our proposed method for simultaneously maximizing speed and sensitivity in multiresidue analysis involves the following steps.

1. Sampling is performed with the ChromatoProbe sample introduction device for intra-injector thermal desorption of pesticides, after simply blending the food sample with a solvent [5,19,35,44–46] (no clean-up or evaporation steps are needed).
2. Fast Supersonic GC–MS analysis of a broad range of pesticides, including thermally labile ones, is performed using a short GC column with high column flow-rate.
3. Improved pesticide identification combined with reduced matrix interference is achieved through the enhanced M^+ provided by cold EI of the compounds in the SMB.

The mass spectrometer is used in full scan mode with reconstructed chromatograms obtained at the M^+ and a second major ion only for targeted analytes. Pesticide identification with STIM requires the co-elution of the appropriate ions in the correct ion ratio at the proper narrow retention time window. For every pesticide that is found in this way, a standard library search and identification is performed with automated background subtraction (such as AMDIS). If such full library identification fails due to extended low mass matrix interference, the STIM approach can still provide sufficient evidence for confirmation. Pesticide identification through its M^+ and one additional major high mass fragment is claimed to be equivalent or superior to its identification with 3 ions that do not include the M^+ due to the unique contribution of the M^+ to positive pesticide identification. As shown in Table 3, the use of full scan MS with STIM data analysis enables GC–MS analysis with considerably reduced matrix interference by an average factor of 90. This major reduction of matrix interference can be achieved for potentially unlimited number of pesticides, unlike selected ion monitoring or MS–MS in which specific conditions are required in time segments for targeted pesticides. Data analysis with STIM still needs targeted pesticides, but it can be a much longer list and accomplished with a single injection.

The reduced matrix interference provided by Supersonic GC–MS enables faster chromatographic analysis with reduced chromatographic separation requirements (still with GC peak widths of 1.2–1.8 s). Accordingly, in our approach, the GC column length was reduced to 6 m and the column flow velocity was increased about 12 times above the U_{opt} from the van Deemter plot so that a speed enhancement factor of 60 was achieved [17] (column length reduction factor time the column velocity increase factor). An SEF of 60 results in up to 8 times poorer chromatographic separation efficiency, and thus, 8 times greater degree of matrix interference. However, the use of Supersonic GC–MS and STIM results in a MIRF of 90, and the combination of use of Supersonic GC–MS with short column, high carrier gas flow-rate and STIM data analysis provides a unique combination of both reduced degree of potential matrix interference by a factor of 11 in our example and much faster analysis as demonstrated in Fig. 4. The SEF of 60 is translated into a combination of 5-fold faster chromatographic analysis and considerably lower pesticide elution temperatures by about 60 °C.

The three goals of lower degree of matrix interfer-

ence, faster analysis, and broader range of pesticides amenable for analysis are simultaneously achieved with the Supersonic GC–MS. Naturally, one can still choose to use a standard column and carrier gas flow-rate and have no gain in chromatographic analysis time, and only standard GC–MS pesticides will be analyzed. In this case a major reduction in matrix interference can be achieved with the consequence of lowest possible identification limits and highest confidence level in the identification at a given low level of pesticides.

Fig. 10 demonstrates how STIM data analysis was used for 13 pesticides spiked at 70 ng/g in a difficult coriander matrix. The 13 pesticides are the same as in Fig. 4 but now at lower levels in coriander matrix. As the figure shows, each pesticide was isolated in RSIM chromatograms of its M^+ and a prominent high mass fragment. All 13 pesticides were identified, and although some matrix interference was observed at other retention times, only the pesticide of interest gave co-elutions of both ions in the correct peak height ratios at the correct retention times.

