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Analysis of pesticide residues in food using gas chromatography–tandem mass spectrometry with a benchtop ion trap mass spectrometer

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Abstract

Recent developments on the Saturn GC–MS system offer enhanced capabilities for both the ion isolation and collision-induced dissociation steps in GC–MS–MS. With these improvements, benchtop GC–MS–MS can improve the detection limit of mass spectrometers for low-level target compounds in complex matrices. With this brief study of malathion, we demonstrate (1) excellent sensitivity, (2) wide dynamic range and (3) effective elimination of chemical background. GC–MS–MS is compared to standard GC–EI-MS and GC–CI-MS techniques for the analysis of malathion in orange extract. GC–MS–MS is the most selective and sensitive technique, virtually eliminating the background interference. Product ion mass spectra are invariant across the chromatographic peak and excellent linearity is observed over a range from 2.2 pg/μl to 2.2 ng/μl for malathion. Detection limit by GC–MS–MS was estimated at 0.5 μg/kg in the orange oil matrix ($S/N = 5$).

1. Introduction

The analysis of pesticides in food receives much attention because of concern over the possible long-term effects of exposure to even low levels of pesticides. Because analytical protocols are beginning to focus on health-based limits, detection and quantitation at extremely low levels is desirable. Following solvent extraction of a food sample, background matrix components are present at levels many times those of the target pesticides, complicating the detection of these minor components. Sample cleanup steps prior to analysis can reduce interference problems in part; capillary gas chromatography (GC) then provides a further separation step for the analysis. GC coupled to mass spectrometry (MS) can be used to achieve selective detection of target pesticide components in the presence of the complex matrix. However, the detection limits attainable are set by the levels of interfering ions from the matrix, which can obscure the signal from the target compound. Tandem mass spectrometry (MS–MS) is an approach which reduces the background due to the complex matrix by excluding all ions except the parent ion, which can then be dissociated to produce a unique product ion mass spectrum [1]. Because the number of ions which can be stored in an ion trap is limited, this reduction in background makes it possible to increase the ionization time and store more of

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the desired ion, thus achieving dramatic reductions in the detection limit.

Ion-trap MS–MS offers several advantages compared to multi-sector or multi-quadrupole instruments, since the steps are carried out tandem-in-time rather than tandem-in-space [2]. Because it is not necessary to transmit the ions from one sector (or quadrupole) to another, there are no transmission losses. Also, the MS–MS efficiency is generally higher in the ion trap. In the collision-induced dissociation (CID) process in the ion trap, the translational kinetic energy of an ion is increased by coupling in external energy. As this energetic ion undergoes elastic collisions with the background gas, some of the kinetic energy will be converted to sufficient internal energy to cause fragmentation. In the ion trap, this energy is usually supplied by applying external voltages. The first workers used resonant excitation, where a single frequency matching the secular frequency of the parent ion was applied [3]. With the radio frequency (RF) trapping field held constant, difficulties arose in maintaining the excitation frequency exactly at the resonant frequency of the parent ion. The frequency had to be empirically tuned and required adjustments as the secular frequency varied with changing concentration due to space charging in the ion trap [2,4]. In order to reduce the stringent frequency requirements, subsequent approaches have applied more than one frequency through various means [5–10].

Recent developments in ion isolation in the ion trap provide very precise isolation of the parent ion and thereby lessen the requirements for selective ion excitation [11]. By modulating the RF trapping field, the secular frequency of the ion may be modulated in and out of resonance with a constant supplementary frequency applied to the endcaps. This modulated resonant excitation alleviates the strict frequency-tuning requirements previously encountered. Another approach to less selective excitation is non-resonant excitation [12], where the trapping field is rapidly changed, instantaneously increasing the kinetic energy of all ions in the trap. These recent developments in isolation and excitation greatly enhance the performance of ion-trap MS–MS. These techniques use MS–MS control parameters which are virtually independent of sample or matrix concentration and which may be calculated rather than optimized experimentally. These developments have simplified GC–MS–MS to the point that it can become a routine analytical technique. As an example, this work demonstrates the applicability of GC–MS–MS to the analysis of the pesticide malathion in orange extract.

