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Ruggedness and other performance characteristics of low-pressure gas chromatography–mass spectrometry for the fast analysis of multiple pesticide residues in food crops☆

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Abstract

Low-pressure gas chromatography–mass spectrometry (LP-GC–MS) using a quadrupole MS instrument was further optimized and evaluated for the fast analysis of multiple pesticide residues in food crops. Performance of two different LP-GC–MS column configurations was compared in various experiments, including ruggedness tests with repeated injections of pesticides in matrix extracts. The tested column configurations employed the same 3 m × 0.15 mm i.d. restriction capillary at the inlet end, but different analytical columns attached to the vacuum: (A) a 10 m × 0.53 mm i.d., 1 μm film thickness RTX-5 Sil MS column; and (B) a 10 m × 0.25 mm i.d., 0.25 μm film thickness DB-5MS column. Under the optimized conditions (compromise between speed and sensitivity), the narrower analytical column with a thinner film provided slightly (<1.1-fold) faster analysis of <5.5 min separation times and somewhat greater separation efficiency. However, lower detection limits for most of the tested pesticides in real extracts were achieved using the mega-bore configuration, which also provided significantly greater ruggedness of the analysis (long-term repeatability of analyte peak intensities, shapes, and retention times). Additionally, the effect of the increasing injection volume (1–5 μl) on analyte signal-to-noise ratios was evaluated. For the majority of the tested analyte–matrix combinations, the increase in sensitivity caused by a larger injection did not translate in the same gain in analyte detectability. Considering the costs and benefits, the injection volume of 2–3 μl was optimal for detectability of the majority of 57 selected pesticides in apple, carrot, lettuce, and wheat extracts.

Keywords: Pesticide residue analysis; Ruggedness; Matrix effects; Injection volume; Food analysis

1. Introduction

In all routine analytical applications, sample throughput is one of the most important considerations in choosing an analytical method or technique for practical use. In this respect, fast gas chromatography–mass spectrometry (GC–MS) offers increased speed of the determinative step (which may or may not translate into a significant increase in sample throughput, depending on time effectiveness of other parts of the overall analytical process). As compared to GC with element selective detectors, MS provides an additional adjustable degree of control in selectivity, thus potentially compensating for reduced selectivity in GC caused by a sacrifice in separation efficiency made for the increase in speed using most fast GC techniques.

A recently published review [1] discusses practical considerations related to fast GC–MS and describes the main current approaches, which all employ short capillary columns in addition to: (i) reduced column inner diameter using micro-bore capillary GC columns coupled with time-of-flight (TOF)-MS or other high duty cycle detectors for analysis; (ii) fast temperature programming using resistive heating or conventional GC ovens; (iii) sub-ambient pressure in the analytical column in low-pressure (LP)-GC–MS; (iv) supersonic molecular beam (SMB) for MS at rather high carrier gas flow rates; and (v) pressure-tunable (also called stop-flow) GC–GC

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for improved selectivity with respect to the utilization of time.

In LP-GC–MS, lower column pressures lead to higher diffusivity of the solute in the gas phase, which shifts the optimum carrier gas velocity to a higher value, resulting in faster GC separation as compared to the use of the same column operated at atmospheric outlet pressure conditions [2–6]. The gain in speed becomes pronounced mainly for shorter and wider columns because they can be operated at lower pressures along the entire column length. The use of a short, narrow restriction capillary connected to the front part of the analytical column can elegantly solve the problems associated with the sub-ambient pressure conditions extending to the injector [7,8].

In our previous study [9], we evaluated the LP-GC–MS approach for a fast analysis of 20 representative pesticides in food matrices using a restriction capillary connected to a short mega-bore column and a quadrupole GC–MS instrument. As compared to conventional GC–MS, the LP-GC–MS method provided several benefits including a 3-fold gain in speed, heightened peaks with peak widths for normal MS operation, reduced thermal degradation of thermally labile pesticides, and due to larger sample loadability lower limits of detection (LODs) for compounds not limited by matrix interferences. A LP-GC–MS column configuration has been also evaluated for analysis of 72 pesticides using ion-trap (ITD)-MS–MS [10–12]. The authors reported a 2-fold gain in speed of their GC–MS analysis, but the resulting, more than 30 min long GC runs can be hardly called fast in terms of the "fast GC" terminology [13,14].

The main objective of the present study was to further optimize and evaluate quadrupole LP-GC–MS for the routine analysis of a larger number of pesticide residues in food crop extracts. In addition to the previously employed column configuration, a narrower analytical column with a thinner film was also tested for comparison purposes. Apart from other experiments, the evaluations involved mainly ruggedness tests with repeated injections of matrix samples. Ruggedness (here, and in many other chromatographic applications, expressed as long-term repeatability of analyte peak intensity, shape, and retention time) is a highly important factor in routine analysis of real-world samples, but its evaluation is usually neglected in other fast GC studies.

2. Experimental

2.1. Chemicals and materials

For comparison purposes, the same 20 representative pesticides (acephate, captan, carbaryl, chlorpyrifos, deltamethrin, dichlorvos, dimethoate, endosulfan I, endosulfan II, endosulfan sulfate, heptachlor, lindane, methamidophos, methiocarb, permethrins, pirimiphos-methyl, procymidone, propargite, and thiabendazole) as selected in the previous study [9], were tested. A composite stock standard solution (10 μg/ml) was prepared in toluene, and a test solution (1 μg/ml) and working standard solutions std1–std3 (100, 50, and 10 ng/ml) were prepared by diluting the stock solution with toluene. Carrot matrix-matched standards P-I/cmstd*m* and P-II/cmstd*m* (where *m* = 1–3) were obtained by reconstituting the residue remaining after evaporation of carrot extracts (prepared by P-I and P-II sample preparation methods, respectively) in working standard solutions.

Table 1
Concentrations of each of the 20 pesticides in matrix-matched standards prepared in blank carrot extracts (reconstituted in toluene after procedures P-I or P-II)

Matrix-matched standard	Pesticide concentration		
	(ng/ml)	(ng/g)	
		z = P-I	*z* = P-II
z/cmstd1	100	100	20
z/cmstd2	50	50	10
z/cmstd3	10	10	2

The P-I procedure was based on the ethyl acetate extraction [15], followed by a high-performance gel permeation chromatography (HPGPC) clean-up of crude extracts. An automated HPGPC system (Gilson, France) was equipped with a PL gel (600 mm × 7.5 mm, 50 Å) high-performance column (PL Labs, UK). Two milliliters of crude extract (0.5 g sample/ml cyclohexane–ethyl acetate, 1:1, v/v) were injected onto a HPGPC column, under conditions as follows: cyclohexane–ethyl acetate (1:1, v/v) mobile phase, flow rate 1 ml/min, collected fraction 15.5–31 ml. This collected "pesticide" fraction was taken to dryness and dissolved in 1 ml of working standard solutions. The final carrot content of the matrix-matched standards P-I/cmstd1–P-I/cmstd3 was 1 g carrot/ml toluene, and the pesticide concentrations in these standards appear in Table 1.

In the P-II procedure, the carrot sample was extracted with acetone and partitioned with a 1:1 mixture of dichloromethane and petroleum ether according to method 303 used by the U.S. Food and Drug Administration [16] and Dutch Inspectorate for Health Protection [17]. No clean-up steps were conducted and the extracts were taken to dryness and dissolved in working standard solutions. The final carrot content of the matrix-matched standards P-II/cmstd1–P-II/cmstd3 was 5 g carrot/ml toluene, for the pesticide concentrations see Table 1.

To further demonstrate the applicability of the LP-GC–MS approach, a more complex mixture of 57 pesticides in toluene was also prepared. For the pesticide list and their concentrations in composite working standard solution std, see Table 2 (a 5-fold more concentrated test solution was also used in some experiments). Apple, lettuce, carrot and wheat extracts were prepared according to the P-I procedure and the respective matrix-matched standards were obtained by reconstituting the residue remaining after evaporation of the extracts in the working standard solution. The final sample content of the matrix-matched standards was 1 g sample/ml toluene, therefore the pesticide concentrations in these standards (in

Table 2
MS conditions for the LP-GC–MS analysis of 20 pesticides using column configurations A and B (start times of windows and ions selected in SIM mode, quantitation ions in bold)

Pesticide	Start time (min)		SIM ions (*m/z*)		
	A	B			
Methamidophos	1.10	1.10	94	95	**141**
Dichlorvos			109	**185**	220
Acephate	1.50	1.45	94	**136**	142
Dimethoate	2.00	1.83	87	93	**125**
Lindane			**181**	183	219
Carbaryl	2.40	2.24	115	**144**	
Heptachlor			**272**	274	
Pirimiphos-methyl			**290**	305	
Methiocarb			153	**168**	
Chlorpyrifos			197	**314**	
Captan	2.70	2.56	**79**	149	
Thiabendazole			174	**201**	
Procymidone			**283**	285	
Endosulfan I and II			195	241	**339**
Endosulfan sulfate	3.22	3.00	272	274	**387**
Propargite			135	173	**350**
Phosalone	3.44	3.27	182	184	**367**
Permethrins	3.67	3.47	163	165	**183**
Deltamethrin	4.10	4.00	**181**	253	255

μg/g) were numerically the same as concentrations in the toluene standard solution std (in μg/ml).