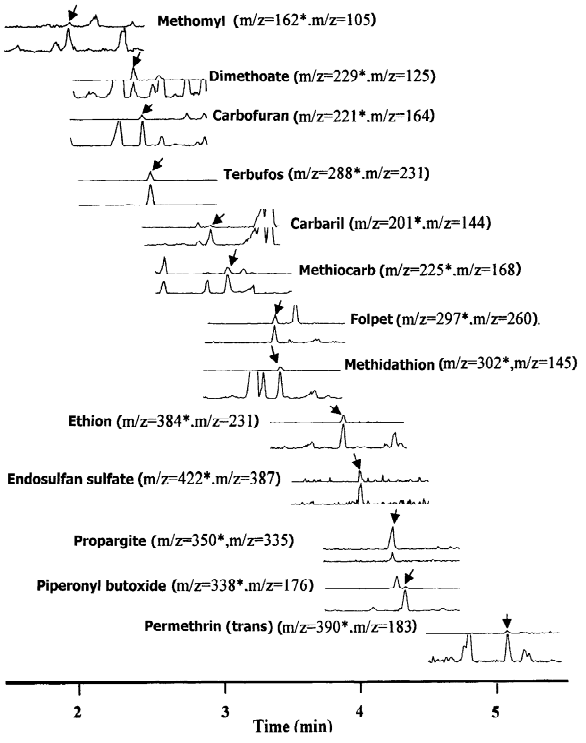

Fig. 10. Pesticide analysis of the indicated 13 pesticides spiked at 70 ng/g in coriander. Data analysis is performed as shown according to the supersonic 2-ions method (STIM), and thus each pesticide is analyzed with two mass chromatograms consisting of its M^+ and one additional high-mass fragment ion. Correct retention time, elution time overlap of ions, and correct peak height ratio is also required for confirmation. Arrows indicate the expected retention times. Note that all 13 pesticides were detected in this way, and that, except for endosulfan sulfate, matrix interference is the limiting source of noise. The optic injector initial temperature was 100 °C and programmed to 260 °C at a rate of 8 °C/s. The GC oven started at 80 °C for 30 s following by temperature programming rate of 35 °C/min up to 310 °C.

Similar experiments were performed with mustard garlic (a spice known as Rocket) and tomato extracts. Rocket exhibited a very complex matrix typical of a spice whereas tomato was easier to analyze due to its lower degree of matrix interference. Fig. 10 also shows how instrumental sensitivity was not the limiting factor, which is common in GC–MS of complex samples, and even with STIM, matrix interference is still the bottleneck. However, endosulfan sulfate in Fig. 10 showed instrumental sensitivity limitations since its mass spectrum has many weak peaks rather than few strong ones. In an easier matrix such as tomato, instrumental sensitivity is the limiting factor more frequently than in more complex matrices.

We note that other soft ionization methods such as chemical ionization (CI) [47–49] can be used for pesticide analysis with the benefit of an enhanced M^+. However, CI is incompatible with mass spectral matching and identification of pesticides using the common EI-based libraries. Also, only certain pesticides give sufficient responses in CI (some require positive CI whereas others work better in negative CI). In addition, CI is less sensitive than EI, and it requires a closed ion source that is less robust than EI. For these reasons, CI is not as useful as our cold-EI approach for achieving universal pesticide analysis. In some routine pesticide analysis laboratories, CI is applied only for certain pesticides or applications for which EI alone is not adequate. It is not unusual for a laboratory to run GC–MS using EI followed by a second run in CI to provide better overall results, but CI has not been adopted as a central ionization method for pesticide analysis.

Low electron energy EI is another potentially soft ionization method that is possible on certain commercial instruments. However, this feature is seldom

used and is incompatible with universal pesticide analysis requirements. The use of 20 eV electron energy EI results with eight times lower EI cross section [15]. In addition, the maximum obtainable electron emission current is reduced by a factor of about 6 due to increased space charge between the filament and ion cage, resulting in an unacceptable pesticide signal loss factor of about 50.

We investigated low electron energy pesticide analysis in the Supersonic GC–MS and found that many pesticides did not show any increase in the relative abundance of the M^+. Even with low electron energies such as 10–14 eV in which the pesticide signal almost disappears, we found only limited increase in the relative abundance of the M^+ for several pesticides. We explain this observation by assuming that for certain molecules only a limited range of electronic states are excited in the electron ionization process while other ions electronic states are inaccessible due to unfavorable Frank Condon factors. Consequently, the EI mass spectra of several pesticides are practically independent on the electron energy, and in our experience, low electron energy EI is not a viable option for pesticide analysis.