2. Experimental

A Saturn 3 ion-trap GC–MS system (Varian, Walnut Creek, CA, USA) equipped with a Wave-Board was used for all experiments. The Wave-Board platform generates user-created, time-programmed wave forms for application to the ion-trap electrodes. For these studies, breadboard MS–MS software was used to create and insert intermediate segments between ionization and mass analysis steps in the scan function to achieve isolation and CID on stored electron impact (EI) ions from malathion (see Fig. 1). These segments were applied to both the automatic gain control (AGC) prescan and the analytical scan. Broadband isolation wave forms were applied to the endcap electrodes during and after ionization to eject unwanted ions above and below the parent ion and to selectively store a narrow range of ions around the parent ion [11]. In a two-step process, resonant ejection then removed all remaining ions below and then above the parent ion mass. The energy for CID was supplied by either non-resonant excitation [12] or modulated resonant excitation [11]. Non-resonant excitation causes an instantaneous shift in the equilibrium position of stored ions, with an accompanying increase in kinetic energy due to the restoring force of the applied fields. The modulated resonance approach applies a calculated near-resonant frequency to the endcap electrodes while the RF voltage on the ring electrode is modulated over a narrow range. With this approach, a range of resonant frequencies is effectively applied to the ions in the

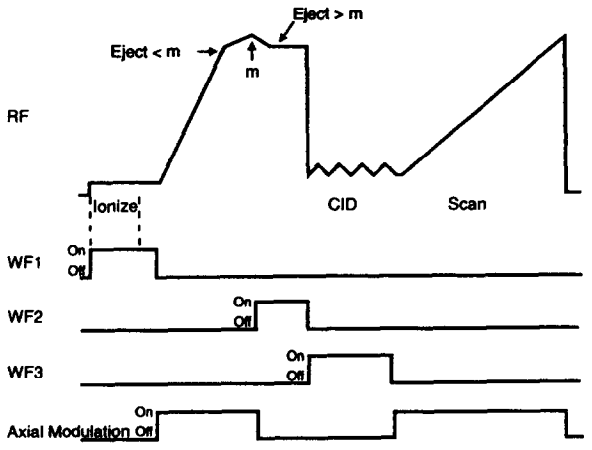

Fig. 1. Scan function showing timing of applied wave forms for modulated resonant excitation. The curve labeled RF is applied to the ring electrode. The timing diagram for the other wave forms shows when they are applied to the end caps. WF1 is an ion ejection wave form, applied during and after ionization. WF2 is a broadband ion ejection wave form, applied during ejection of masses higher than the parent mass. WF3 is a sine wave matching the secular frequency of the parent ion, applied during the CID step. The axial modulation wave form is applied during low mass ejection and also during the analytical scan.

trap. Since application of the exact resonant frequency is not required, the modulated resonant CID approach is not sensitive to shifts of secular frequency of parent ions with concentration. Thus, a greater linear dynamic range can be achieved. Whether the non-resonant or modulated resonant approach is used, the optimal excitation voltage for the CID step must be determined experimentally.

Malathion (PolyScience, Niles, IL, USA) was dissolved in methylene chloride (J.T. Baker, Phillipsburg, NJ, USA) to prepare standards over the concentration range from 2.2 pg/μl to 22 ng/μl. Two oranges were pureed and then extracted for several hours in 500 ml of methylene chloride. The entire extract was filtered into a separatory funnel. After settling, the methylene chloride phase was drawn off and evaporated to give a final solution in which 1 μl was equivalent to 5 mg of the original fruit. This final extract was then spiked with malathion at the 10 pg/μl level and at the 75 pg/μl level. Injections were 1 μl.

Capillary column: DB-5 (J & W, Folsom, CA, USA), 30 m × 0.25 mm I.D., 0.25 μm film thickness. Column program: hold at 70°C for 1 min. Ramp to 270°C at 10°C/min. Hold for 4 min. Injector: temperature-programmable, cold on-column injector (Varian SPI) with high-performance insert, programmed as follows: hold at 25°C for 0.5 min. Ramp to 260°C at 180°C/min. Hold for 21 min. Helium was used as the carrier gas at a linear velocity of approximately 35 cm/s. The transfer line was held at 260°C and the ion-trap manifold was set to 195°C. The mass spectrometer analytical scan rate was 1 s/scan.

