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Optimization and evaluation of low-pressure gas chromatography–mass spectrometry for the fast analysis of multiple pesticide residues in a food commodity

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Abstract

A fast method of analysis for 20 representative pesticides was developed using low-pressure gas chromatography–mass spectrometry (LP-GC–MS). No special techniques for injection or detection with a common quadrupole GC–MS instrument were required to use this approach. The LP-GC–MS approach used an analytical column of 10 m×0.53 mm I.D., 1 μm film thickness coupled with a 3 m×0.15 mm I.D. restriction capillary at the inlet end. Thus, the conditions at the injector were similar to conventional GC methods, but sub-atmospheric pressure conditions occurred throughout the analytical column (MS provided the vacuum source). Optimal LP-GC–MS conditions were determined which achieved the fastest separation with the highest signal/noise ratio in MS detection (selected ion monitoring mode). Due to faster flow-rate, thicker film, and low pressure in the analytical column, this distinctive approach provided several benefits in the analysis of the representative pesticides versus a conventional GC–MS method, which included: (i) threefold gain in the speed of chromatographic analysis; (ii) substantially increased injection volume capacity in toluene; (iii) heightened peaks with 2 s peak widths for normal MS operation; (iv) reduced thermal degradation of thermally labile analytes, such as carbamates; and (v) due to larger sample loadability lower detection limits for compounds not limited by matrix interferences. The optimized LP-GC–MS conditions were evaluated in ruggedness testing experiments involving repetitive analyses of the 20 diverse pesticides fortified in a representative food extract (carrot), and the results were compared with the conventional GC–MS approach. The matrix interferences for the quantitation ions were worse for a few pesticides (acephate, methiocarb, dimethoate, and thiabendazole) in LP-GC–MS, but similar or better results were achieved for the 16 other analytes, and sample throughput was more than doubled with the approach.

Keywords: Matrix effects; Gas chromatography, fast; Gas chromatography, low-pressure; Food analysis; Pesticides

1. Introduction

In the 1960s, Giddings [1] demonstrated that applying a vacuum at the column outlet would result in greatly reduced analysis times in gas chromatography (GC). However, much of the research conducted since then concerning fast GC has focused on the use of small diameter (micro-bore) capillary columns [2–5]. The micro-bore approach has a major limitation in many real-world analyses due to

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the low sample capacity, and injection/detection concerns with split injections and narrower peaks also limit the usefulness of the approach. Multi-pesticide residue analysis in foods is one common application in which the use of micro-bore columns (<0.25 mm I.D.) is essentially impractical, thus other approaches to obtain fast GC separations should be considered for this purpose.

A rather promising approach is fast temperature programming of relatively short (5–6 m) capillary columns (0.25–0.32 mm I.D.) through the use of resistive heating [6–14] or conventional GC ovens [14]. This concept does not require special injection techniques or significant changes in the instrument design, and a variety of GC detectors may still be used. GC–mass spectrometry (MS) using supersonic molecular beams is another approach that permits rapid separations and analysis at high flow-rates without losses in injection capacity [15–17].

Another approach, as Giddings first demonstrated [1], is to conduct GC at sub-ambient pressure conditions, or low-pressure gas chromatography (LP-GC). For many years, this technique was not practical due to the prevalence of packed columns and selective detectors in GC applications. In those days, complicated instrument designs or external apparatuses were needed to conduct LP-GC, and injection/detection problems remained. However, the use of MS for detection, which requires low pressure for optimal analysis, conveniently can provide the vacuum for LP-GC without the need for an additional external vacuum system. GC–MS is a continually improving and growing technique with several advantages over GC with selective detectors (universal detection with high selectivity, confirmatory, sensitive, quantitative, able to distinguish co-eluting peaks, use of spectral libraries), and its applicability for LP-GC is yet another benefit.

Interest in vacuum outlet operation was renewed in the 1980s when a series of theoretical studies discussing its advantages for improving the speed of analysis was published [18–21]. It was demonstrated that lower column pressures led to higher diffusivity of the solute in the gas phase and increased the optimum carrier gas linear velocity (U_{opt}) in the Van Deemter plot. Consequently, faster GC separations could be achieved under reduced pressure at the same column dimensions as atmospheric column outlet pressure operation with a disproportionately smaller loss of separation power (the column efficiency was calculated to decrease by 12.5%, however, experimental results showed no loss of theoretical plates) [18]. In any approach to fast GC, some sacrifice must be made in sample capacity, detection limit, and/or separation power for the increase in speed. LP-GC–MS makes a sacrifice only in separation efficiency. According to theory, the gain in speed becomes more pronounced for short and/or wide-bore capillary columns [21–25]. Thus, contrary to high-speed micro-bore capillary GC, LP-GC provides *increased* sample capacity through the use of mega-bore columns.

Until recently, however, the practical use of short and/or wide-bore columns under vacuum outlet conditions was still associated with certain difficulties: (i) sub-ambient pressures extended to the column inlet and required special injection techniques; and (ii) the higher GC gas flow-rate can lead to excessive pressure at the mass spectrometer and increase detection limits [26].

Different injection techniques for sub-atmospheric pressure conditions have been applied with varying degrees of success [27,28], but the simplest and most applicable approach is to connect the mega-bore analytical column to a short, narrow restriction column at the injection end [28,29]. In this way, the analytical column is kept under low-pressure conditions, but the inlet remains at usual column head pressures in GC and common injectors can be used with conventional injection methods and typical injection volumes. An added benefit of this approach is that the restriction column also serves as a retention gap (or guard column) in the analysis of relatively dirty samples.

Increased sample capacity and speed of analysis are two main advantages of LP-GC–MS, but other advantageous features [15,29] include: (i) peak widths are similar as in traditional GC methods, thus MS scan rate does not have to be faster than that commonly used in GC–MS; (ii) peak heights are increased which can lead to higher signal/noise ratios and lower detection limits; (iii) lower column temperatures may be used to still achieve a rapid separation which reduces oven cool-down time and improves analysis of thermally labile compounds; and (iv) higher flow-rates can be used which may

help to reduce residence times of analytes in the injector and reduce interaction with active sites or avoid degradation. Due to the use of shorter, mega-bore columns, the number of theoretical plates in LP-GC–MS cannot match those in long-column and/or narrow-bore separations, but MS detection does not require complete separation of all analytes for analysis. Furthermore, MS scan speed with typical quadruple instruments is still capable of generating several data points across a relatively narrow peak [15].

A main goal of this study was to explore LP-GC–MS through the optimization of the speed and sensitivity of LP-GC–MS applied to the analysis of 20 representative pesticides. Additionally, a dozen sets of analyses of real samples (carrot extracts) were performed and results compared with those obtained by the conventional GC–MS technique. This study was intended to determine the feasibility of LP-GC–MS for the possible routine analysis and/or screening of hundreds of pesticide residues in foods by monitoring laboratories.

2. Experimental

2.1. Chemicals and materials

Pesticide standards (acephate, captan, carbaryl, chlorpyrifos, deltamethrin, dichlorvos, dimethoate, endosulfan I, endosulfan II, endosulfan sulfate, heptachlor, lindane, methamidophos, methiocarb, permethrins, pirimiphos-methyl, procymidone, propargite, and thiabendazole), all 95% or higher purity, were obtained from the US Environmental Protection Agency's Pesticide Repository (Fort Meade, MD, USA), Chemservice (West Chester, PA, USA), Ultra Scientific (North Kingstown, RI, USA), or Dr. Ehrenstorfer (Augsburg, Germany). These pesticides were chosen carefully based on their diverse range of volatility, chemical classes, quality of their analysis in GC, and thermal lability. For example, acephate, methamidophos, and thiabendazole are notorious for giving tailing peaks in GC and GC–MS. Also, dichlorvos and deltamethrin are quite volatile and nonvolatile, respectively, and the carbamates (methiocarb and carbaryl) are more commonly analyzed by liquid chromatography (LC) than GC. The analysis of these 20 representative pesticides was challenging, and they included the extremes for determining the feasibility of the approach.