3.7. Fast pesticide sample preparation and large volume injection of 'dirty' samples with the ChromatoProbe

Without doubt, the most time-consuming aspect of pesticide analysis is sample preparation. Because agricultural product cannot be injected as is into the GC, it must be extracted with a solvent. This generally entails the need for removal of co-extracted water, clean-up of interfering matrix components, and solvent evaporation steps. This time-consuming, expensive, and laborious procedure is needed in order to reduce matrix interference and prevent excessive injector liner and analytical column degradation due to their contamination with low-volatility components. Sample introduction by thermal means is ideal for GC since it can be used to selectively introduce only volatile and semivolatile compounds into the GC column. The ChromatoProbe direct sample introduction device [5,19,22,35,44–46] is a simple tool that converts a standard temperature programmable GC injector into an intra-injector thermal desorption device with excellent inertness and GC integrity.

With the ChromatoProbe, typically 5–15 μl of sample extract is placed in a small glass microvial, which is then placed in a probe. The probe is then inserted into the GC liner that is kept at reduced temperature long enough for the solvent to evaporate (about 1 min). The inlet temperature is then rapidly increased to volatilize the analytes, which are focused at the head of the analytical column. Then, the GC analysis proceeds normally. Afterwards, the spent microvial is removed along with the non-volatile matrix components that normally would contaminate the GC system. Thus, the approach is more rugged than even traditional injection methods. The ChromatoProbe has been extensively tested and evaluated for use with pesticide analysis [19,44–46].

The ChromatoProbe serves to significantly reduce the sample preparation time through the elimination of clean-up and solvent evaporation steps and/or it can serve for large concentrated extract volume injection to increase the instrumental sensitivity. However, unlike the use of standard large volume introduction, large volume injection with the ChromatoProbe eliminates the introduction of low-volatility matrix components into the column and liner since these components remain in the microvial, which is thrown away after every analysis.

Fig. 11 shows RSIM chromatograms of ethion and terbufos in coriander obtained involving the use of the ChromatoProbe for large volume injection. In the analysis, 20 μl of a 5 g/ml acetone extract of 20 ng/g pesticides were added to the microvial. Thus, an equivalent of 100 mg coriander, which contained 2 ng ethion and terbufos, was introduced with the ChromatoProbe.

Fig. 11 clearly demonstrates the effectiveness of STIM. We chose ethion and terbufos because they share the same low mass fragment ions and have very similar mass spectra in conventional MS analysis. The mass chromatograms of the molecular ions, $m/z = 384$ for ethion and $m/z = 288$ for terbufos, have no interfering matrix peaks. The mass chromatogram at the $m/z = 231$ high-mass fragment is also clean around the elution time of terbufos whereas some nearby eluting matrix peaks are observed around the elution time of ethion, but they do not interfere in the analysis. In contrast, the RSIM