Pesticide standards, all 95% or higher purity, were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All solvents used in experiments were analytical grade (Merck, Germany). Carrots, apples, lettuce and wheat grains (none of which contained pesticide analytes) were obtained at a retail market.

2.2. GC–MS conditions

GC–MS experiments were performed using an Agilent (Little Falls, DE, USA) 6890 gas chromatograph combined with a 5973 MSD. The system was equipped with electronic pressure control (EPC), a split/splitless injector, and a 7673A autosampler; Chemstation software was used for instrument control and data analysis. Samples were injected into 4 mm i.d. double taper liners with internal volume of 800 μl (No. 5181-3315, Agilent, USA).

Two column configurations—A and B—were used for the experiments. In the column configuration A, a 10 m × 0.53 mm i.d. × 1 μm film thickness RTX-5 Sil MS capillary column (Restek, USA) was connected to a 3 m × 0.15 mm i.d. non-coated restriction column (Restek) at the inlet end. A stainless steel union (Agilent 0101-0594) in which the restriction column fit inside the mega-bore column was used for a true zero-dead-volume connection. In the column configuration B (LP-GC–MS approach B), a 10 m × 0.25 mm i.d. × 0.25 μm film thickness DB5-MS capillary column (J&W Scientific, USA) was connected to the same non-coated restriction column as in the configuration A. A stainless steel column connector (Agilent 5061-5801) was used in this case.

The optimized conditions for the analyses of the 20 pesticides using both column configurations were as follows: He carrier gas, pressure pulse 40 psig for 0.5 min, then constant pressure 20 psig for the rest of the analysis (1 psig = 6894.77 Pa), 1–5 μl (pulsed splitless) injection volume, 250 °C inlet temperature, 280 °C MSD interface temperature, 150 °C ion source temperature, 230 °C quadrupole temperature and an oven temperature program of 90 °C for 0.5 min, then a 80 °C/min ramp to 180 °C followed by a 60 °C/min ramp to 290 °C (held for 3 min). Total GC run time was 6.5 min and retention times (t_R) of the last eluting analyte deltamethrin were 4.73 and 4.34 min using the column configurations A and B, respectively. The MS conditions in the selected ion monitoring (SIM) mode are given in Table 3. The fastest ion monitoring possible, i.e. the minimum "dwell 10" setting in the Agilent Chemstation software, was used for recording of all selected ions in all experiments.

The optimized conditions for the analysis of the 57 selected pesticides were the same as in the case of the 20 pesticides with the exceptions of the oven temperature program and MS SIM settings. The temperature program started at 90 °C (held for 0.5 min), then the temperature was ramped at 80 °C/min to 180 °C followed by a 40 °C/min ramp to 250 °C and a 60 °C/min ramp to 290 °C (held for 3 min). Total GC run time was 7 min and t_R of the last eluting analyte deltamethrin were 5.49 and 5.22 min using the column configurations A and B, respectively. The MS conditions for the analysis of the 57 pesticides in the SIM mode are given in Table 2.

2.3. Comparison of the column configuration A and B performances—sequence of samples

To compare the performance of the LP-GC–MS column configurations A and B, the 20 selected pesticides were repetitively analyzed in 10 sequences (a–j), between which no GC system maintenance was performed. The order of the injections in the sequences was as follows: (1) toluene; (2–4) std1*n*–std3*n*; (5) carrot blank; (6–8) cmstd1*n*–cmstd3*n* (where *n* = a–j). For each column configuration, this set of 10 sequences was analyzed three times testing: (i) 5 μl injections of toluene solutions and carrot extracts prepared by the procedure P-I; (ii) 1 μl injections of toluene solutions and carrot extracts prepared by the procedure P-II; and (iii) 5 μl injections of toluene solutions and carrot extracts prepared by the procedure P-II. The system maintenance, involving replacement of the liner and the restriction capillary and cutting about 5–10 cm of the front part of the analytical column, was performed between these experiments (sets of 80 injections).

2.4. Determination of the influence of the injection volume on signal-to-noise (S/N) ratio

To determine the effect of the injected volume on the analyte detectability (S/N ratio), 1–5 μl of blank extracts

Table 3
List of 57 pesticides including their concentrations in the working standard solution std (in toluene) and MS conditions for their LP-GC–MS analysis using column configurations A and B (start times of windows and ions selected in SIM mode, quantitation ions in bold)

Pesticide	Concentration in std (ng/ml)	Start time (min)		SIM ions (*m*/*z*)		
		A	B			
Methamidophos	391	1.10	1.10	94	95	**141**
Dichlorvos	178			109	**185**	220
Mevinphos	391	1.50	1.55	**127**	192	
Acephate	404			94	**136**	142
Propham	950			93	137	**179**
Methacriphos	298	1.82	1.80	180	208	**240**
Heptenophos	285	1.93	1.89	109	124	**250**
Omethoate	487			110	141	**156**
Chlorpropham	1280	2.13	2.11	154	171	**213**
Monocrotophos	286			109	**127**	192
Dimethoate	309	2.32	2.28	87	**93**	125
Diazinon	115	2.43	2.37	179	**304**	
Lindane	94			**181**	219	
Phosphamidon I	76			127	**264**	
Etrimfos	118			153	**292**	
Chlorothalonil	67			**266**	268	
Pirimicarb	218			**166**	238	
Phosphamidon II	205	2.63	2.56	109	127	**264**
Chlorpyrifos-methyl	238			197	286	
Parathion-methyl	205			233	**263**	
Tolclofos-methyl	208			**265**	267	
Vinclozolin	39			212	**285**	
Carbaryl	478			115	**144**	
Pirimiphos-methyl	164	2.80	2.70	279	**290**	
Fenitrothion	162			125	**277**	
Malathion	259			**173**	256	
Dichlofluanid	181			123	**224**	
Chlorpyrifos	221			197	**314**	
Fenthion	205			125	**278**	
Parathion-ethyl	196			139	**291**	
Chlorfenvinphos	306	3.03	2.94	**267**	323	
Tolylfluanid	47			137	**238**	
Captan	126			**79**	149	
Thiabendazole	369			174	**201**	
Procymidone	121			**283**	285	
Folpet	277			**260**	297	
Methidathion	229			125	**145**	
Endosulfan I	39	3.31	3.20	195	241	**339**
Imazalil	613			173	**215**	
Bupirimate	312			208	**273**	
Ethion	142	3.56	3.44	153	**231**	384
Endosulfan II	35			195	241	**339**
Triazophos	374			161	**172**	257
Endosulfan sulfate	43	3.77	3.65	272	274	**387**
Bifenthrin	91	3.88	3.78	165	**181**	
Fenoxycarb	609			116	186	**255**
Bromopropylate	85			185	**341**	
Phosmet	223			133	**160**	317
Tetradifon	43	4.08	3.98	159	229	**356**
Phosalone	266			**182**	367	
Azinphos-methyl	119			77	132	**160**
λ-Cyhalothrin	69			181	**197**	208
Azinphos-ethyl	118	4.28	4.14	105	132	**160**
Permethrin	206	4.38	4.24	163	165	**183**
β-Cyfluthrin	79	4.57	4.42	**206**	226	
Cypermethrin	103			163	**181**	208
Fenvalerate	93	4.93	4.74	**167**	225	419
Deltamethrin	238	5.32	5.08	181	**253**	255

prepared by the procedure P-I were injected into the GC–MS system along with 1–5 μl of the same extracts spiked with 57 selected pesticides (matrix-matched standards). This procedure was repeated for each of the following matrices: apples, wheat, lettuce, and carrots. S/N was determined for each analyte as the ratio of analyte peak height (obtained in the analysis of matrix-matched standards) to the value of root mean square (RMS) noise obtained in the chromatogram of the corresponding blank extract (the same injection volume and matrix) at the elution time (taken from the beginning to the end) of the given analyte peak.