3. Results and discussion

3.1. Full-scan and MS–MS spectra of malathion

The full-scan EI mass spectrum of malathion is shown in Fig. 2A, as are the product ion spectra resulting from CID of parent ions m/z 158 (B) or m/z 173 (C). These parent ions correspond to the ester and phosphate fragments in the malathion molecule, as shown in Fig. 3. Note that the molecular ion at m/z 330 is not observed in the full-scan EI mass spectrum; therefore the MS–MS approach for malathion requires the isolation and CID of a malathion fragment ion. The product ion spectra for the two chosen parent ions consist of a subset of fragment ions from the complete malathion mass spectrum. Thus, one can identify fragmentation pathways in MS–MS by the successive isolation and CID of different ions from the full-scan mass spectrum. After investigating the m/z 158 and m/z 173 fragment ions, we chose the m/z 173 ion for quantitative studies because it exhibited the least chemical background interference and the most intense signals.

3.2. Isolation and CID

The m/z 173 ion was isolated using a two-step process, first eliminating ions below m/z 173 then removing those ions above m/z 173 [11]. The excitation voltage near the resonant fre-

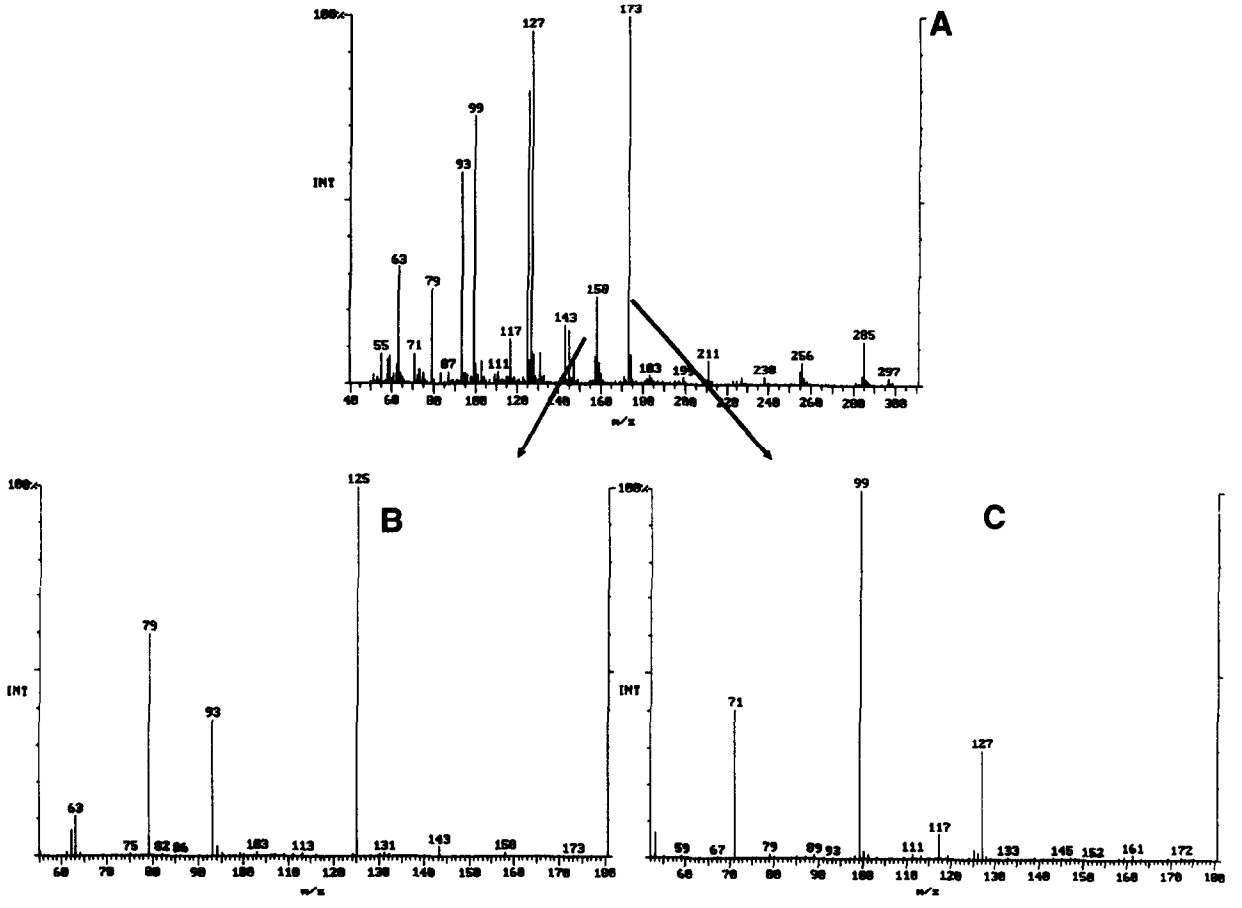

Fig. 2. EI-MS mass spectrum (A) of malathion standard with CID product ion spectra from m/z 158 (B) and m/z 173 (C) ions. Non-resonant excitation was used for CID; 40 V for 20 ms. Note that the product ions arising from m/z 158 form a distinct subset from those coming from m/z 173.