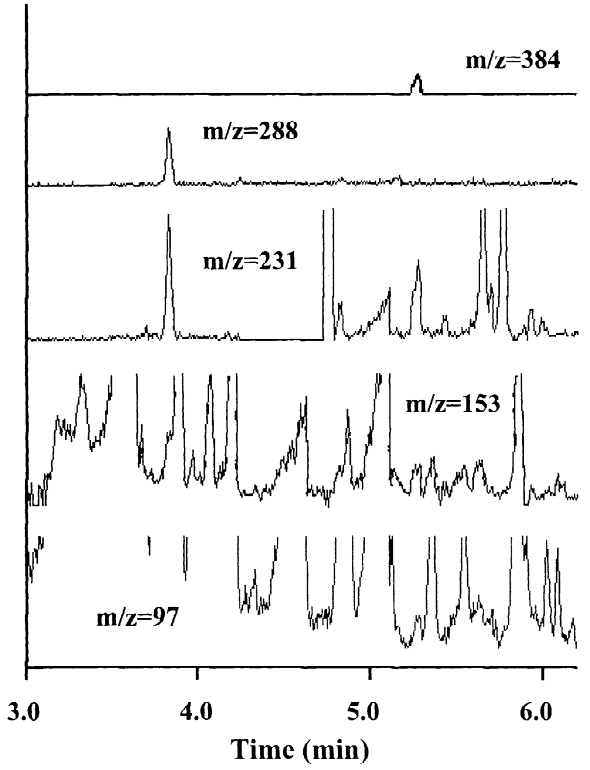

Fig. 11. Large extract volume injection with the ChromatoProbe for obtained improved instrumental concentration sensitivity. A 20-μl coriander extract of 5 g/ml spiked with 20 ng/g terbufos and ethion was introduced into the ChromatoProbe microvial (equivalent of 2 ng pesticide in 100 mg coriander). The mass chromatograms at the molecular ions $m/z=384$ and 288 are shown as well as the mass chromatograms at the lower mass fragments $m/z=231$, 153 and 97. Note the large increase of matrix interference in the mass chromatograms of the low mass fragments and the high instrumental sensitivity obtained with the 'clean' mass chromatograms at the molecular ions.

mass chromatograms at $m/z=153$ and $m/z=97$, that are needed in the standard 3-ion confirmation method, are very complex and show an uneven baseline. Thus, the analysis with the 3-ion method would fail for these two pesticides in coriander, even at higher concentrations than 20 ng/g.

As in the case of Fig. 3, Fig. 11 also demonstrates the substantial increase of matrix interference even with the M^+ enhancement in Supersonic GC–MS. The exponential relationship between matrix interference and mass is retained in cold EI, but the mass from which this effect begins is expected to shift slightly to higher masses than in standard thermal EI.

Clearly, the use of the ChromatoProbe for large volume injection enables lower instrumental detection limits and thus can be used with easier matrices if <10 ng/g detection limits are needed. However, for the majority of analytical needs, the ChromatoProbe should be used with blended only samples in order to save sample preparation time [5,19,44–46].

Large volume injection with the Supersonic GC–MS is more beneficial than with standard GC–MS for two reasons: (a) STIM data analysis reduces the degree of matrix interference, and thus large volume injection can lower the detection limits when matrix is not the limiting source of noise; (b) a megabore (0.53 mm I.D.) column can be used with the Supersonic GC–MS. Megabore columns have about an order of magnitude higher tolerance for the semivolatile matrix components that thermally desorb along with the pesticide analytes than 0.25 mm I.D. columns (the matrix tolerance increases at about the third power of the column diameter since it relates to the film volume per plate). Thus, with a megabore column without the use of the ChromatoProbe for sampling, either 10 times larger volumes can be repeatedly injected with similar column lifetime as of a standard 0.25 mm I.D. column, or its lifetime will be 10 times longer when the same equivalent sample amount is introduced. The lower separation power of the wider column is countered by improved selectivity of the Supersonic GC–MS with its enhanced M^+ and STIM data analysis. The use of megabore columns in pesticide analysis with the Supersonic GC–MS was previously demonstrated for the analysis of diazinon in chervil [19].

4. Conclusions

A new technique was developed based on the enhancement of the M^+ achieved with the Supersonic GC–MS. The pesticides are identified through mass chromatograms on their M^+ and an additional high mass fragment, the co-elution requirement of these two mass chromatograms and their pesticide specific peak height ratio. If a pesticide is identified this way further confirmation is attempted with standard full library search identification but the two ions method as above enables the lowest identification concentration. Furthermore, unlike MS–MS or

SIM this method has no limitation to the number of pesticides that can be analyzed with it and thus it is a true multipesticide residue method.

We found that the degree of matrix interference is exponentially reduced with the fragment mass by a factor of over 20 per 100 amu. As a result, the Supersonic GC–MS two-ion method (STIM) was found to substantially reduce the matrix interference by an average factor of 90 due to the enhancement of the M^+. In all, 88 pesticides were analyzed with the Supersonic GC–MS and a M^+ was observed in every case. In contrast, with standard GC–MS only 57% of the pesticides showed a M^+ with over 1% relative abundance. The average relative abundance of the M^+ with the Supersonic GC–MS was 50% and it was typically higher by about an order of magnitude in comparison with that observed with standard GC–MS.