3. Results and discussion

3.1. Optimization of conditions for the LP-GC–MS column configuration A

In the previous study [9], we optimized and evaluated the LP-GC–MS technique using a HP 5890 Series II Plus GC combined with a 5972 MSD. In this study, we took advantage of a more advanced Agilent 6890/5973 GC–MSD system to further improve the analysis of the selected pesticides. For comparison purposes, the same mixture of the 20 pesticides and the same column configuration as in the previous study were used for the initial experiments. With this column combination (described in Section 2.2 as the column configuration A), a constant column inlet pressure of 20 psig provided the maximum sensitivity (peak heights) for most of the analytes. Lower column inlet pressures resulted in wider, thus smaller peaks; whereas at the pressures >20 psig, the effect of MS response decrease with increasing flow rates prevailed over the peak sharpening effect at the same pressure conditions [9]. Fig. 1A shows this effect along with the influence of the column inlet pressure setting on t_R in the case of the last eluting analyte deltamethrin (note that a ≈10% reduction in the analysis time would result in a ≈30% decrease of sensitivity).

The oven of an Agilent 6890 GC offers higher temperature programming rates with a maximum setting of 120 °C/min versus a 70 °C/min maximum setting in the case of a HP 5890 GC instrument. However, these maximum rates are reached only at low temperatures. For example, the Agilent 6890 (240 V) GC provides a ramp of 120 °C/min only for heating from 50 to 70 °C, whereas in temperature ranges of 70–115, 115–175, and 175–300 °C, the actual rates of only 95, 65, and 45 °C/min can be achieved, respectively [18]. This is caused by increasing heat losses from the oven to the surrounding air as the temperature increases [19], which may lead to larger time lags between the actual and set column temperatures and less reproducible heating at higher temperature programming rates. The former effect lengthens the analysis time versus the expected one, whereas the latter effect decreases the t_R precision, which is a crucial parameter in fast GC–MS with narrow SIM time-window settings.

According to our experience with an Agilent 6890 system, when using a single ramp for heating from 70 to 325 °C, a

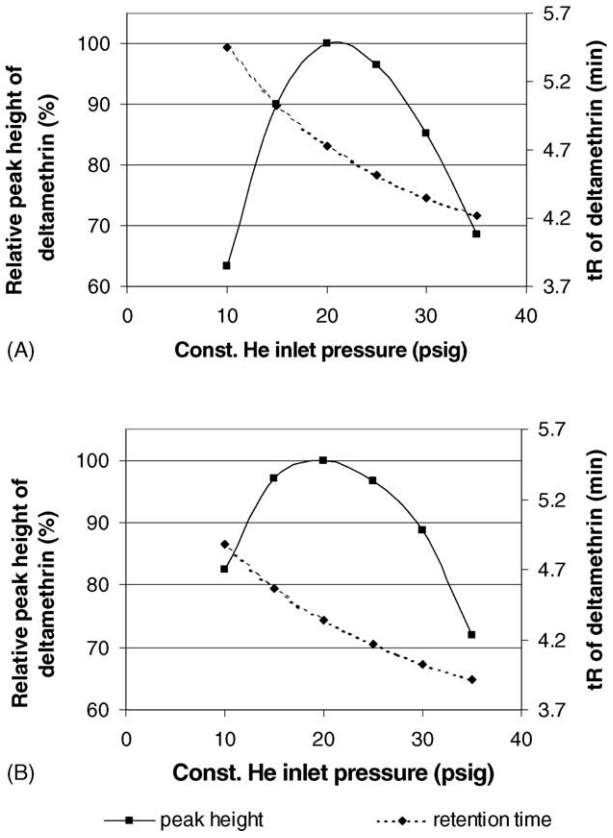

Fig. 1. Influence of the column inlet pressure (10–35 psig) on the peak height (given relatively vs. the greatest peak height) and retention time of deltamethrin obtained with the LP-GC–MS column configurations A and B using the same oven temperature program of 90 °C for 0.5 min, then a 80 °C/min ramp to 180 °C followed by a 60 °C/min ramp to 290 °C (held for 3 min).

temperature programming rate of about 60 °C/min provides acceptable t_R reproducibility for all analytes, including the late eluting ones [20,21]. In this study, we attempted to increase the speed of the analysis using two temperature ramps: (i) a higher ramp at the beginning of the temperature program combined with (ii) a 60 °C/min ramp for programming to higher temperatures. In comparison with a 60 °C/min single ramp temperature program from 90 to 290 °C, a combination of a 80 °C/min ramp from 90 to 180 °C followed by a 60 °C/min ramp to 290 °C reduced t_R of the last eluting deltamethrin by 0.4 min and still provided acceptable heating repeatability (analyte t_R relative standard deviations, R.S.D. < 0.1%, n = 10).

Another advantageous feature of the Agilent 6890 EPC system and newer version of the software involves a pulsed splitless option, i.e. the possibility to apply a pressure pulse during the analyte transfer to the column followed by an immediate adjustment to the initial column pressure conditions optimal for the analysis. The increased pressure during the injection leads to the faster analyte transfer, which results in reduced losses of susceptible analytes due to absorption and/or degradation on actives sites in the inlet [22,23]. Moreover, it also enables injections of larger sample volumes due

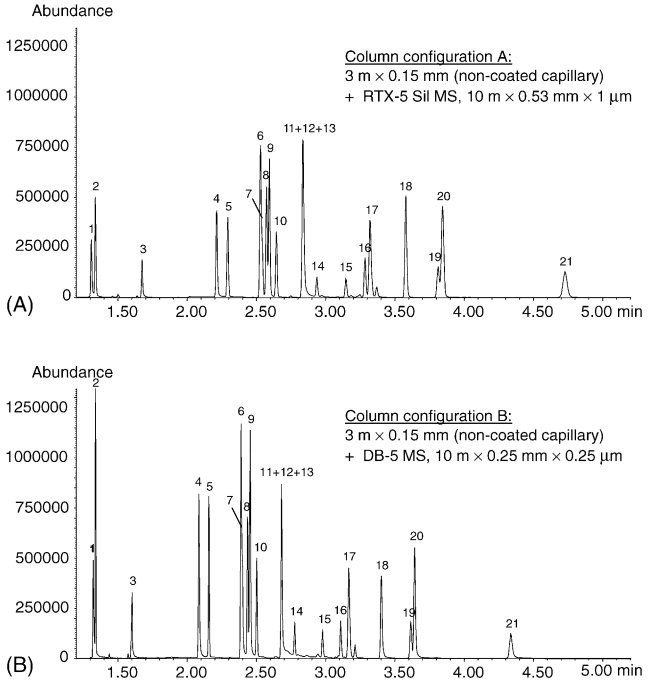

Fig. 2. Chromatograms of 5 μl injection of the mixture of 20 pesticides at 1 μg/ml in toluene obtained with the LP-GC–MS column configurations A and B at the optimized conditions: (1) methamidophos; (2) dichlorvos; (3) acephate; (4) dimethoate; (5) lindane; (6) carbaryl; (7) heptachlor; (8) pirimiphos-methyl;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

to lower expansion volumes at the higher pressure. Generally, injection volumes that generate vapor volumes, which are ≤75% of the liner volume, are considered safe. Thus, at the inlet temperature of 250 °C, maximally 4.5, 5.5, 6.5, and 7.5 μl of a sample in toluene could be safely injected into the used 800 μl liner at the inlet pressures of 30, 40, 50, and 60 psig, respectively. These pressure pulses were tested for 1–5 μl injection of the 20 pesticides in toluene. The 50 and 60 psig pressure pulses caused peak distortion of early eluting analytes, which appeared mainly at larger injection volumes. Thus, the 40 psig pressure pulse was used in further experiments because it permitted a safe injection of up to 5 μl without peak deformations. Fig. 2A shows a chromatogram of 5 μl injection of the 20 pesticides at 1 μg/ml in toluene using the column configuration A at the optimized conditions.

3.2. Optimization of conditions for the LP-GC–MS column configuration B

Separation efficiency and/or sample capacity are usually partially sacrificed in fast GC for an increase in speed. The combination of fast GC with MS detection can compensate for both decreased GC selectivity and sensitivity [1], but analysis of some complex samples still requires a certain degree of chromatographic separation. The LP-GC–MS approach using a short, mega-bore column with a relatively thick film (1 μm) offers high sample capacity, but the separation efficiency is relatively low. For that reason, we decided to compare the performance of the column configuration A with another column set-up (column configuration B).

For comparison purposes, we used the same temperature program as in the case of the column configuration A and performed the other optimization experiments described earlier. Interestingly, a constant column inlet pressure of 20 psig provided the overall highest analyte sensitivity (measured as peak heights) as with the mega-bore column (see Fig. 1B). This would suggest that the flow rate is dictated mainly by the restriction capillary in both cases. However, when connected to a vacuum, the actual pressure in a 0.53 mm i.d. capillary is lower than in the case of a 0.25 mm i.d. capillary of the same length [7], resulting in a somewhat higher column flow rate in the former case if the same pressure is applied to the restriction capillary at the same oven temperature. On the other hand, the mega-bore column with a relatively thick film retains the analytes longer even at the higher column flow rates (as shown by t_R of deltamethrin in Fig. 1A and B). Thus, using the same oven temperature program, the analytes elute from the narrower column at slightly lower temperatures, potentially resulting in similar flow rate conditions (or better: a similar outcome of the antagonistic GC peak sharpening and MS effects) at the time of analyte elution from both column configurations at the constant column inlet pressure of 20 psig.