quency for m/z 173 (95 kHz) was applied for 10 ms at an amplitude of 1 V while the RF voltage was modulated over a range of ± 0.8 u, corresponding to ± 5 digital to analog converter (DAC) steps. The product ion spectrum for malathion under these conditions is shown in Fig. 4. The MS–MS process is an ion filter for the reduction of background —only those m/z 145, 127, 117 and 99 ions produced from the m/z 173 parent ion will appear in the malathion MS–MS spectrum.

The early ion-trap MS–MS approach of empirically tuning the excitation frequency exactly to the resonant frequency of the parent ion is of limited value for GC–MS–MS because the resonant frequency of parent ions varies with concentration during elution of GC peaks. Using the Wave-Board approach of modulating the RF trapping field during the excitation step, the calculated (not experimentally determined) frequency is close enough to cause dissociation. In addition, variation in resonant frequency with sample concentration does not affect the efficiency of the CID step. Also, because unit mass isolation is achieved during the isolation step, the frequency can be varied without creating unwanted product ions and the spectrum is cleaner, containing only undissociated parent ions and product ions. Fig. 4 shows the MS–MS mass spectrum from the m/z 173 parent ion of malathion at the apex and near the baseline of the chromatographic peak (1.7 ng on-column).

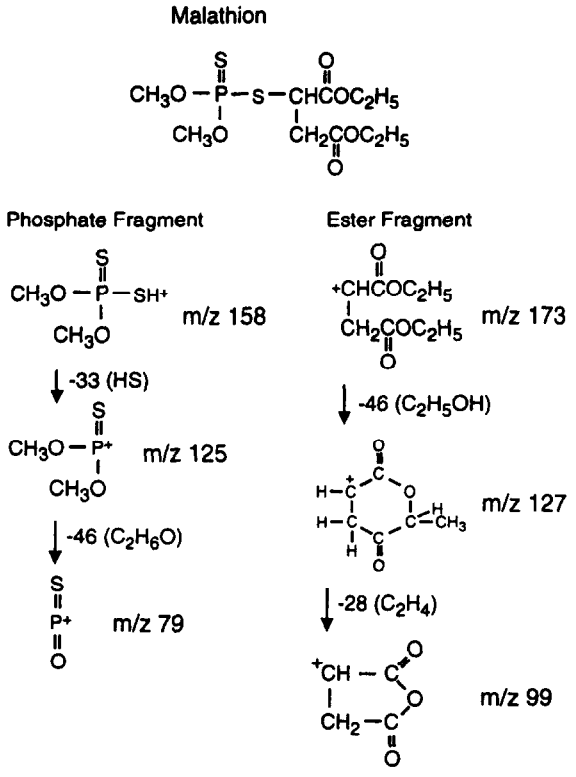

Fig. 3. Proposed fragmentation pathways for the phosphate fragment (m/z 158) and for the ester fragment (m/z 173) of malathion (M_r 330).

The ratios of the product ions are virtually identical.