Since matrix interference is the bottleneck in pesticide analysis with GC–MS, its reduction enables faster chromatography through the use of short columns with high flow-rate and rapid temperature programs. The sacrifice in chromatographic separation efficiency is made up by the reduction of matrix interference at the M^+ and high mass fragment ion. An important additional major benefit of this approach is the increased capability of analysis of thermally labile compounds such as methomyl and other carbamate pesticides. Thus, greater speed is achieved together with lower identification limits and broader range of pesticides that can be simultaneously analyzed. Furthermore, due to the possibility of high column flow-rate, a megabore column can be used with large volume injection to provide larger sample capacity and increased instrumental sensitivity, if needed. A ChromatoProbe device is also compatible for sample introduction in Supersonic GC–MS, and it can eliminate the need for clean-up and solvent evaporation steps and/or be used for large volume injection without introducing non-volatile matrix residue into the column.

While we described in this paper a new approach with several new features and their combinations, clearly the use of the Supersonic GC–MS with its enhanced M^+ is the central new ingredient of this method. This feature enables considerable reduction of matrix interference (including column bleed) and thus identification at lower concentrations with improved confidence. This method can either be used with a standard column and column flow-rate or with a shorter column and or higher column flow and temperature programming rates for the achievement of faster chromatographic separation and improved analysis capability of thermally labile pesticides. These capabilities of faster analysis of broader range of pesticides are coupled with some offset of lower degree of reduction of matrix interference due to the reduction of the separation power of the GC. We feel that the combination of faster analysis of broader range of pesticides with only limited reduction of matrix interference is beneficial and superior over the achievement of only substantial reduced matrix interference. However, the decision is flexible and may depend on the specific needs. Similarly applies for the choice of column and while we performed this research with a 0.2 mm I.D. short column, a megabore (or any other) column can be used.

The approaches described in this paper clearly require further exploration and validation. The purpose of this paper was to demonstrate the features of Supersonic GC–MS in difficult analyses and the possibilities that the new approach brings to providing better pesticide analysis and improving food safety.

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References

[1] M. Dressler, Selective Gas Chromatographic Detectors, Elsevier, Amsterdam, 1986.
[2] T. Cairns, J. Sherma, Emerging Strategies For Pesticide Analysis, CRC Press, Boca Raton, FL, 1992.