Table 1
Concentrations of each of the 20 pesticides in standards in toluene

Solvent standard	Pesticide concentration (μg/ml)
Std 1	5.000
Std 2	0.500
Std 3	0.250
Std 4	0.050
Std 5	0.005

A composite stock standard solution (10 μg/ml) was prepared in toluene, and working standard solutions (std1–std5) were prepared by diluting the stock solution with toluene (see Table 1 for concentrations). Carrot matrix-matched standards (cmstd1–cmstd5) were obtained by reconstituting the residue remaining after evaporation of carrot extracts in working standard solutions. The carrots were extracted with acetone and partitioned with a mixture of dichloromethane–light petroleum (1:1) according to method 303 used by the US Food and Drug Administration [30] and the Dutch Inspectorate for Health Protection [31]. No clean up steps were conducted and the extracts were taken to dryness and dissolved in toluene. The final carrot content of the matrix-matched standards was 5 g carrot/ml toluene, and the pesticide concentrations in these extracts appear in Table 2. All solvents used in experiments were analytical grade from Fisher (Fair Lawn, NJ, USA). Carrots (labeled to have been organically grown) were obtained at a retail market.

2.2. GC–MS conditions

GC–MS experiments were performed using a

Table 2
Concentrations of each of the 20 pesticides in standards in carrot extracts (reconstituted in toluene)

Matrix-matched standard	Pesticide concentration	
	μg/ml	μg/g
Cmstd 1	5.000	1.000
Cmstd 2	0.500	0.100
Cmstd 3	0.250	0.050
Cmstd 4	0.050	0.010
Cmstd 5	0.005	0.001

Hewlett-Packard (HP) 5890 Series II Plus gas chromatograph combined with a 5972 mass-selective detector. The GC system was equipped with an electronic pressure control (EPC), a split/splitless injector and a HP 7673A autosampler. To decrease to the effective size of the oven and permit more rapid and reproducible GC temperature control, an oven insert (HP kit G2646-60500) was used in both LP-GC–MS and conventional GC–MS.

After some initial experimentation with other column dimensions in LP-GC–MS, nearly all experiments were conducted with a 10 m×0.53 mm I.D., 1 μm film thickness RTX-5 Sil MS capillary column (Restek, Bellefonte, PA, USA) which was connected to a 3 m×0.15 mm I.D. non-coated restriction column (Restek) at the inlet end. A stainless steel union in which the restriction column fit inside the mega-bore column was used for a true zero-dead-volume connection. The analyses of real samples were performed at the optimized conditions, which were as follows: He carrier gas (constant inlet pressure 20 p.s.i.g., 1 p.s.i.g.=6894.77 Pa above atmospheric pressure), 250°C inlet temperature, 1 or 2 μl (splitless) injection volume, 280°C mass-selective detector interface temperature, and a temperature program of 90°C for 0.5 min, then 60°C/min ramp to 290°C (held for 3 min). Total GC run time was 6.8 min, and 3.5 min elapsed between injections accounting for cool-down (1.75 min), stabilization (0.5 min), and injection (1.25 min). The MS conditions in the selected ion monitoring (SIM) mode are given in Table 3.

For comparison purposes, an effort was also made to achieve a rapid separation with a similar separation pattern of the 20 pesticide analytes using conventional GC–MS. For the GC–MS analyses of real samples, a 30 m×0.25 mm I.D., 0.25 μm film thickness RTX-5MS capillary column (Restek) was used. The analyses were performed at the following conditions: He carrier gas, 250°C inlet temperature, 1 ml/min constant flow (12 p.s.i.g. initial inlet pressure), 1 μl (splitless) injection volume, 280°C mass-selective detector interface temperature, and a temperature program of 90°C for 0.5 min, then 20°C/min ramp to 220°C followed by 5°C/min ramp to 240°C and 20°C/min ramp to 290°C (held for 6.5 min). This method took 20 min, which is at least twice as fast as a typical GC analysis of pesticides ranging in volatility from dichlorvos to deltamethrin [30–32]. The same type of 4 mm I.D. double taper

Table 3
MS conditions for the LP-GC–MS and conventional GC–MS methods (start times of windows, ions selected in SIM mode, and dwell times)

Pesticide	Start time (min)		SIM ions (m/z)			Dwell time (ms)
	LP-GC–MS	GC–MS				
Methamidophos	1.30	4.25	94	95	141	20
Dichlorvos			109	185	220	
Acephate	1.68	4.80	94	136	183	30
Dimethoate	2.20	7.00	87	93	125	20
Lindane			181	183	219	
Carbaryl	2.85	8.30	115	144		10
Heptachlor			272	274		
Pirimiphos-methyl			276	290		
Methiocarb			153	168		
Chlorpyrifos			197	314		
Captan	3.20	9.70	79	149		10
Thiabendazole			174	201		
Procymidone			283	285		
Endosulfan I			195	241		
Endosulfan II			195	241		
Endosulfan sulfate	3.78	12.20	272	273	387	20
Propargite			135	173	350	
Phosalone	4.00	13.20	182	185	367	30
Permethrins	4.30	14.20	163	165	183	30
Deltamethrin	4.80	16.00	181	208	253	30

liners was used in both LP-GC–MS and conventional GC–MS, and MS SIM conditions with the exception that time window settings were the same in both cases (see Table 3).

2.3. Long-term stability study – sequence of samples

Once the LP-GC–MS and conventional GC–MS conditions were optimized, the pesticides in toluene and the carrot extracts were repetitively analyzed in 12 sequences (a–l), between which no column maintenance was performed. In the LP-GC–MS approach, two injection volumes, 1 and 2 μl, were tested, whereas in conventional GC–MS analysis only 1 μl injection was possible due to peak fronting at higher injection volumes. This peak fronting was caused by an incompatibility of the greater amount of toluene with the stationary phase in the narrow-bore column. The order of the injections in the sequences was as follows: (1) toluene, (2–6) std1*n*–std5*n*, (7) toluene, (8) carrot blank, (9–13) cmstd5*n*–cmstd1*n* (where *n*=a–l). Thus, 156 injections in all were made in each set of sequences, and 72 of those injections were carrot extracts. For each 1 μl injection, 5 mg equivalent carrot was being introduced into the system, and for each 2 μl injection, 10 mg sample equivalent was injected.

3. Results and discussion

3.1. Initial experiments to set column parameters

Initially, a 15 m×0.53 mm I.D., 1 μm film thickness analytical column was tested in LP-GC–MS. After a set of experiments, 5 m was cut from this column because the 15 m length did not allow for the desired gains in speed of analysis. For the restriction column, 0.15 mm I.D. deactivated capillaries of different lengths (4.5, 3, and 1.5 m) were tested in conjunction with the 10 m analytical column. In essence, the restriction column diameter and length controlled the necessary head pressure to yield the desired flow-rate. Nearly any restriction column, could give the desired flow conditions provided that the maximum inlet pressure of 100 p.s.i.g. for the instrument was not exceeded. The 3 m×0.15 mm I.D. column was ultimately chosen because, in part, its use led to moderate pressure at the inlet for the final flow conditions employed. Furthermore, the non-coated restriction column also served as a retention gap, thus the longer, wider column also better helped to focus the analytes and protect the analytical column against the deposition of less volatile matrix components. After running a long sequence of ''dirty'' sample extracts, this column could be easily cut or replaced if needed.

3.2. Optimization of speed and signal/noise ratios

The LP-GC–MS technique enables faster GC analysis due to the increased U_{opt} under reduced column pressure. Experiments by De Zeeuw et al. [29] determined that 90 cm/s was the U_{opt} for a 10 m×0.53 mm I.D. column in LP-GC. However, the vacuum system of the MS used for LP-GC–MS limits flow-rate that can be achieved before the pumping capacity of the vacuum system affects performance of the detection. In conventional GC–MS, typical column outlet gas flow is 1 ml/min and instruments are designed to handle an optimal flow range of 1–2 ml/min. The 5972 mass-selective detector used in this study used a diffusion pump (60 l/s) backed by a roughing pump that automatically shuts down if the pressure exceeded a preset maximum value. In a similar system as used in this study, Wylie and Uchiyama found that an 8.8 ml/min He flow-rate was the maximum that the GC–MS system could tolerate before it would automatically shut down [33]. However, in this study, detection sensitivity and mass-selective detection tuning problems were significantly affected before that pressure was reached. Thus, in our experiments, not only speed of separation, but also detection capabilities had to be optimized. For this purpose, experiments were performed to optimize the column inlet pressure, oven temperature program, splitless time period, and injection volume.