3.3. Optimization of resonant and non-resonant CID

Breakdown curves show the intensity of product ions versus the applied excitation voltage. They offer a convenient means for selecting the optimum excitation voltage. The analyst might prefer to choose a CID voltage which produces primarily one product ion to achieve maximum sensitivity. Alternatively, a different voltage might be chosen so that additional product ions are present for increased confidence in identification. The breakdown curve for CID of the m/z 173 ion of malathion by modulated resonant excitation is shown in Fig. 5A. There is a wide voltage range over which the intensity of product ions is fairly constant. In a comparable breakdown curve for m/z 173 by non-resonant excitation (Fig. 5B), the maxima for the various product ions are much better defined and show less overlap. Resonant excitation at an applied voltage of 1.0 V was chosen for the quantitative aspects of this study using an excitation time of 10 ms and a modulation range of ± 0.8 u.

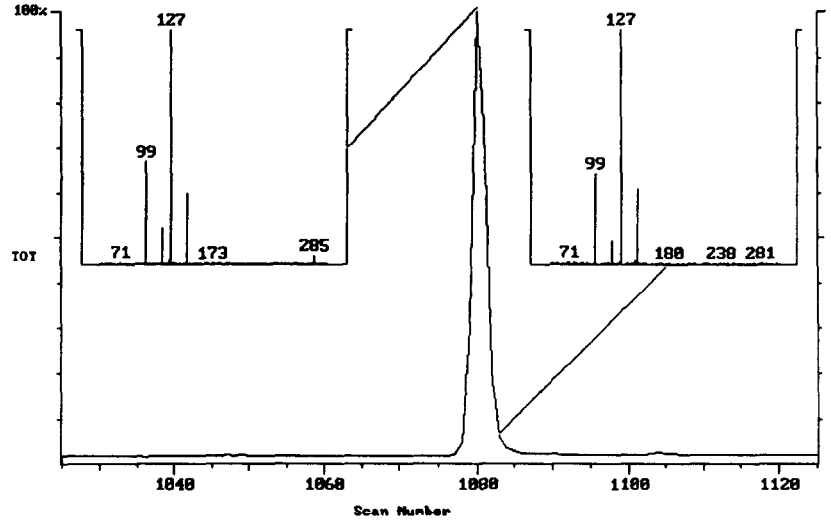

Fig. 4. CID product ion spectrum for malathion standard (1.7 ng injected) showing constant ion ratios across the chromatographic peak. Resonant excitation, 1 V for 10 ms, modulate RF ± 0.8 u at 1000 μs/DAC step.