The use of a 3 m long restriction capillary serving as a retention gap along with the application of a 40 psig pressure pulse during the sample introduction permitted the splitless injection of 1–5 μl of samples in toluene without peak distortions also in the case of the narrower analytical column with a thinner film in the column configuration B. Fig. 2B shows a chromatogram of 5 μl injection of the 20 pesticides at 1 μg/ml in toluene using the column configuration B at the optimized conditions. A slightly faster GC analysis is achieved with this column set-up as compared to the column configuration A employed at otherwise the same conditions (t_R of deltamethrin 4.34 min versus 4.73 min).

3.3. Peak characteristics in the LP-GC–MS analysis using two different column configurations

In addition to the slightly shorter analysis time, the use of the column configuration B resulted in narrower analyte peaks, which generally means: (i) increased sensitivity due to higher peaks; (ii) improved separation efficiency; and (iii) less data points across the peaks as compared to the column configuration A. It should be noted that increased sensitivity does not necessary translate into lower detection limits in real samples if the matrix components represent the limiting source of noise and/or the limiting factor in the ruggedness of the GC method (limitations in injection volume size and/or matrix concentration).

In terms of the separation efficiency (number of theoretical plates), the use of the column configuration B gave 1.3–2-fold more number of theoretical plates depending on the particular analyte. The 2-fold improvement was achieved for the last eluting analyte peak (deltamethrin) with full width at half maximum (FWHM) of 2.22 and 1.44 s in the LP-GC–MS approaches A and B, respectively.

For the same data acquisition rate, peak width dictates the number of data points across the peak. As discussed elsewhere [1], there are many discrepancies in the literature concerning how many data points are needed to define a chromatographic peak. Depending on opinions of different authors, 15–20 or as little as 3–4 points are required or claimed to meet quantitation needs. Moreover, other issues further complicate this situation. For instance, it is not always clear if FWHM of full peak widths at baseline ($w_b = 6\sigma$) are used in the discussions or if the baseline points at the beginning and end of the peak should be counted or not.

In SIM, two factors determine the data acquisition rate: (i) the number of ions in the given time window; and (ii) the dwell time, i.e. the time spent monitoring a single ion. In all of our experiments, we used the fastest ion monitoring (minimum dwell time) possible with the 5973 instrument. In this setting ("dwell 10"), it takes 25 ms to record one ion and other 5 ms per each cycle. For example, it takes 255 ms to record 10 ions in one cycle, resulting in a data acquisition rate of 3.92 data points/s in this case. For comparison with the full scan mode, the rate of 3.92 scans/s corresponds to the maximum scanning speed over the range of 589 amu and a data acquisition rate of 42 scans/s can be achieved with a 10 amu scan range. Thus, in addition to a more simple operation than SIM, the full scan mode generally offers faster data acquisition rates and/or increased spectral information in applications where sensitivity can be sacrificed. In residue analysis, however, sensitivity is often the paramount factor; therefore one of our objectives was to demonstrate that a quadrupole instrument in SIM mode is also capable of being applied in a fast GC–MS analysis.

Fig. 3A-1 and B-1 compare peak widths and number of data points across a peak obtained for chlorpyrifos in the LP-GC–MS analysis of the 20 pesticides using the column configurations A and B, respectively. Chlorpyrifos elutes in the middle region of the chromatogram (t_R of 2.65 or 2.50 min in the LP-GC–MS approach A or B, respectively), which is commonly more "crowded" with pesticide peaks as compared to the beginning and end of a typical chromatogram in the GC analysis of pesticides. Thus (even in a conventional GC analysis), ions for several medium-volatile pesticides are usually included in one time window, resulting in slower data acquisition rates for these analytes. In our case, we monitored four other analytes together with chlorpyrifos in one time window, which entailed 10 ions to be recorded in one cycle (2 ions per each analyte). The calculation based on w_b and the data acquisition rate of 3.92 data points/s gave ≈10 and 8 data points across chlorpyrifos peak in the LP-GC–MS approaches A and B, respectively. When this kind of calculation

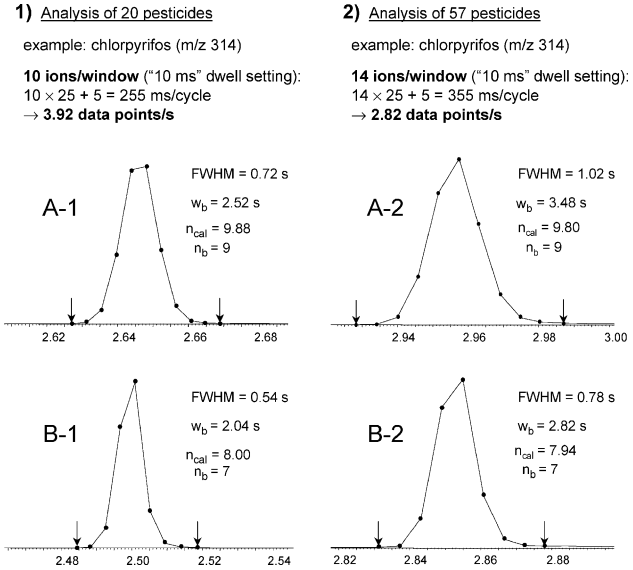

Fig. 3. Peak width and number of points across the peak obtained for chlorpyrifos (*m/z* 314) in the LP-GC–MS analysis of the (1) 20 pesticides (5 ng of chlorpyrifos injected) and (2) 57 pesticides (5.5 ng of chlorpyrifos injected) in toluene using the column configurations A and B. Peak characteristics: full width at half maximum (FWHM), peak width at 50% of the peak height; w_b, peak width at baseline; n_{cal}, calculated number of points across the peak: (1) $n_{cal} = 3.92 w_b$ and (2) $n_{cal} = 2.82 w_b$; n_b, number of points across the peak that are above the baseline. Arrows indicate the beginning and end of the peak (the first data points before and after the peak elevates from the baseline).

is performed, then one of the baseline points is included in the resulting rounded number. We prefer to count only the points that occur above the baseline, because they actually define the peak. This approach gives nine and seven data points as shown in Fig. 3A-1 and B-1, respectively.

In terms of precision of measurements of peak areas and heights, no significant difference between nine and seven points across the peak was observed, provided that the column contamination by non-volatile matrix components did not cause the response diminishment effect as discussed in the following section. Using the same instrument, Dallüge et al. [24] experimentally determined that five to six data points across a peak provides acceptable peak height and area R.S.D. (their number is calculated, thus it corresponds to four to five data points above the baseline). Considering 4 data points across the chlorpyrifos peak at our conditions, up to 21 or 17 ions can be included in one window (corresponding to data acquisition rates of 1.89 and 2.33 data points/s at the shortest dwell time setting possible) when using the column configuration A or B, respectively. Thus, one strategy to include more analytes into a fast GC–MS SIM method (at minimum dwell time) involves reducing the data acquisition rate and, consequently, the number of points across peaks without sacrificing the speed of the analysis.

Another possibility is to somewhat slow-down the separation of the medium-volatile analytes, resulting in slightly wider analyte peaks in this region. Our attempt to include almost three times as many analytes (57 pesticides) in a fast

LP-GC–MS method serves as an example of this approach. The 57 selected pesticides had the same volatility range (from dichlorvos to deltamethrin) as the 20 analytes, therefore we used the same GC conditions except for the temperature program, which employed a slower temperature programming rate of 40 °C/min from 180 to 250 °C (see Section 2). As a result (demonstrated in Fig. 3A-2 and B-2), we obtained the same number of data points across chlorpyrifos peaks as in the analysis of the 20 pesticides, although 14 ions were included in one time window this time (see Table 2). As a penalty, the analysis times were ≈1.2-fold longer in both LP-GC–MS column configurations (deltamethrin t_R 5.49 and 5.22 min in the LP-GC–MS approaches A and B, respectively) when the slower temperature program was used.