Fig. 1 displays the influence of the column inlet pressure from 10 to 60 p.s.i.g. on the response (peak height) and retention time of 10 ng injected deltamethrin (other experimental parameters were set at their optimized values as described below). Deltamethrin was most suitable for the evaluation of both speed and detectability because: (i) as the last

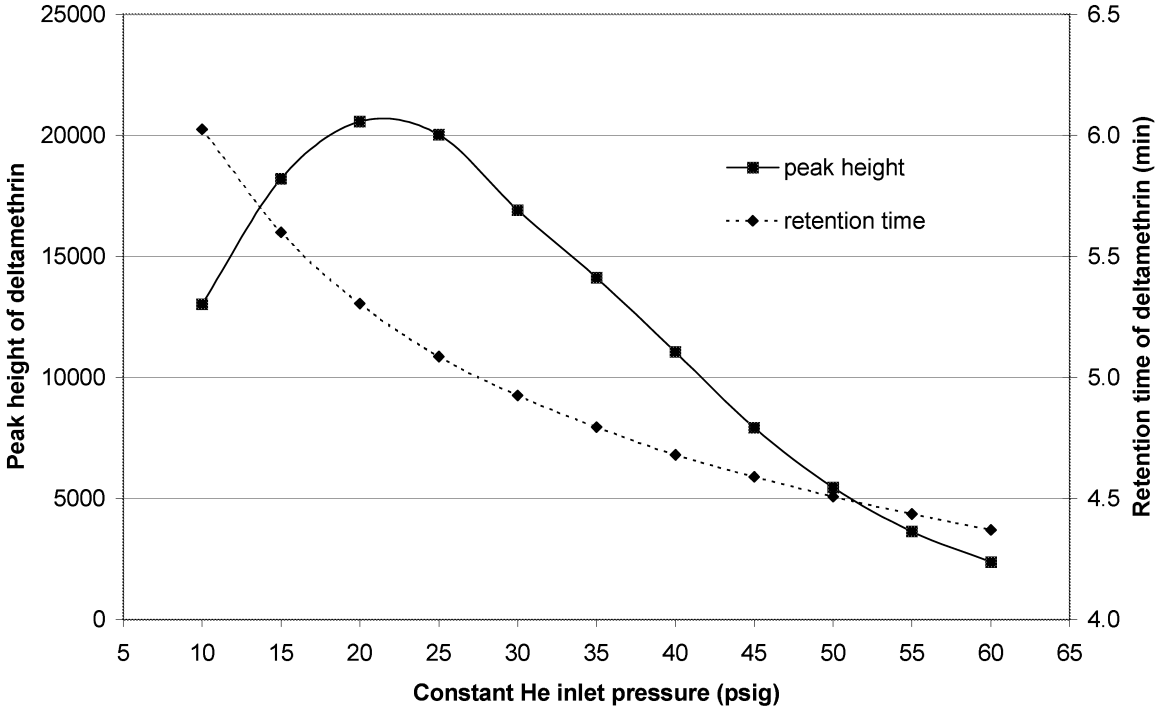

Fig. 1. Influence of the column inlet pressure (10–60 p.s.i.g.) on the response (peak height) and retention time of 10 ng injected deltamethrin.

analyte to elute, it practically determines the analysis time; and (ii) it has lower sensitivity in GC–MS than many of the other pesticides. As Fig. 1 shows, maximum peak height of deltamethrin was achieved at 20 p.s.i.g. column inlet pressure (noise was essentially constant in SIM vs. pressure thus maximum signal correlated to maximum signal/noise ratio).

The curve representing the influence of the column inlet pressure on peak height resulted from two opposing effects: (i) increases in the pressure (flow-rate) led to taller peaks; and (ii) lower MS responses were achieved as pressure at the MS system increased. The shape of this curve was the same for all analytes tested, except for the early-eluting analytes, methamidophos and dichlorvos. In their cases, maximum detectability was obtained when the column inlet pressure was 15 p.s.i.g. Nevertheless, the differences in response between 15 and 20 p.s.i.g. were rather small for these compounds, and therefore, 20 p.s.i.g. was used as the constant column inlet pressure.

As Fig. 1 shows, the retention time of deltamethrin was 5.3 min at the maximum signal/noise ratio. To gain a min in speed (approx. 20% faster analysis time), the cost in limit of detection was nearly 10-fold.

The coupling of two columns of different diameters complicated the estimations of flow-rate in the LP-GC–MS method. To estimate the linear velocities and flow-rates in LP-GC–MS, two experimental methods were employed: (i) the elution times of injected air were monitored (m/z 28); and (ii) the gauge and foreline pressures were correlated with known flow-rates (via Chemstation calculations) in conventional columns which were then used to determine flow-rates in LP-GC–MS. In the former case, an ‘‘average’’ column internal diameter was calculated by inputting the column length (13 m) and determined linear velocity into the Chemstation software. This fictitious I.D. was then used to calculate the flow-rate. In the latter method, single columns of known diameter and length were installed, and the Chemstation software was used to

calculate flow-rates at given inlet pressures in GC–MS. The foreline and gauge pressure readings were monitored at the known flow-rates, and a highly reproducible correlation of calculated flow-rate occurred with respect to foreline and gauge pressure readings. The results from both approaches were in close agreement. Thereafter, the flow-rate and linear velocity in LP-GC–MS could be determined from the foreline and/or gauge pressure readings at the MS system.

In our system, the flow-rate was approximately 2.6 ml/min (linear velocity 90 cm/s) at 90°C, and 1.2 ml/min (65 cm/s) at 290°C. These values were close to the U_{opt} of 90 cm/s previously determined in LP-GC for an analytical column of the same dimensions [29]. EPC was also attempted in the LP-GC–MS approach, but complications due to the coupling of two columns of different dimensions confounded the software. In theory, only the 3 m×0.15 mm I.D. column needed to be entered into the software with vacuum outlet to attain accurate EPC settings, but flows were not constant based on measurements. No ''false'' column dimensions entered to fool the software accurately accounted for the differences in flows at low and high column temperatures.

3.3. Oven temperature program and retention time reproducibility

A fast oven temperature program contributed to the fast GC separation in the LP-GC–MS method. However, speed alone is not the paramount consideration in designing the analysis; the consistency of the retention time is also a critical factor. Even in GC–MS, retention time is critical to help confirm the presence or absence of an analyte of interest.

An oven insert (simply a rectangular pad) to reduce oven size was found to improve temperature control performance. The insert also provided faster cool-down after the analysis (1.75 min from 290 to 90°C vs. 2.35 min without the insert), which contributed to increased sample throughput. In an experiment, 10 repeated injections of std1 using a 60°C/min ramp rate were made with and without the oven insert. With the insert, the average retention time of deltamethrin was 5.325±0.006 min, whereas the retention time was 5.816±0.010 min without the oven insert. Thus, a shorter analysis time and improved GC performance was achieved using the insert.

The maximum temperature program rate permitted by the HP 5890 gas chromatograph is 70°C/min. However, this rate was not actually reached as indicated by the instrument displays, even with the use of the oven insert, but reproducibility was the more important consideration. Deltamethrin was again a suitable analyte for this comparison – not only because it essentially determined the analysis time, but also because problems with retention time repeatability are more pronounced in the case of late eluting compounds in fast GC [14]. In another experiment using the insert, three settings of fast temperature programming rates (50, 60 and 70°C/min) were tested and analyte retention times and their variability were monitored. Both 50 and 60°C/min gave very good retention time repeatability of all analytes, but 70°C/min gave less consistent results. Fig. 2 shows this comparison of repeated analyses of 10 ng injected deltamethrin at the different conditions. Relative standard deviations (RSDs) of the retention times (n=5 in each case) were 0.015, 0.024, and 0.353% for 50, 60, and 70°C/min ramp rates, respectively.

3.4. Injection

In splitless injection, the time that the split vent is closed influences retention times (linked with initial column temperature hold time) and peak heights of

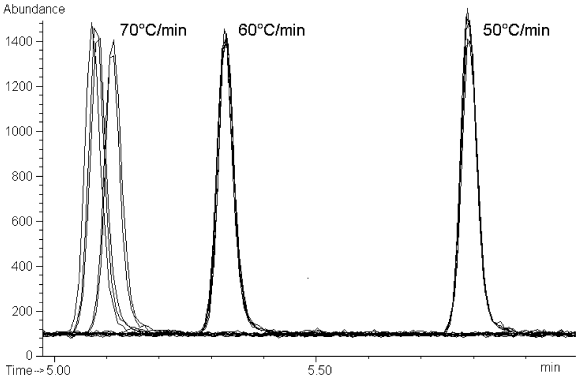

Fig. 2. Overlay of five repeated injections of 10 ng injected deltamethrin (m/z 253) using three settings of fast temperature programming rates: 50, 60 and 70°C/min (using oven insert).

the analytes (due to analyte transfer efficiency to the column). For a given flow-rate and liner volume, shorter splitless time periods speed the analysis and less solvent tailing is likely to occur. Conversely, a splitless time that is too short does not provide complete transfer of analytes into the GC column.