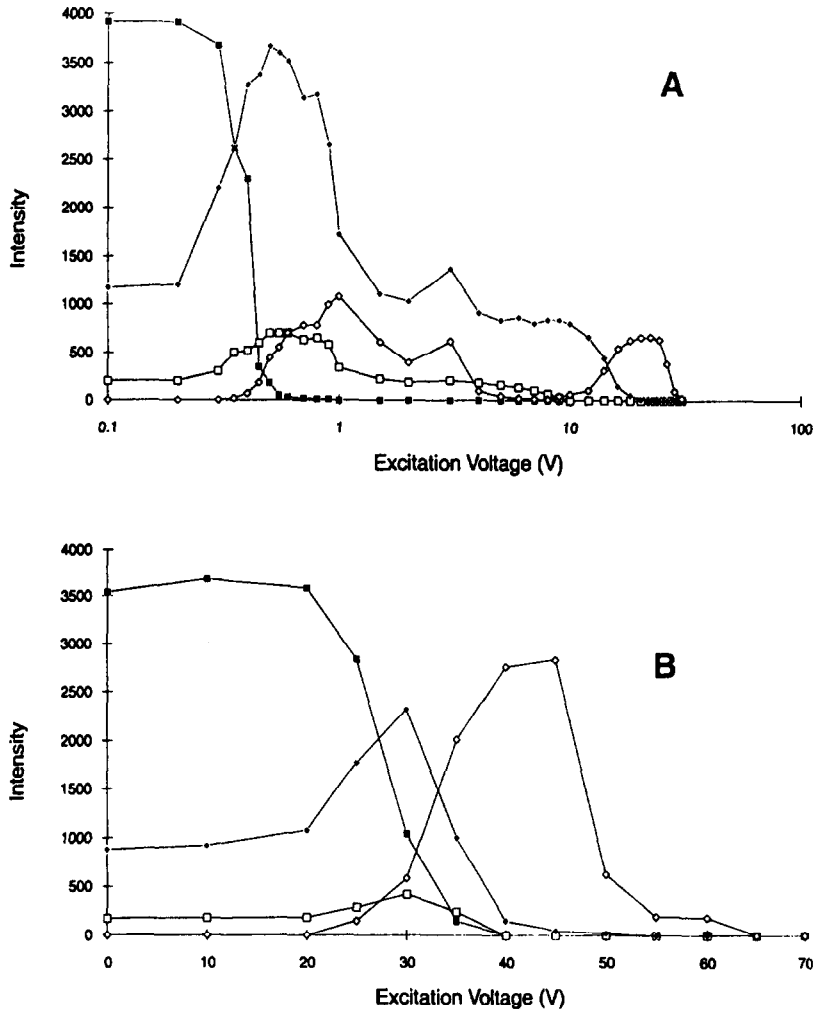

Fig. 5. Breakdown curves for malathion. (A) Resonant excitation, 5 ms, modulate ± 0.8 u at 28 μs/DAC step. (B) Non-resonant excitation, 5 ms, RF = 300. ■ = *m/z* 173; □ = *m/z* 145; ◆ = *m/z* 127; ◇ = *m/z* 99.

3.4. Linearity

To examine the linearity and dynamic range of the MS–MS approach, an external standard calibration curve was prepared based on the peak area of the quantitation ion at *m/z* 127 vs. concentration between 2.2 pg/μl and 22 ng/μl. The calibration curve for 11 points is linear between 2.2 pg/μl and 2.2 ng/μl with a correlation coefficient of 0.9997, a slope of 221 ± 1.4 area counts/pg and an intercept of 1960 ± 1240 area counts. This large linear range is evidence that the efficiency of the CID process does not change with analyte concentration. It also shows how well AGC maintains a constant amount of charge within the ion trap, regardless of the concentration.

3.5. Detection limit in the orange matrix

The whole-orange extract provides a rich matrix to evaluate the EI-MS, CI-MS and EI-MS–MS approaches. The results of this comparison are shown in Figs. 6–8. The spike levels for malathion were at 75 pg/μl (15 μg/kg orange) for the EI-MS and CI-MS runs, and at 10 pg/μl

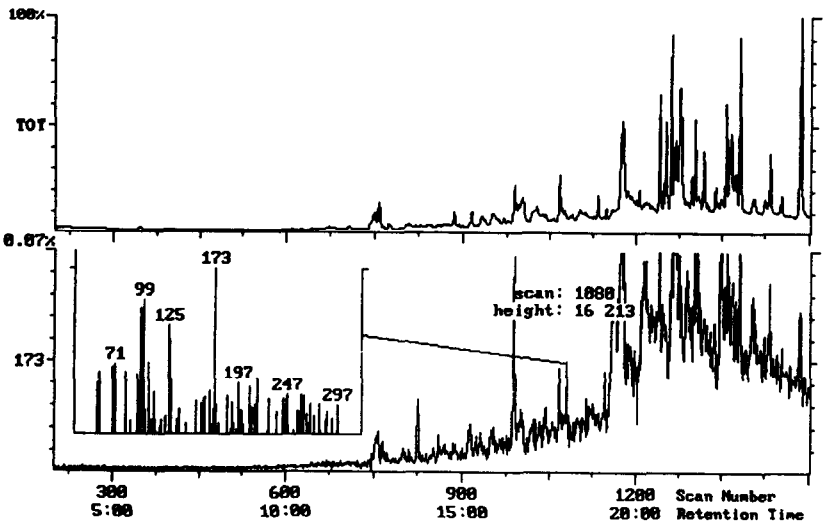

Fig. 6. Standard EI-MS chromatogram of 75 pg/μl malathion in orange extract. Top trace is total ion chromatogram, 100% = 44 221 629. Lower trace is selected ion chromatogram for m/z 173; $S/N = 5.2$. Inset mass spectrum is background subtracted. Time in min.