3.4. Analysis of real samples using the LP-GC–MS column configurations A and B

The optimization experiments were performed with solvent (toluene) solutions of pesticides. However, to evaluate the feasibility of any analytical approach for routine practice, analyses of real-world samples must be conducted because co-extracted matrix components usually have a great impact on method performance characteristics. In GC analysis, this impact may be both immediate and long-term. The immediate symptoms mainly include lower analyte detectability (due to co-elutions of analyte and matrix component peaks) and matrix-induced response enhancement [25]. The long-term problems are caused by non-volatile matrix components, which gradually contaminate the GC inlet and front part of the column, resulting in formation of new active sites and gradual decrease of analyte responses in both solvent and matrix solutions. This is an effect sometimes called matrix-induced response diminishment [26]. Therefore, to demonstrate ruggedness of a GC method in real-life analyses, a long-term study of the GC system performance, involving repeated injections of matrix samples, should be an essential part of the overall evaluation.

In the previous study [9], we compared the performance of conventional GC–MS and LP-GC–MS methods in relatively long GC sequences consisting of injections of pesticide solutions in toluene and carrot extracts (prepared by the procedure P-II described in Section 2). The conventional GC–MS approach employed a 30 m × 0.25 mm i.d., 0.25 μm film thickness RTX-5MS capillary column and the column configuration A was used in the LP-GC–MS method. Without the use of a retention gap, only 1 μl injection of a sample in toluene was possible in conventional GC–MS, whereas 1 and 2 μl were tested in LP-GC–MS. Using the same injection volume, comparable matrix effects (enhancement and diminishment) were observed in both approaches. Due to lower separation efficiency, direct matrix interferences for the quantitation ions were worse for 4 out of the 20 tested pesticides in LP-GC–MS, but similar or better LODs were achieved in all other cases. The larger injection volume of 2 μl generally improved detectability of analytes with the LP-GC–MS approach, but the response diminishment effect was more pronounced for susceptible analytes as more non-volatile matrix components were introduced into the GC system.

To compare the ruggedness and other performance characteristics of the two LP-GC–MS set-ups discussed in this study, we conducted experiments described in Section 2.3. In addition to 1 μl injections of carrot extracts prepared by the procedure P-II, both column configurations were subjected to repeated 5 μl injections of: (i) the same, rather dirty P-II carrot extracts (a sample equivalent of 25 mg injected each time); and (ii) cleaner and less concentrated carrot extracts prepared by the procedure P-I (corresponding to a 5 mg sample equivalent in one injection). Using this experimental design, not only we could test the performance of the column configurations A and B, but also evaluate two different injection volumes and sample preparation procedures.

Table 4 presents ruggedness results of these experiments (expressed as R.S.D. of peak heights, areas, and t_R) obtained for heptachlor in both toluene solutions (std1*n*–std3*n*, *n* = a–j) and carrot extracts (cmstd1*n*–cmstd3*n*, *n* = a–j). The organochlorine pesticide heptachlor represents a relatively stable analyte, which is generally not prone to matrix effects in GC [9,27]. Indeed, very good ruggedness was observed when sample equivalents of 5 mg of the carrot matrix were introduced into the GC system, with slightly better results obtained in the case of the extracts subjected to the GPC cleanup in the sample preparation procedure P-I. These results also demonstrate that comparable precision of measurements of peak areas and heights can be achieved with nine or seven data points across the heptachlor peak (heptachlor elutes in the same time window as above discussed chlorpyrifos) with the LP-GC–MS set-ups A and B, respectively.

Nevertheless, even in the case of normally non-problematic heptachlor, the repeated, 5-fold higher matrix introductions (5 μl injections of P-II carrot extracts) resulted in decreased ruggedness, characterized by a gradual peak height diminishment (due to peak broadening and distortion) and also a gradual increase in t_R in both matrix and matrix-free pesticide solutions. In this respect, the mega-bore analytical column provided significantly better results as compared to the narrower column with the thinner film in the LP-GC–MS set-up B, which is indicated by the approximately 5-fold better precision of the t_R measurements and 1.4–1.9-fold lower R.S.D. of peak heights. This is further demonstrated in Figs. 4 and 5, which show overlaid chromatograms of another organochlorine pesticide, lindane, and a carbamate pesticide carbaryl, respectively, obtained in the analysis of toluene solutions std1a–std1j in the experiments with 5 μl injections of P-I and P-II carrot extracts. We preferred to present the overlays of the toluene solutions over the carrot extracts because matrix components eluting in the proximity of the analyte peaks would complicate the figure in the case of the P-II extracts. Also, the signal diminishment effect is usually more pronounced in solvent solutions (compare the results for heptachlor in Table 4), thus providing

Table 4
Repeatability of peak heights, areas and t_R (expressed as R.S.D. in %, n = 10) obtained for heptachlor (m/z 272) in solvent standards (std1n–std3n, n = a–j) and carrot matrix-matched standards (cmstd1n–cmstd3n, n = a–j) in the 10 sequences using the LP-GC–MS column configurations A and B, injection volumes 1 or 5 μl, and sample preparation methods P-I or P-II for preparation of carrot extracts (see Section 2 for a detailed description)

Column configuration	Experiment	Solvent standards			Matrix-matched standards		
		std1	std2	std3	cmstd1	cmstd2	cmstd3
(a) R.S.D. (%) of peak heights							
A	P-I 5 μl	3	4	3	3	3	4
	P-II 1 μl	3	4	4	5	5	9
	P-II 5 μl	21	23	24	19	19	16
B	P-I 5 μl	4	6	4	7	9	5
	P-II 1 μl	7	8	9	4	6	9
	P-II 5 μl	40	37	39	26	30	30
(b) R.S.D. (%) of peak areas							
A	P-I 5 μl	3	4	3	2	2	3
	P-II 1 μl	3	3	4	6	5	8
	P-II 5 μl	13	16	15	15	14	14
B	P-I 5 μl	4	3	4	5	6	5
	P-II 1 μl	5	5	8	4	5	8
	P-II 5 μl	4	4	10	7	8	14
(c) R.S.D. (%) of t_R							
A	P-I 5 μl	0.03	0.04	0.03	0.02	0.02	0.03
	P-II 1 μl	0.04	0.05	0.03	0.04	0.04	0.05
	P-II 5 μl	0.23	0.23	0.22	0.18	0.17	0.18
B	P-I 5 μl	0.03	0.05	0.06	0.03	0.02	0.04
	P-II 1 μl	0.03	0.03	0.02	0.04	0.04	0.04
	P-II 5 μl	1.16	1.18	1.16	0.91	1.00	1.02

better indication of the column tolerance to increasing number of matrix injections.

Similarly to heptachlor, the 5 μl injections of the P-I extracts (and 1 μl injections of the P-II extracts, for which the results are not shown in the figures) did not cause signal diminishment or t_R shift of the lindane peaks in the respective sequences of GC runs using both LP-GC–MS set-ups. Again, the column configuration A proved to handle the increased matrix injections (5 μl of P-II extracts) significantly better, considering the more rapid decrease in lindane peak heights

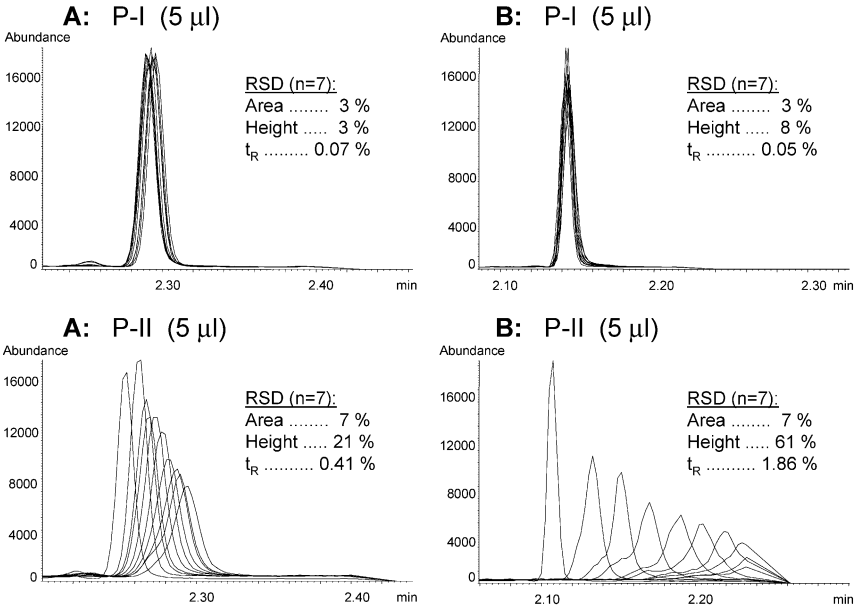

Fig. 4. Overlay of 10 extracted ion chromatograms of lindane (m/z 181) obtained in the analysis of toluene solutions std1a–std1j in the experiments with repeated 5 μl injections of P-I and P-II carrot extracts using the LP-GC–MS column configurations A and B (see Section 2.3 for the sequence of GC injections).