In the LP-GC–MS approach, an experiment was conducted to determine the effect of splitless time (from 0.1 to 1 min) on the analyte responses. The results showed that the responses increased as splitless time increased up to 0.5 min, but longer splitless times protracted the analysis unnecessarily. The higher flow-rate in LP-GC–MS served to sweep the analytes faster from the injector than in conventional GC–MS. Fig. 3A exhibits a chromatogram of the 20 pesticides in std1 at the optimized LP-GC–MS conditions (constant inlet pressure 20 p.s.i.g., temperature program rate 60°C/min, splitless time period 0.5 min, 1 μl injection, SIM). For comparison, Fig. 3B shows the conventional GC–MS chromatogram.

As stated previously, an advantage in the use of wide-bore columns is their high sample capacity. In this study, a mega-bore column (0.53 mm I.D.) with stationary phase film thickness of 1 μm was used. Van Deursen et al. [28] calculated that 1–1.5 μm is the maximum film thickness recommended for the LP-GC technique before thicker films reduce the benefits of the approach by extending retention times. Furthermore, the higher bleed from thick-film columns is detrimental to MS performance.

To evaluate sample loadability, injection volumes of 1 to 5 μl were tested for LP-GC–MS. As injection volume increased, peak heights of analytes also increased whereas peak widths (full width at half-maximum, FWHM) remained constant (2.2 s), as shown in Fig. 4. Therefore, no significant adverse effect on chromatographic separation was observed at injection volumes as high as 5 μl in the case of standards in toluene.

In the case of the conventional GC–MS column, 2 μl injections of standards in toluene gave significant peak fronting due to solvent overloading in the column (condensed toluene was incompatible with the stationary phase). There may be more than one reason for the ability to inject larger volumes in LP-GC–MS. Firstly, the LP-GC–MS set-up used a retention gap (restriction capillary), and the analytical column had a much thicker film. Secondly, the low pressure in the LP-GC–MS analytical column affected the vaporization temperature of the toluene and reduced or eliminated condensation of the solvent. Thus, larger volume injections in solvent could be made without affecting chromatography in LP-GC–MS.

There are several approaches to large volume injection in GC that typically require expensive injection systems. The ability to inject relatively large volumes in LP-GC–MS without the need for a special injector is another advantage of the approach. However, an inherent factor with large volume injection is that the cleanliness of the injected sample limits injection volume more than the technical ability to inject larger volumes. Gains in detectability are made as more sample is injected only to the extent that matrix interferences are not the limiting source of noise. Furthermore, the ruggedness of the method nearly always suffers as injection volume increases.

3.5. Analysis of real samples – matrix effects

To evaluate the feasibility of the LP-GC–MS approach for routine analysis of pesticide residues in real samples and to compare it with conventional GC–MS, a number of analyses were conducted for the pesticides in toluene (solvent standards) and in the carrot extracts (matrix-matched standards) as described in Experimental. In conventional GC–MS analysis (see Fig. 3B for a chromatogram), only 1 μl injection was possible. In the LP-GC–MS approach, although 5 μl was possible for solvent standards, 1 μl was used in one experiment for better comparison with conventional GC–MS, and 2 μl was evaluated in a second experiment to test a higher injection volume.

Figs. 5 and 6 show the long-term stability of responses for selected pesticides, heptachlor and dimethoate, at two concentration levels in both solvent and matrix-matched standards which were obtained by all three tested alternatives (conventional GC–MS, and 1 and 2 μl injection in LP-GC–MS). Heptachlor represents a relatively stable, non-problematic analyte in a GC analysis whereas dimethoate often gives peak tailing and is strongly affected by the matrix enhancement effect [33–41]. In the matrix

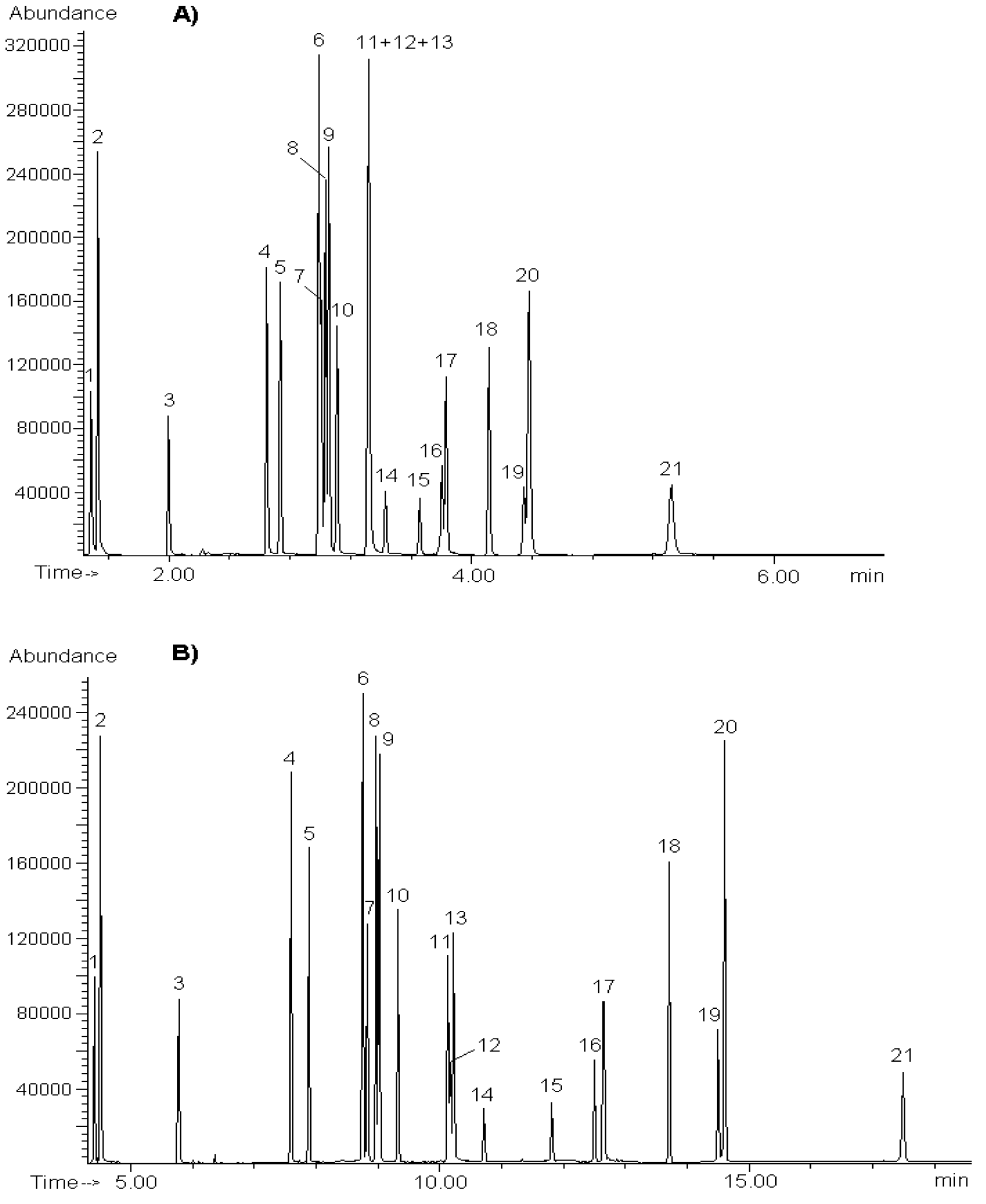

Fig. 3. Chromatogram of std1 (1 μl injection of 5 μg/ml pesticide mixture in toluene) at: (A) the optimized LP-GC–MS conditions, (B) conventional GC–MS conditions. (1) Methamidophos, (2) dichlorvos, (3) acephate, (4) dimethoate, (5) lindane, (6) carbaryl, (7) heptachlor, (8) pirimiphos-methyl, (9) methiocarb, (10) chlorpyrifos, (11) captan, (12) thiabendazole, (13) procymidone, (14) endosulfan I, (15) endosulfan II, (16) endosulfan sulfate, (17) propargite, (18) phosalone, (19) *cis*-permethrin, (20) *trans*-permethrin, (21) deltamethrin.

enhancement effect, the matrix components fill active sites in the liner and reduce the adsorption and/or degradation of susceptible analytes in the inlet, thus allowing their higher transfer to the column.