(2 μg/kg orange) for the EI-MS–MS run. Each figure shows the total ion current chromatogram as well as the mass chromatogram for the chosen quantitation ion. A variety of oil components coelutes with and obscures the malathion peak in standard EI-MS (Fig. 6). One qualitative measure of the level of sample contamination is the ion count for the tallest chromatographic peak, which is listed in the caption for each figure. The result for the EI-MS run is in excess of 40 000 000 counts. A conventional approach to reduce matrix interference is CI-MS when the analyte of interest is more likely to be ionized that the matrix components. As shown in Fig. 7, another advantage of CI is the appearance of the M + 1 ion at m/z 331. Significant reduction of

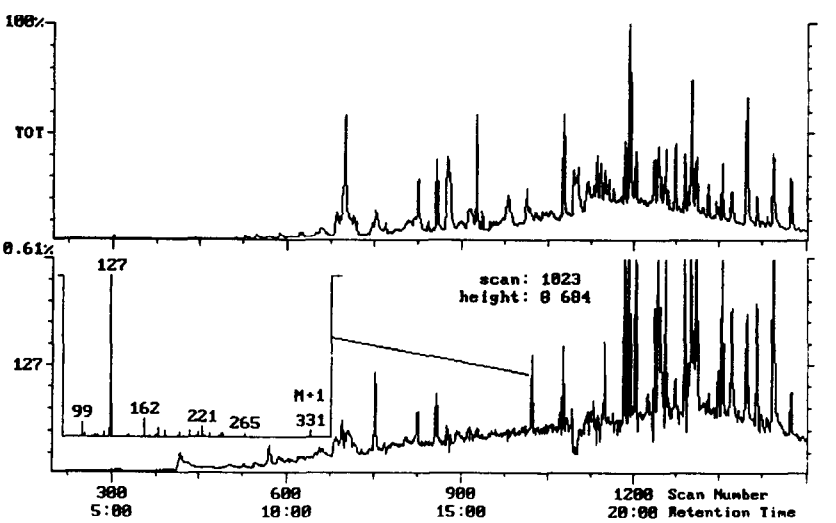

Fig. 7. CI-MS chromatogram of 75 pg/μl malathion in orange extract. Top trace is total ion chromatogram, 100% = 2 593 448. Lower trace is selected ion chromatogram for m/z 127; $S/N = 17.5$. Inset mass spectrum is background subtracted. CI reagent gas is methane. Time in min.

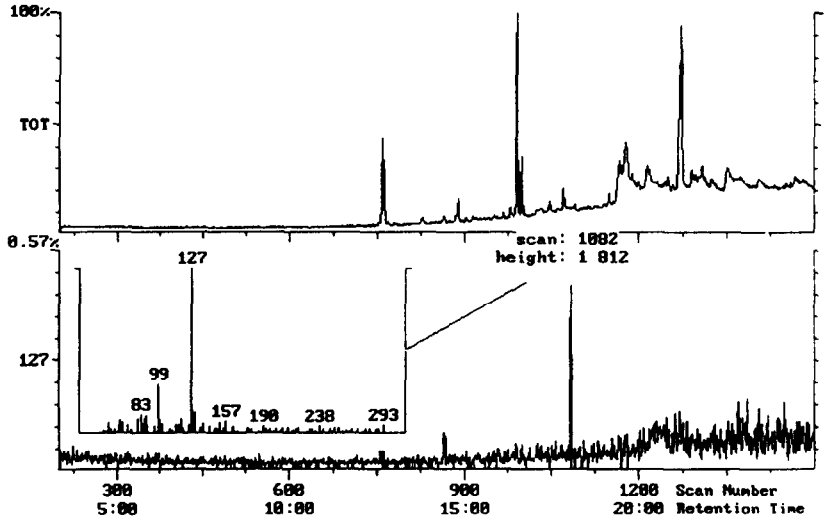

Fig. 8. MS–MS chromatogram of 10 pg/μl malathion in orange extract. Top trace is total ion chromatogram, 100% = 317 739. Lower trace is selected ion chromatogram for m/z 127; $S/N = 23.6$. Inset mass spectrum is background subtracted. CID: resonant excitation, 1 V, 10 ms, modulate ± 0.8 u at 1000 μs/DAC step. Time in min.