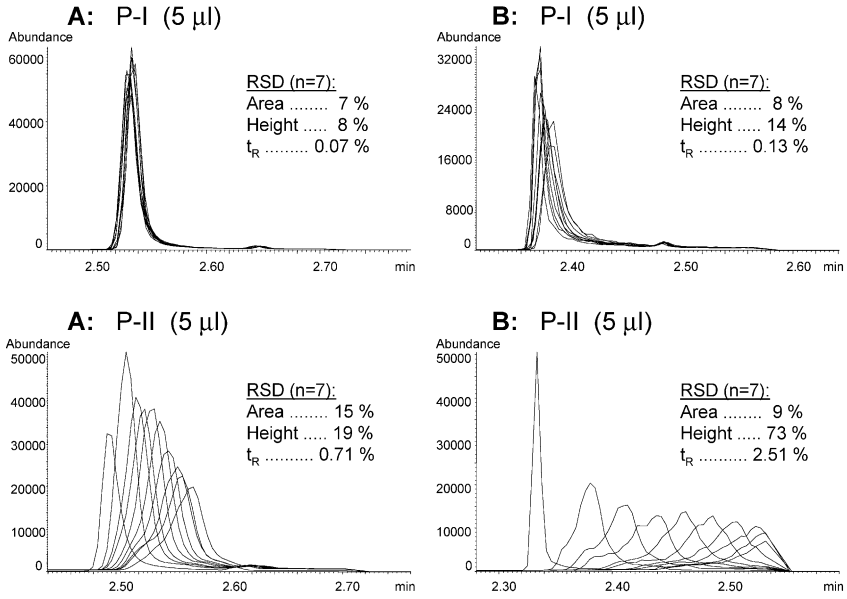

Fig. 5. Overlay of 10 extracted ion chromatograms of carbaryl (*m/z* 144) obtained in the analysis of toluene solutions std1a–std1j in the experiments with repeated 5 μl injections of P-I and P-II carrot extracts using the LP-GC–MS column configurations A and B (see Section 2.3 for the sequence of GC injections).

and substantial t_R shifts with the increasing number of matrix introductions into the column ensemble B (the same time scale is shown in the figures). The shifts of lindane to longer t_R led to dropping its peak from the respective time window (set for monitoring of lindane and dimethoate ions) after 28 matrix injections. Thus, after this point, lindane could not be detected using the initial method settings, which further indicates rather low method ruggedness in the LP-GC–MS approach B.

Carbaryl represents an analyte prone to losses and tailing in the GC system [27,28]. For this and similarly susceptible pesticides included in our test mixture (e.g. methamidophos, dimethoate, methiocarb, etc.), the column configuration A provided superior results also for the injections of 5 mg equivalents of the carrot matrix (compare the upper parts of Fig. 5). As compared to lindane and other less problematic analytes, the injections of the 25 mg matrix equivalents caused faster deterioration of the carbaryl detectability when the LP-GC–MS column configuration B was used. The carbaryl peak moved out of its respective SIM window also after 28 matrix injections, thus for comparison purposes, the peak height, area and t_R R.S.D. are given only for $n = 7$ repetitions, although all 10 measurements are shown in Figs. 4 and 5.

The above examples illustrate the importance of method ruggedness in GC–MS SIM analysis, especially if the analyte ions are monitored in rather narrow windows as in the case of a fast GC–MS analysis. Another important consideration involves analyte LODs, which are also influenced by method ruggedness (peak height diminishment with increasing number of matrix injections) in addition to the separation efficiency, injection volume and matrix content in the injected sample. Table 5 gives average LODs of the 20 pesticides estimated from the matrix-matched calibration curves in the third sequence in the respective experiments. The LODs were calculated by extrapolating the S/N ratios (signals obtained as peak heights in cmstd1c–cmstd3c analyses divided by RMS noise at the analyte elution times obtained in the carrot blank chromatogram from the third sequence) at the chosen quantitation ions to determine the concentrations at which S/N = 3.

The results in Table 5 shows that the column configuration A provided lower LODs in about 60% of the cases, whereas the column configuration B gave lower LODs in only 20% of the overall results (and 20% of the results were comparable for both column configurations, i.e. LOD ratios were within the range of 0.8–1.2). Thus, due to the increased tolerance towards matrix injections, overall better analyte detectability was obtained with the column configuration A in spite of the greater separation efficiency and slightly taller peaks achieved in the LP-GC–MS approach B. Generally, the 5 μl injections of cleaner P-I extracts led to lower analyte LODs in carrot samples as compared to both 1 and 5 μl injections of the P-II extracts, although the pesticide concentrations related to the matrix content (in ng/g) were lower in the P-I extracts (see Table 1). With the same (5 mg) matrix equivalent injected, the 5-fold higher amount of pesticides introduced to the column configurations A and B in 5 μl injections of the P-I extracts resulted in lower LODs in 20 and 13 cases, respectively, out of the 20 tested pesticides as compared to the 1 μl injections of the P-II extracts. With the same amount of pesticides injected in 5 μl injections, the 5-fold higher matrix concentration in the P-II provided better LODs for only rarely (in the case of heptachlor in the configuration A and in 3 other cases with the configuration B), but had detrimental

Table 5
Average estimated LODs (in ng/g) of the pesticides analyzed in the carrot extracts from the third sequence (cmstd1c–cmstd3c) using the LP-GC–MS column configurations A and B, injection volumes 1 or 5 μl, and sample preparation methods P-I or P-II (see Section 2 for a detailed description)

Pesticide	*m/z*	Column configuration A			Column configuration B		
		P-I	P-II		P-I	P-II	
		5 μl	1 μl	5 μl	5 μl	1 μl	5 μl
Methamidophos	141	0.3	0.8	4	0.9	3	>20
Dichlorvos	185	0.1	0.1	0.1	0.4	0.2	0.1
Acephate	136	5	>20	>20	2	>20	>20
Dimethoate	125	5	7	5	2	3	7
Lindane	181	0.3	2	1	3	6	3
Carbaryl	144	0.5	0.9	1	0.6	1	11
Heptachlor	272	0.2	0.3	0.1	0.2	0.2	0.2
Pirimiphos-methyl	290	0.1	0.2	0.1	0.3	0.2	0.2
Methiocarb	168	3	12	3	1	5	7
Chlorpyrifos	314	0.1	0.4	2	0.1	0.9	0.2
Captan	79	2	10	15	2	9	>20
Thiabendazole	201	2	10	3	31	11	>20
Procymidone	283	0.3	1	0.4	0.5	0.5	1
Endosulfan I	339	0.6	5	6	0.8	1	7
Endosulfan II	339	0.5	9	10	0.8	2	10
Endosulfan sulfate	387	0.3	0.3	0.3	1	0.5	0.5
Propargite	350	0.2	0.4	0.2	0.2	0.4	0.8
Phosalone	367	0.3	0.4	0.4	0.2	0.4	0.9
Permethrins	183	0.2	1	0.4	0.7	2	3
Deltamethrin	181	0.7	4	5	1	2	11

effect on detectability for the majority of the tested pesticides (14 cases in both LP-GC–MS approaches).

Using the column configuration A, a comparison of the LODs obtained for 1 and 5 μl injections of the P-II extracts shows that the larger volume improved detectability for nine analytes, whereas higher LODs were observed in four instances. The situation was more than reversed with the column configuration B, where the larger injection volume improved LODs for only 2 analytes, but had negative impact on detectability of 14 out of the 20 pesticides as determined in the third sequence. This comparison further underlines the adverse impact of the decreased ruggedness on the overall analytical performance.

3.5. Evaluation of injection volume for maximized pesticide detectability in various food crops

The above evaluation of LODs demonstrates that larger injection volumes do not always lead to improved analyte detectability. As discussed, one of the reasons is the potentially lower ruggedness caused by a larger amount of non-volatile matrix components introduced into the GC system as the injection volume increases. However, even if the ruggedness is not the main issue, the (semi-)volatile matrix components may still play an important role in analyte detectability, dictating the level of chemical noise in the analysis.

The previous experiments with the 20 selected pesticides in carrot samples showed that the overall lowest LODs and best ruggedness were achieved with 5 μl injections of P-I carrot extracts using column configuration A. To evaluate the effect of (semi-)volatile matrix interferences and determine the most suitable injection volume, we tested 1–5 μl injections of four different matrix extracts prepared by the P-I procedure, spiked with the 57 pesticides and analyzed using the LP-GC–MS approach A. Apples, wheat, and lettuce were selected in addition to carrots, as samples representing different matrix co-extractives. In each experiment, S/N ratio was determined for the 57 tested pesticides as described in Section 2.4.