No clean-up (except for a liquid–liquid partition) was used in our study to test the feasibility of the LP-GC–MS approach using a rather concentrated, complex sample. Of course, clean-up of the raw extracts would decrease matrix build-up, but it would

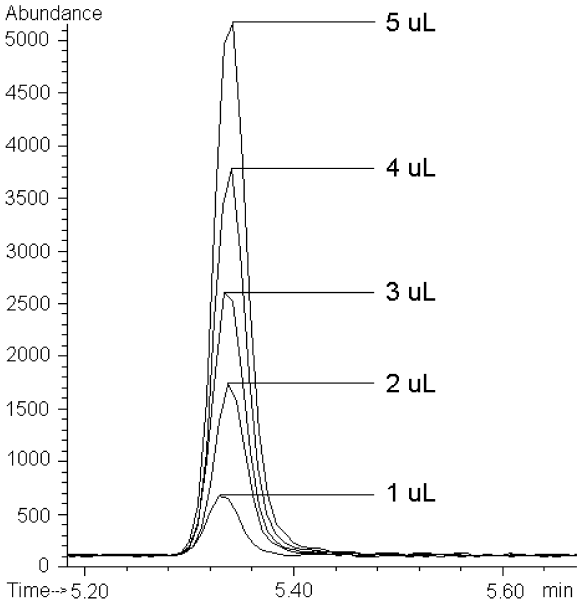

Fig. 4. Overlay of peaks for 5 μg/ml deltamethrin (*m*/*z* 253) using 1–5 μl injection.

not necessarily avoid the matrix enhancement effect [39]. Moreover, a time-consuming sample preparation procedure would reduce sample throughput gains by using fast GC analysis in an overall analytical method.

Using both conventional GC–MS and LP-GC–MS methods, the matrix enhancement effect was observed for susceptible pesticides. A greater enhancement was observed at lower analyte concentrations in this study, as demonstrated in the comparison between Fig. 5 (B1 and B2) and Fig. 6 (B1 and B2).

In the analysis of real samples, the most straightforward way of compensation for this matrix effect is to prepare calibration standards in blank matrix extract rather than in pure solvent [39,40]. However, one of the drawbacks of this approach relates to the increased amount of injected matrix in an overall sequence. The contamination of the inlet and front part of the column increases, which results in the decrease in response of all analytes in both matrix-matched and solvent standards (matrix diminishment effect) [35].

As expected, this signal reduction due to matrix build-up increased when a greater amount of matrix was injected, as shown in the comparison of 1 and 2 μl injections in Fig. 5 (B2 and B3). After 50 2 μl injections of the carrot extracts (500 mg equivalent matrix), dimethoate was no longer detected in the 0.05 μg/ml solvent standard. The use of an internal standard would help compensate for the matrix diminishment effect and improve quantitation of real samples. Also, no clean-up of extracts was conducted in this study, and the use of an appropriate solid-phase extraction step would be beneficial in routine monitoring. Of course, simple maintenance of the GC inlet should also be performed on a more frequent basis as more sample matrix is injected into the GC system.

As shown in Figs. 5 and 6, the LP-GC–MS method gave a more rapid decline in response than the conventional GC–MS method. This is probably due to the narrow, 3 m restriction capillary and not the mega-bore analytical column. The restriction capillary was acting like a guard column and it should have been cut or changed after approximately 300 mg of equivalent carrot sample was injected in this application. The 0.25 mm I.D. coated conventional column was able to accept more material before the inlet end of the column needed to be cut.

3.6. Analysis of real samples – detectability and separation

Fig. 7 compares peak height to peak area (*H*/*A*) ratios obtained by both tested methods after a few sets of analyses. This ratio was used because the direct comparison of peak heights would not be correct due to the different GC flow-rates, column lengths, film thicknesses, and column diameters. In all comparisons made, the results of LP-GC–MS are given with respect to conventional GC–MS as commonly practiced [31,32], not vs. fast GC at normal GC pressure conditions. Features of LP-GC–MS solely due to increased flow-rate and use of a shorter column can also be achieved by increasing flow-rate in a shorter column in conventional GC–MS.

Using conventional GC–MS, pesticides notorious for giving tailing peaks (e.g., methamidophos, acephate, and thiabendazole) gave remarkably lower *H*/*A* ratios in comparison with many of the other analytes. The LP-GC–MS approach, presumably due to the higher flow-rate and thicker film, reduced peak

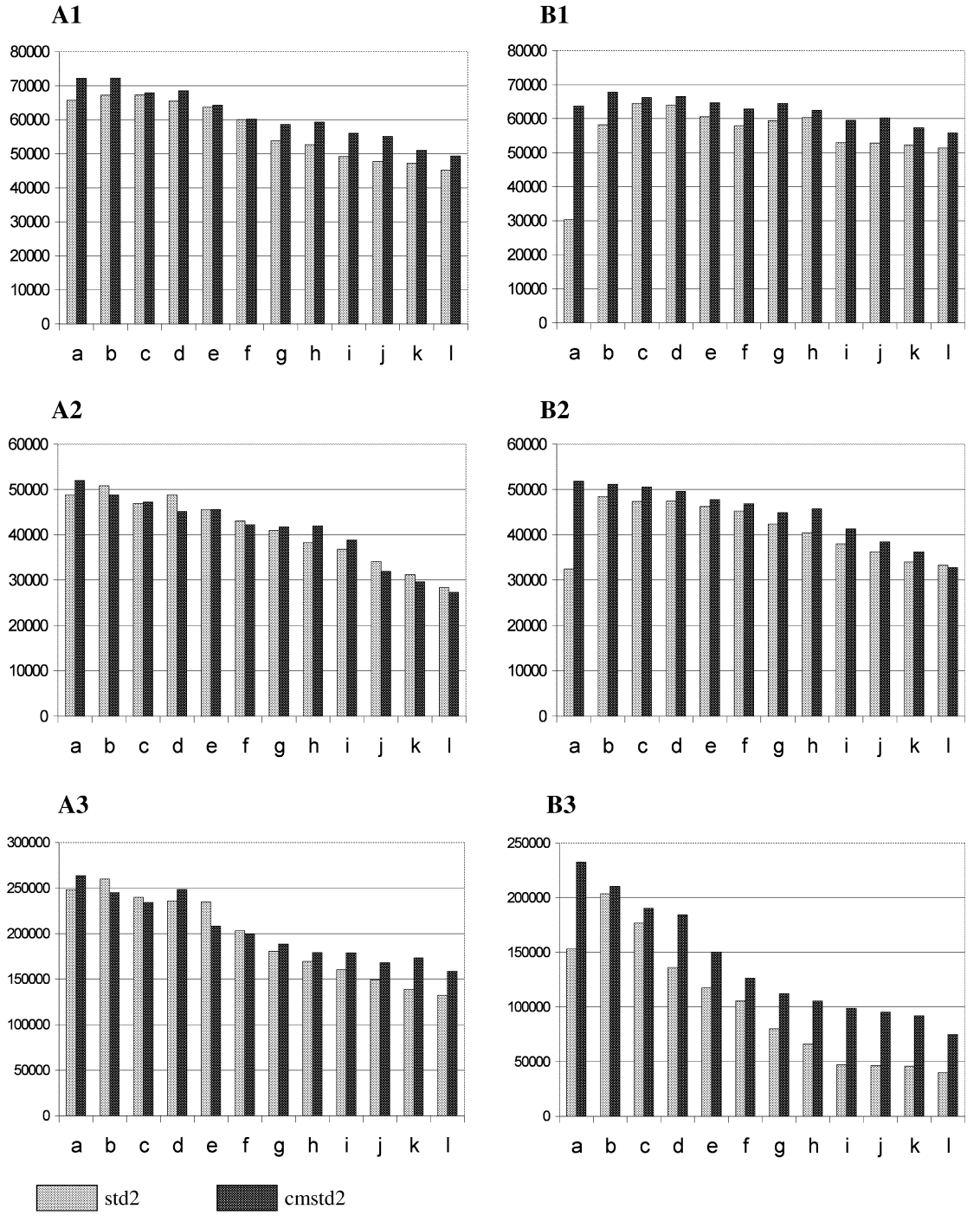

Fig. 5. Long-term stability of responses (peak areas) of (A) heptachlor and (B) dimethoate, at 0.5 μg/ml in solvent standards (std2*n*) and matrix-matched standards (cmstd2*n*) in the 12 sequences (*n* = a–l) for (1) conventional GC–MS technique, 1 μl injection, (2) LP-GC–MS technique, 1 μl injection, and (3) LP-GC–MS technique, 2 μl injection. See Section 2.3 for a description of the stability study.