[3] S. Cheskis, E. Atar, A. Amirav, Anal. Chem. 65 (1993) 539.
[4] A. Amirav, H. Jing, Anal. Chem. 67 (1995) 3305.
[5] A. Amirav, H. Jing, J. Chromatogr. A 814 (1998) 133.
[6] J. Fillion, F. Sauve, J. Selwyn, J. AOAC Int. 83 (2000) 698.
[7] M. Gamon, C. Lleo, A. Ten, F. Mocholi, J. AOAC Int. 84 (2001) 1209.
[8] R.S. Sheridan, J.R. Meola, J. AOAC Int. 82 (1999) 982.
[9] S.E. Stein, J. Am. Soc. Mass. Spectrom. 10 (1999) 770.
[10] J.M. Halket, A. Przyborowska, S.E. Stein, W.G. Mallard, S. Down, R.A. Chalmers, Rapid. Com. Mass. Spectrom. 13 (1999) 279.
[11] S. Dagan, J. Chromatogr. A 868 (2000) 229.
[12] J.A. Sphon, J. Assoc. Off. Anal. Chem. 61 (1978) 1247.
[13] T. Cairns, R.T. Baldwin. Anal. Chem. 552A–557A (1995).
[14] R. Baldwin, R.A. Bethem, R.K. Boid, W.L. Budde, T. Cairns, R.D. Gibbons, J.D. Henion, M.A. Kaiser, D.L. Lewis, J.E. Matusik, J.A. Sphon, R. Stephany, R.K. Trubey, J. Am. Soc. Mass. Spectrom. 8 (1997) 1180.
[15] R.A. Bethem, R.K. Boyd, J. Am. Soc. Mass. Spectrom. 9 (1998) 643.
[16] S. Dagan, A. Amirav, Int. J. Mass. Spectrom. Ion Proc. 133 (1994) 187.
[17] S. Dagan, A. Amirav, J. Am. Soc. Mass. Spectrom. 7 (1996) 737.
[18] A. Amirav, S. Dagan, International Laboratory, (1996) 17A–17L.
[19] A. Amirav, S. Dagan, T. Shahar, N. Tzanani, S.B. Wainhaus, Fast GC–MS with Supersonic Molecular Beams, in: Advances in Mass Spectrometry, Vol. 14, Elsevier, 1998, p. 529.
[20] S. Dagan, A. Amirav, Eur. Mass. Spectrom. 4 (1998) 15.
[21] A. Amirav, N. Tzanani, S.B. Wainhaus, S. Dagan, Eur. Mass. Spectrom. 4 (1998) 7.
[22] S.B. Wainhaus, N. Tzanani, S. Dagan, M.L. Miller, A. Amirav, J. Am. Soc. Mass. Spectrom 9 (1998) 1311.
[23] A. Amirav, A. Danon, Int. J. Mass Spectrom Ion Proc. 97 (1990) 107.
[24] A. Amirav, Org. Mass. Spectrom. 26 (1991) 1.
[25] S. Dagan, A. Amirav, J. Am. Soc. Mass. Spectrom. 6 (1995) 120.
[26] A. Danon, A. Amirav, J. Chem. Phys. 86 (1987) 4708.
[27] A. Danon, A. Amirav, J. Phys. Chem. 93 (1989) 5549.
[28] A. Danon, A. Amirav, Israel J. Chem. 29 (1989) 443.
[29] A. Danon, A. Amirav, Int. J. Mass. Spectrom. Ion. Proc. 96 (1990) 139.
[30] A. Danon, A. Amirav, Int. J. Mass. Spectrom. Ion. Proc. 125 (1993) 63.
[31] S. Dagan, A. Amirav, T. Fujii, Int. J. Mass. Spectrom. Ion. Proc. 151 (1995) 159.
[32] S.C. Davis, A.A. Makarov, J.D. Hughes, Rapid. Com. Mass. Spectrom. 13 (1999) 237.
[33] S.C. Davis, A.A. Makarov, J.D. Hughes, Rapid. Com. Mass. Spectrom. 13 (1999) 247.
[34] A. Amirav, A. Gordin, N. Tzanani, Rapid. Com. Mass. Spectrom. 15 (2001) 811.
[35] A. Amirav, S. Dagan, Eur. Mass. Spectrom. 3 (1997) 105.
[36] F.W. McLafferty, R.H. Hertel, R.D. Villwock, Org. Mass Spectrom. 74 (1974) 690.
[37] A. Fialkov, A. Gordin, A. Amirav. Extending the Range of Compounds Amenable for GC–MS Analysis (in preparation).
[38] A. Maccoll, Org. Mass. Spectrom. 26 (1991) 235.
[39] W.A. Chupka, J. Chem. Phys. 54 (1971) 1936.
[40] W. Genuit, N.M.M. Nibbering, Int. J. Mass. Spectrom. Ion. Proc. 73 (1986) 61.
[41] L.R. Crawford, Int J. Mass. Spectrom. Ion. Phys. 10 (1972/73) 279.
[42] J.E. Evans, N.B. Jurinski, Anal. Chem. 47 (1975) 961.
[43] P.E. Kavanagh, Org. Mass. Spectrom. 15 (1980) 334.
[44] H. Jing, A. Amirav, Anal. Chem. 69 (1997) 1426.
[45] S.J. Lehotay, J. AOAC. Int. 83 (2000) 680.
[46] S.J. Lehotay, A.R. Lightfield, J.A. Herman-Fetch, D.J. Donoghue, J. Agric. Food. Chem. 49 (2001) 4589.
[47] T. Cairns, K.S. Chiu, D. Navarro, E. Siegmund, Rapid. Commun. Mass. Spectrom. 7 (1993) 971.
[48] C.H. Liu, G.C. Mattern, G.M. Singer, J.D. Rosen, J. Assoc. Anal. Chem. 72 (1989) 984.
[49] G.C. Mattern, J.B. Louis, J.D. Rosen, J. Assoc. Anal. Chem. 74 (1991) 982.