Fig. 6 demonstrates the effect of the increasing injection volume on S/N ratios obtained in four different matrices, showing for what percentage of the tested 57 pesticides the S/N ratios were increased, decreased or remained without a significantly change as the injection volume increased in 1 μl increments from 1 to 5 μl. For purpose of this trend presentation, a significant change, increase or decrease, was considered if the percent difference in S/N ratios (ΔS/N) for two subsequent injection volumes $n-1$ and n (relative to the S/N ratio obtained for 1 μl) was equal or greater than +20% or lower than −20%, i.e. $[S_n/N_n - S_{n-1}/N_{n-1}]$: $S_1/N_1 \geq +0.2$ or $S_1/N_1 \leq -0.2$, respectively. Fig. 6 shows that, as the injection volume increased above 2 μl (for wheat and carrot extracts) or 3 μl (for apple and lettuce extracts), the S/N ratios of a fewer number of pesticides were improved, resulting in either practically unaffected analyte detectability in most cases or even increasing number of pesticides with higher LODs as compared to the smaller injection volumes.

This is just a general evaluation because it is also important for which pesticides the gain in detectability was achieved or lost. The gain is beneficial mainly for the most troublesome pesticides (“weakest links”) in GC–MS, such as methamidophos, acephate, dimethoate or captan, which typically have

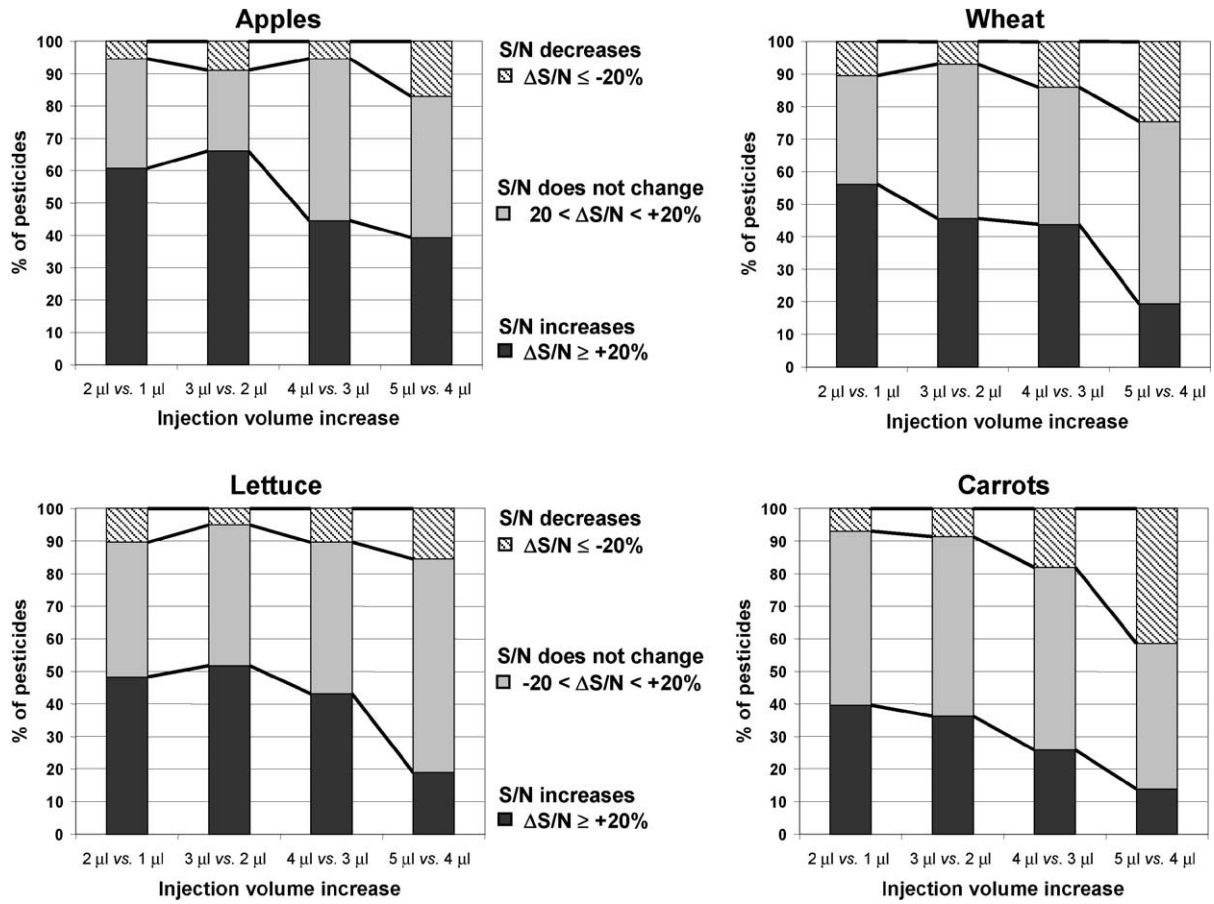

Fig. 6. Percentage of the tested pesticides (57 analytes = 100%), for which the S/N ratio was (i) increasing, (ii) without significant change, and (iii) decreasing as the injection volume of the investigated matrix extracts was increased in 1 μl increments from 1 to 5 μl. A significant change, increase or decrease, is considered if the percent difference in S/N ratios (ΔS/N) for two subsequent injection volumes $n-1$ and n (relative to the S/N ratio obtained for 1 μl) is greater than +20% or lower than −20%, i.e. $[S_n/N_n - S_{n-1}/N_{n-1}]$: $S_1/N_1 \geq +0.2$ or $S_1/N_1 \leq -0.2$, respectively.

higher LODs than other analytes. In these cases, the injection volumes larger than 2 or 3 μl led mainly to reduced analyte detectability because these problematic pesticides are usually highly influenced by matrix interferences due to their low *m*/*z* ions in GC–MS. Thus, the injection volume of 2–3 μl can be really considered optimal for the analysis of the selected group of pesticides in the given matrices.

For sensitivity increasing linearly with the injection volume n (n-fold increased signal versus 1 μl injection), one would expect approximately n-fold gain in detectability versus 1 μl, i.e. $(S_n/N_n):(S_1/N_1) \approx n$. This potential gain can be achieved for noise ≈ constant, i.e. when practically no chemical noise is present (no matrix interferences and/or highly specific *m*/*z*). Unfortunately, it is often not the case in real-life pesticide residue analysis, including conventional GC–MS methods with a large volume injection [29] where separation efficiency is not sacrificed for speed as in the LP-GC–MS technique. Fig. 7 gives examples of the influence of the increasing injection volume (1–5 μl) on signal (peak height), RMS noise, and resulting S/N ratio for several pesticides. As demonstrated, the analyte signal was linearly increasing with the increasing volume injected (≈5-fold gain in sensitivity for 5 μl versus 1 μl), thus the noise level was the main factor dictating whether and to what extent the given S/N ratio would increase or decrease. For a gain in detectability, it is important that the signal increases faster than the noise, i.e. the ratio a_S/a_N must be greater than S_n/N_n, where a_S and a_N are the slopes of the signal and noise curves, respectively. These curves are given by the equations $S_{n+\Delta n} = a_S\,\Delta n + S_n$ and $N_{n+\Delta n} = a_N\,\Delta n + N_n$, where Δn is the change in injection volume n, thus $\Delta n = 1$ for a 1 μl increment (two-point curves).

In the case of chlorothalonil (*m*/*z* 266) in Fig. 7A, a_S/a_N was significantly greater than S_n/N_n for the entire range of tested injection volumes in apples, lettuce, and wheat, thus resulting in a substantial S/N ratio increase even for 5 μl versus 4 μl. The analysis of chlorothalonil in wheat gives an example of noise ≈ constant, where the increase in detectability was ≈n-fold (ΔS/N ≈ 100% for n = 2–5 μl). In carrot extracts, however, the gain in S/N was insignificant (ΔS/N < 20%) when more than 3 μl were injected. On the contrary, β-cyfluthrin (*m*/*z* 206) in Fig. 7B represents an example when the noise level in carrot, lettuce, and wheat extracts increased with the injection volume faster or about the same as com-