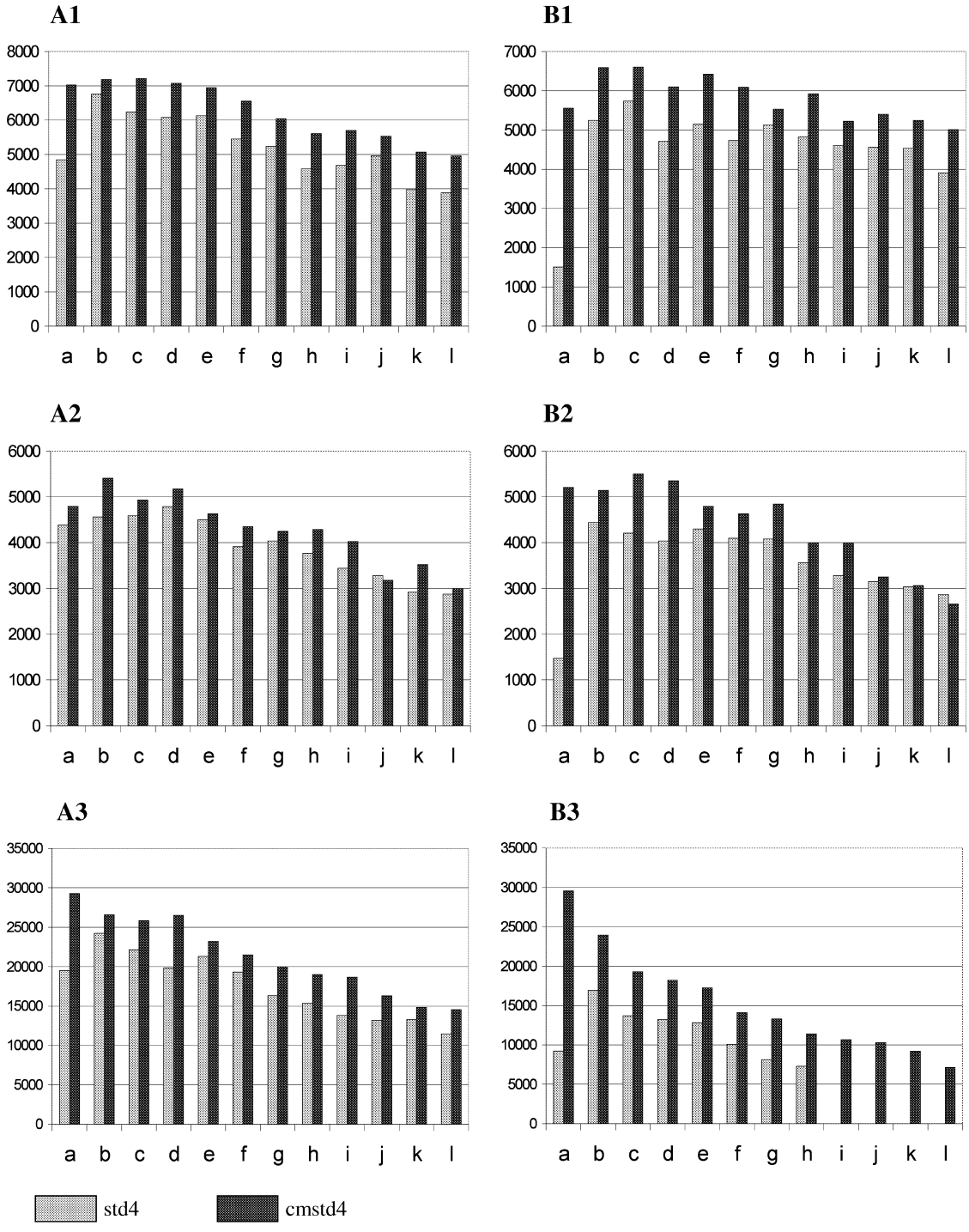

Fig. 6. Long-term stability of responses (peak areas) of (A) heptachlor and (B) dimethoate at 0.05 μg/ml in solvent standards (std4*n*) and matrix-matched standards (cmstd4*n*) for the 12 sets (*n* = a–l) using (1) conventional GC–MS technique, 1 μl injection, (2) LP-GC–MS technique, 1 μl injection, and (3) LP-GC–MS technique, 2 μl injection. See Section 2.3 for a description of the stability study.

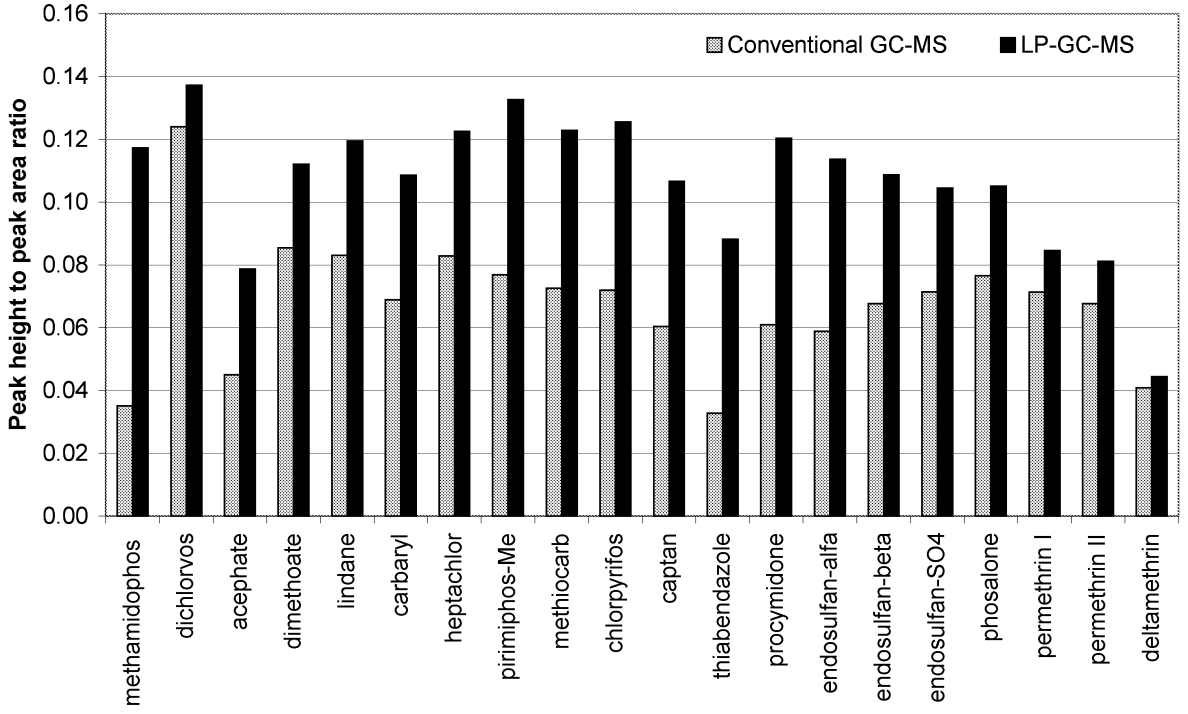

Fig. 7. Peak height to peak area ratios obtained by conventional GC–MS and LP-GC–MS (1 μl injection of std3d).

tailing of these pesticides and thus improved their detection limits in solvent. Fig. 8 shows the difference in peak shapes of thiabendazole obtained in the different methods (procymidone is also shown for comparison of a non-problematic pesticide). The thicker film alone may have been a factor in the better peak shape for thiabendazole, but LP-GC–MS gave undeniably better peak shapes than conventional GC–MS even after many injections of carrot extracts.