matrix interference was achieved for this sample with methane CI, although enough matrix components appear in the mass chromatogram to raise the detection limit. The ion count for the strongest peak is reduced to less than 3 000 000 counts. MS–MS is the most selective technique, virtually eliminating the background, and gives the best detection limit for malathion (Fig. 8). The ion count for the strongest peak in the GC–MS–MS run is reduced to about 300 000 counts, a factor of >100 less than the figure from EI-MS. Table I shows the calculated detection limits for the three techniques. Comparing the area counts for the malathion peak using each technique, MS–MS may produce a lower peak area, but also the lowest contribution from the background (noise), allowing a few pg on-column to be easily detected. Fig. 9 compares the EI-MS (A) and EI-MS–MS (B) mass spectra from the scan at the apex of malathion elution. Both runs are at the 75 pg/μl spike level, and both spectra are shown without background subtraction. Note the increase in ionization time from 185 μs in the EI-MS approach to 8196 μs in the MS–MS approach. As discussed earlier, the improved detection limit of the MS–MS technique in complex samples is a result of two factors: the increase in ionization time allowed when a narrow range of ions is stored during ionization, and the filtering effect of removing all background ions from the masses where the product ions are observed.

Table 1
Results of comparative analyses of malathion in orange extract

Technique	Injected amount (pg)	Quantitation ion	Peak area	S/N	Detection limit for $S/N = 5$ (μg/kg)
EI-MS	75[a]	173	23 786	5.2	14.5
CI-MS	75	127	12 973	17.5	4.3
EI-MS–MS	75	127	16 140	147.1	0.5

[a] 75 pg injected corresponds to 15 μg/kg of orange.

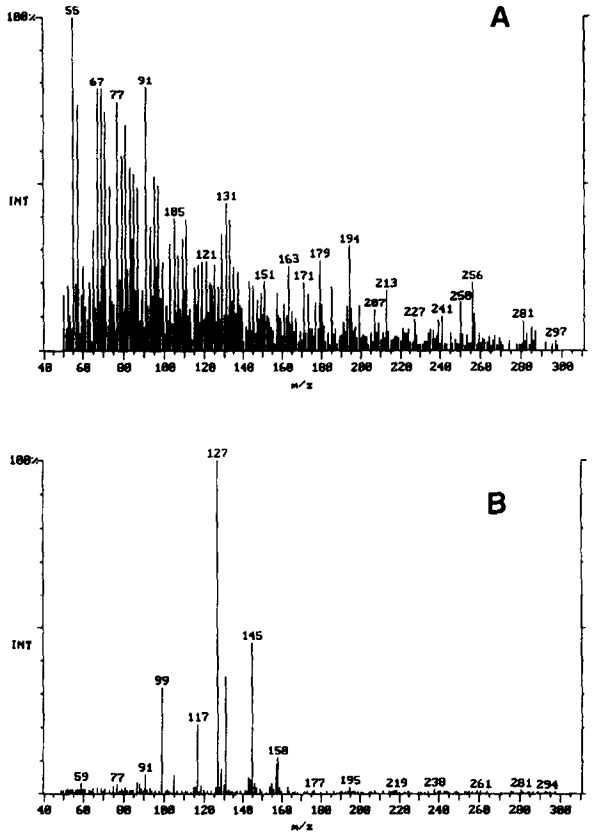

Fig. 9. MS compared with MS–MS for discrimination against chemical matrix. Mass spectra are from the apex of a chromatographic peak of 75 pg of malathion in orange extract and are not background subtracted. (A) EI-MS; ionization time calculated by AGC was 185 μs. (B) MS–MS; ionization time calculated by AGC was 8196 μs.

4. Conclusions

Using resonant methods of ion isolation and either non-resonant or modulated resonant excitation for CID, ion-trap GC–MS–MS is reliable and easy to automate, offering a convenient way to analyze target compounds in a complex sample matrix. Isolation and CID of individual fragment ions also provide fragmentation pathway information to elucidate molecular structures. GC–MS–MS was compared to two other GC–MS techniques for the analysis of malathion in orange extract. Whereas EI-MS and CI-MS are clearly viable techniques, MS–MS provides superior linear response, dynamic range, and detection limit for the analysis of malathion and should also provide a routine analytical technique for a wide variety of samples in many matrices.

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