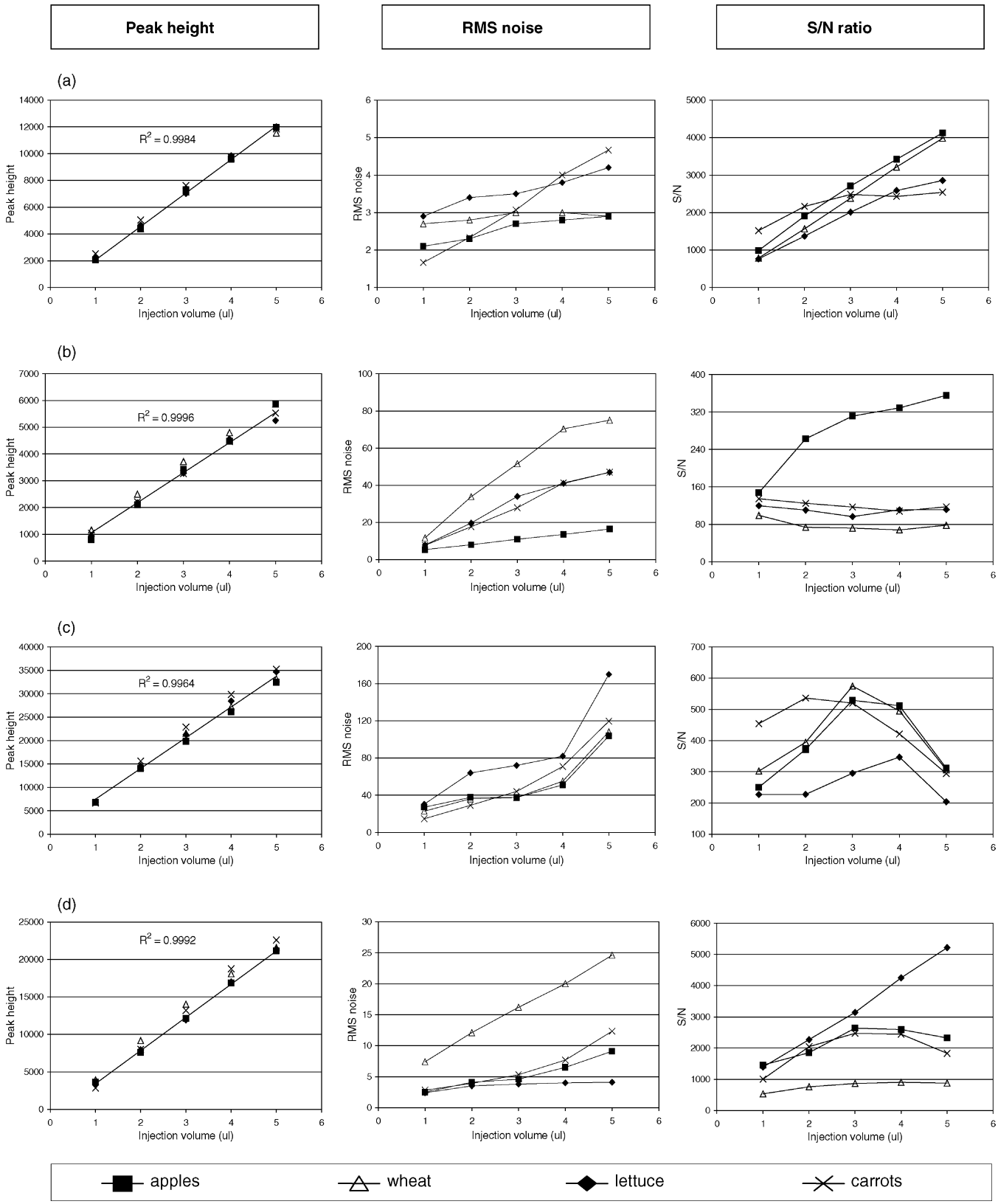

Fig. 7. Signal (peak height), RMS noise, and S/N ratio obtained for (a) chlorothalonil (*m*/*z* 266), (b) β-cyfluthrin (*m*/*z* 206), (c) dimethoate (*m*/*z* 93), and (d) fenitrothion (*m*/*z* 277) in 1–5 μl injections of apple, wheat, lettuce, and carrot extracts.

pared to the analyte signal (a_S/a_N < or ≈S_n/N_n), resulting in a S/N decrease or no significant gain when more than 1 μl of these extracts was injected. In the case of apple extracts, the growth in noise was much less steep, thus the S/N ratio was increasing with the injection volume, with a significant increase ($\Delta S/N \geq 20\%$ for $\Delta n = 1$) observed up to 3 μl.

For dimethoate (*m/z* 93) in Fig. 7C, the noise was increasing exponentially, resulting in an increase of S/N ratio with up to 3 μl injections (2 μl for carrot extract) and insignificant gain ($a_S/a_N \approx S_n/N_n$) or even loss in detectability ($a_S/a_N < S_n/N_n$) with larger injection volumes. Fenitrothion (*m/z* 277) in Fig. 7D represents an example of various trends in the noise growth with increasing injection volume of the tested matrix extracts. As a result, fenitrothion detectability was significantly improving up to 3 μl injections of apple and carrot extracts and, in order to achieve the highest S/N ratio in wheat, it was not necessary to inject more than 2–3 μl in this case. In lettuce, however, a considerable gain ($\Delta S/N \approx 60$–80%) in detectability was observed for all tested injection volumes (up to 5 μl).

The above examples demonstrate that the injection volume providing maximized analyte detectability hinges on the particular analyte–matrix combination. Generally, the selection of an optimal injection volume for a given group of analytes should involve considerations about the gain in detectability for the majority of them (with close attention to the weakest links in the group) and about the impact on method ruggedness, which may be detrimentally affected by a larger amount of matrix injected to the GC system.

4. Conclusions

In this study, we further optimized operating parameters and evaluated performance characteristics of LP-GC–MS for the analysis of multiple pesticide residues in food crops. Two LP-GC–MS column configurations A and B, employing the same restriction capillary at the inlet end, but different analytical columns attached to the vacuum provided by MS, were tested in various experiments. In addition to the pesticide solutions prepared in solvent, which were used for initial optimization and evaluation of speed, separation efficiency and peak characteristics (including the number of points across peaks), the LP-GC–MS systems were subjected to thorough ruggedness tests involving repeated injections of pesticides in matrix extracts.

The optimization compromising speed and sensitivity resulted in similar GC–MS settings (except for the SIM programs) for both column configurations. As compared to the LP-GC–MS approach A, the narrower analytical column with a thinner film in the configuration B provided slightly faster analysis (<1.1-fold) for both tested groups of 20 and 57 pesticides (retention times of the last eluting analyte deltamethrin <5 and 5.5 min, respectively, with both column configurations) and greater separation efficiency (≈1.3–2-fold more theoretical plates depending on the particular analyte), resulting in slightly narrower and taller peaks. Thus, with the same data acquisition rates for both systems, less data points across a peak were obtained with the column configuration B. However, no significant difference in precision of peak area and height measurements was observed even for the peaks defined by the lowest number of points, providing that the analyte response was not affected by matrix injections. Despite of the greater separation efficiency (potentially higher GC selectivity versus matrix components) and slightly better sensitivity (taller peaks) obtained with the column configuration B, generally better detectability of analytes in real samples (carrot extracts) was achieved using the mega-bore analytical column with a thicker film in the LP-GC–MS approach A, which provided substantially better tolerance towards matrix injection and, consequently, significantly greater ruggedness of the analysis.

Decreased ruggedness was exhibited by significantly lower long-term repeatability of analyte peak height and t_R measurements, caused by gradual peak diminishment, distortion, and shifting to longer t_R with the increasing number of matrix injections. Direct sample introduction (DSI) technique (or its automated version called difficult matrix injection) would significantly improve ruggedness because the root cause of the problems—non-volatile matrix components—is being removed after each injection [29–31]. Another possibility may involve the use of analyte protectants [28], which are compounds that strongly interacts with the active sites in the GC system, thus reducing losses and analyte tailing and effectively compensating for both matrix-induced response enhancement and diminishment effects even in long GC sequences of matrix injections [32].

This study clearly demonstrates the importance of ruggedness in real-life analysis, because, if not specifically addressed (by the use of DSI, analyte protectants or some other technique), decreased ruggedness may limit the number of matrix injections and/or amount of matrix injected each time, negatively impacting analyte detectability or the overall throughput (due to down-times for system maintenance). This issue is even more pronounced in fast GC–MS analysis with time-dependent settings, such as SIM windows, because significant t_R shifts may result in omitting of the analyte signal.

However, even if the ruggedness is not a problem, such as in the LP-GC–MS approach A analyzing the relatively clean P-I extracts, the (semi-)volatile matrix components also play an important role in the analysis, dictating the level of chemical noise (interferences) and, consequently, analyte detectability. The evaluation of the injection volumes (1–5 μl) showed that the increase in sensitivity did not translate in the same gain in analyte detectability for the majority of the tested analyte–matrix combinations. The selection of an optimal injection volume for a given situation should involve a balancing act between the improvement of LODs for majority of the analytes (mainly for those least sensitive and highly impacted by matrix interferences), losses of detectability for minimum of them, and the impact on method ruggedness, which may be adversely affected by larger injections of real-life samples. In

this respect, the injection volume of 2–3 μl was optimal for detectability of the majority of the 57 pesticides (including the difficult ones) in the four different matrices tested in our study.

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