Another benefit of the LC-GC–MS approach was improved detectability of the thermally labile carbamates, carbaryl and methiocarb. Due to the faster GC analysis, not only taller peaks are obtained, but also analytes spend less time in the liner and column, and elution temperature for a given separation may be lower. To demonstrate this advantage, relative responses of selected pesticides (their peak heights related to corresponding peak heights of heptachlor) were averaged from 3 sets of analyses of solvent and matrix-matched standards (std3*n* and cmstd3*n*, *n*=d, h, l, i.e., in the fourth, eighth and 12th sequences). Table 4 presents the results from these calculations. The relative responses of carbaryl and methiocarb obtained in LP-GC–MS (numbers in bold in Table 4) were slightly higher than those obtained in conventional GC–MS, whereas relative responses of thermally stable pesticides were very similar (almost identical) in both methods. Note that this enhancement in LP-GC–MS of carbaryl and methiocarb responses was less pronounced in the case of matrix-matched standards because matrix components partly protect these pesticides from thermal degradation in the GC inlet.

Limits of detection (LODs) of the pesticides in matrix were estimated from the matrix-matched calibration curves in the third sequence. The LODs were calculated by extrapolating the signal/noise (S/N) ratios of the pesticide peaks at the chosen quantitation ion(s) to determine the concentration at which $S/N=3$. Table 5 presents the average results for the LODs of the 20 pesticides using the different methods. If no co-eluting mass spectral interferences arose from the matrix, then the 2 μl injections in LP-GC–MS provided the lowest LOD, which was the case for deltamethrin, phosalone, procymidone,

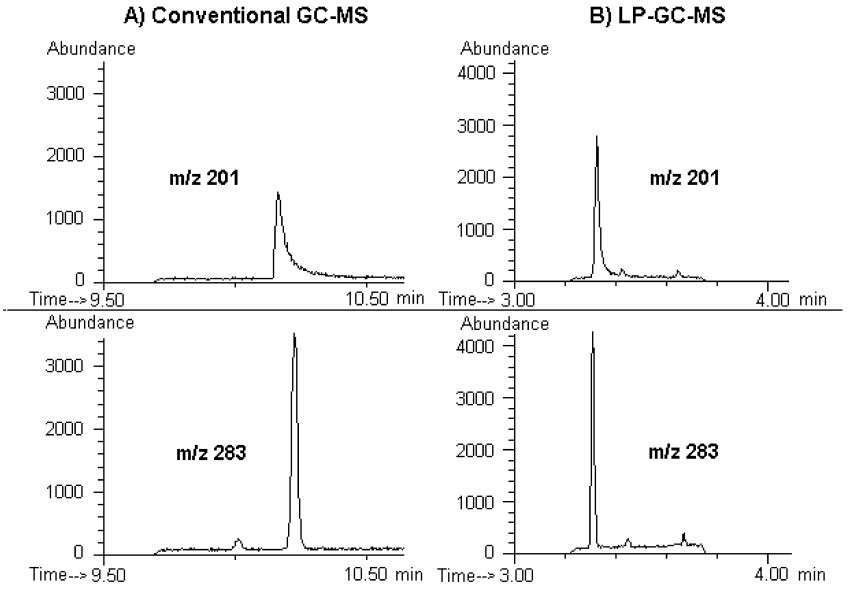

Fig. 8. Comparison of peak shapes of thiabendazole (*m*/*z* 201) and procymidone (*m*/*z* 283) obtained by (A) conventional GC–MS and (B) LP-GC–MS (1 μl injection of std3d).

captan, pirimiphos-methyl, heptachlor, and propargite (propargite's double peak in conventional GC–MS vs. single peak in LP-GC–MS also played a part in this instance). In those cases in which mass spectral interferences from the matrix limited LODs in both conventional GC–MS and LP-GC–MS, then LODs were similar independent of the amount injected. This situation was the case for methamidophos, dichlorvos, lindane, carbaryl, chlorpyrifos, permethrins, and endosulfans I, II, and sulfate.

However, there were some instances (acephate, dimethoate, thiabendazole, and methiocarb) in which a matrix interferant co-eluted in the LP-GC–MS method but not in the conventional GC–MS approach due to the reduced separation efficiency. Fig. 9 shows how acephate retention time fell in a narrow window between interferences in conventional GC–MS but not in LP-GC–MS. Fig. 10 provides a comparison for selected other analytes in the different methods.

It is difficult to directly compare the separation efficiencies of the two methods due to the many

Table 4
Average relative responses±standard deviation of selected pesticides in std3*n* and cmstd3*n* (*n* = d, h, l) obtained by conventional GC–MS and LP-GC–MS (1 μl injection)

Pesticide	*m*/*z*	Relative response in solvent standard[a]		Relative response in carrot standard[a]	
		GC–MS	LP-GC–MS	GC–MS	LP-GC–MS
Dimethoate	125	0.93±0.06	0.93±0.06	1.0±0.0	1.0±0.1
Lindane	181	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Carbaryl	144	2.5±0.1	**3.4±0.1**	3.1±0.2	**3.6±0.3**
Heptachlor	272	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Pirimiphos-methyl	290	2.0±0.0	2.0±0.1	2.0±0.1	2.1±0.2
Methiocarb	168	2.3±0.0	**2.9±0.0**	2.6±0.2	**2.9±0.1**
Chlorpyrifos	314	0.80±0.00	0.80±0.00	0.77±0.06	0.77±0.06

Data in bold demonstrate increased response of the carbamates in LP-GC–MS presumably due to less degradation at the higher flow-rate.
[a] Peak heights of pesticide vs. heptachlor.

Table 5
Average estimated limits of detection (LODs) of the pesticides analyzed in the carrot extracts from the third sequence (cmstds1–5c) in each approach

Pesticide	*m/z*	LOD (ng/g)		
		GC–MS (1 μl injection)	LP-GC–MS (1 μl injection)	LP-GC–MS (2 μl injection)
Methamidophos	141	2	4	2
Dichlorvos	185	0.1	0.3	0.1
Acephate	136	35	203	49
Dimethoate	125	2	6	8
Lindane	181	0.6	1	0.6
Carbaryl	144	0.5	1	0.5
Heptachlor	272	0.4	0.3	0.1
Pirimiphos-methyl	290	0.2	0.2	0.1
Methiocarb	168	0.3	0.9	0.9
Chlorpyrifos	314	0.3	0.4	0.3
Captan	79	17	16	8
Thiabendazole	201	3	11	16
Procymidone	283	0.5	0.4	0.2
Endosulfan I	195	3	4	4
Endosulfan II	241	3	6	5
Endosulfan sulfate	272	0.6	0.8	0.6
Propargite	350	0.5	0.4	0.1
Phosalone	367	0.5	0.3	0.2
Permethrins	183	2	3	2
Deltamethrin	181	4	3	1

differences in oven temperature programs and other parameters. Deltamethrin eluted at 290°C in both methods with retention times and peak widths (FWHM) of 5.35 min and 2.2 s, respectively, in LP-GC–MS and 17.49 min and 2.5 s, respectively, in conventional GC–MS. This corresponds to an 8.2-fold loss in separation power in LP-GC–MS versus the conventional approach (in terms of calculated number of theoretical plates). However, similar calculations for heptachlor, which eluted at approximately 230°C in both methods, showed that the GC–MS method with the 30 m narrow-bore column gave only 3.2-fold more theoretical plates than LP-GC–MS with the 10 m mega-bore column. This reduced separation power in LP-GC–MS led to a loss of detectability for four of the 20 analytes in the carrot extracts. The LODs were still quite low in those instances and this cost resulting from less extensive separation for a few analytes was compensated by the lower LODs for several other pesticides (achieved due to larger sample loadability in LP-GC–MS) and threefold gain in speed of the separation.

4. Conclusions

In this study, the operating parameters of LP-GC–MS were evaluated and optimized for the analysis of multiple pesticide residues in nonfatty foods. No expensive or complicated changes in a common quadrupole GC–MS instrument were required to use this unique approach, and injection, detection, and analysis were conducted as usual in GC–MS (SIM). In ruggedness testing of the LP-GC–MS approach involving the analysis of carrot extracts, the demonstrated general advantages of LP-GC–MS over conventional GC–MS methods included: (i) threefold gain in the speed of chromatographic analysis; (ii) substantially increased injection volume capacity in toluene; (iii) heightened peaks with 2 s peak widths for normal MS operation; (iv) reduced thermal degradation of thermally labile analytes, such as carbamates; and (v) due to larger sample loadability lower detection limits for compounds not limited by matrix interferences. Ruggedness and matrix effects in the LP-GC–MS approach and were not better than conventional GC–MS, but these are common issues

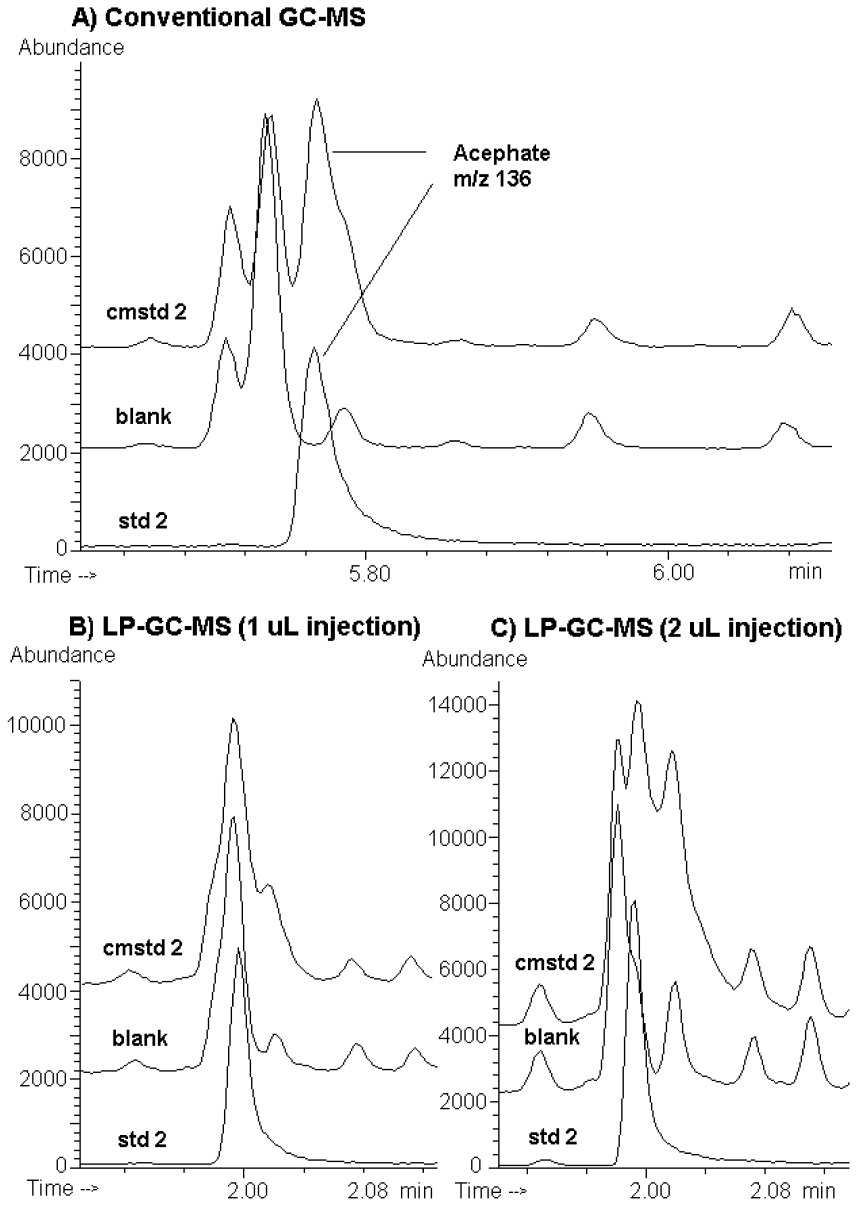

Fig. 9. Overlay of extracted ion chromatograms for acephate (m/z 136) of cmstd2c, blankc and std2c obtained by (A) conventional GC–MS, (B) LP-GC–MS (1 μl injection), and (C) LP-GC–MS (2 μl injection).

with any analytical method and are easily addressed by routine system maintenance or further clean-up of extracts. The trade-off in LP-GC–MS was a lower separation efficiency than conventional methods, which can affect detection of some analytes in a complex matrix.

Other methods to fast GC are also possible, and all must balance the gains in speed with losses in detection capability, sample capacity, and/or separation efficiency. In the LP-GC–MS approach, only loss of separation was sacrificed at a modest level, and gains in detectability, sample capacity, and speed were demonstrated. The use of MS still enabled analysis of the 20 analytes in the complex extracts

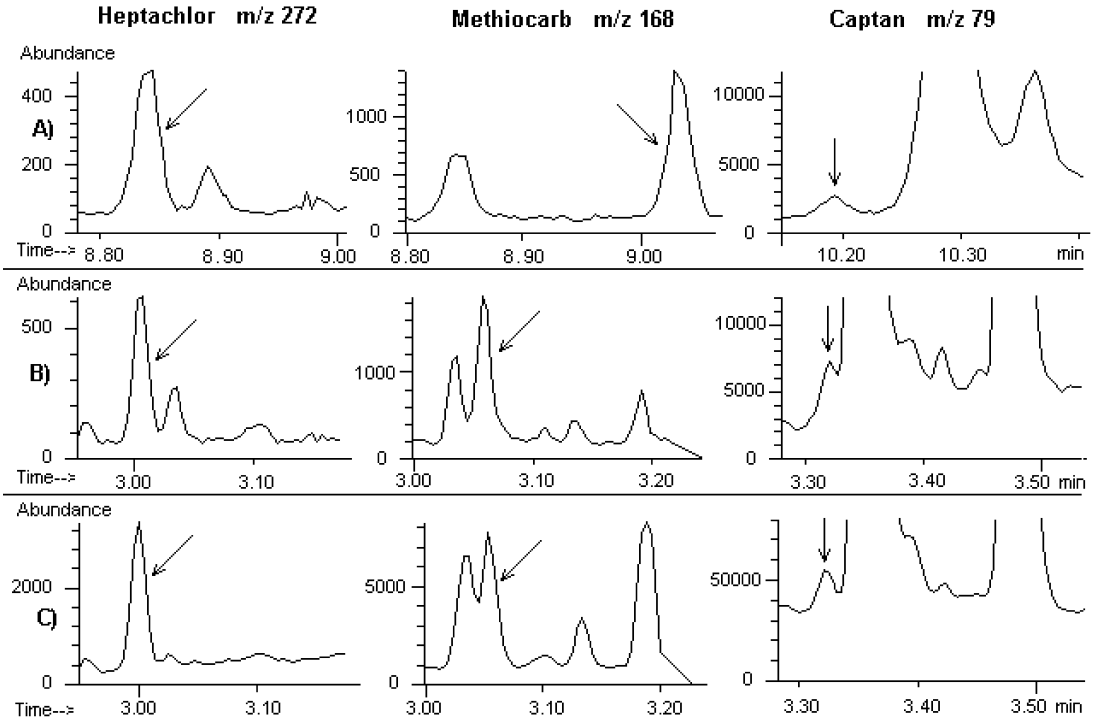

Fig. 10. Separation of selected pesticides from matrix components obtained by (A) conventional GC–MS, (B) LP-GC–MS (1 μl injection), and (C) LP-GC–MS (2 μl injection) for heptachlor in cmstd4c (m/z 272), methiocarb in cmstd4c (m/z 168), and captan in cmstd2c (m/z 79).

when co-elutions occurred, albeit at higher detection limits for a few pesticides.

In the future, the application of direct sample introduction in the LP-GC–MS approach would be very beneficial in that even larger extract volumes could be injected without clean-up or solvent evaporation steps, and no maintenance would be needed because the nonvolatile matrix components would be removed after every injection [42–45]. This fast and easy sample preparation and large volume injection technique is an effective complement in fast GC–MS analysis to increase overall sample throughput [46]. Quantitative and confirmatory analysis for multiple pesticides in a single sample could take as little as 15 min from start to finish with such an approach.

5. Note

Mention of brand or firm name does not constitute an endorsement by the US Department of Agriculture above others of a similar nature not mentioned.

Acknowledgements

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