Pulsed Splitless Injection and the Extent of Matrix Effects in the Analysis of Pesticides

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Summary

The applicability of pulsed splitless injection to the gas chromatographic analysis of pesticide residues in fruit and vegetables has been evaluated. 22 pesticides belonging to different chemical classes, including those known to be liable to matrix induced response enhancement, were selected for the study. The parameters of pressure pulse have been tested for optimum performance of injection. Application of the pressure pulse was found to decrease matrix effects during analyses of real samples. Further decline of matrix effects was obtained using higher sample injection volumes. The installation of a deactivated retention gap was necessary to obtain good peak shapes with injection volumes exceeding 1 µL of sample. Up to 4 µL was then injected without peak distortion and consequent loss of resolution. Using 4 µL pulsed splitless injection, matrix effects were almost completely eliminated even at very low concentration levels of analytes. The highest matrix effects observed for tested compounds at the lowest concentration level tested were in the range of 110–122%.

1 Introduction

Because of its high separation power for complex mixtures and the low limits of detection attainable by conventional detectors, capillary gas chromatography is nowadays the technique most widely used for the analysis of pesticide residues in food crops. However, the accuracy of generated data depends on the overall performance of the gas chromatographic system. In this respect the most important part of the gas chromatograph is the injection port. Many studies concerned with splitless injection have proved that the detector responses of many compounds can be significantly influenced by so-called matrix effects.

Matrix induced response enhancement is a complex phenomenon observed during analyses of real samples containing some matrix components (*e.g.* lipids, waxes, pigments, *etc.*). These cannot always be completely separated from analytes during clean-up of crude extracts, especially when multi-residue methods are used. Molecules of impurities then compete during injection period with analytes for the active sites in the injection chamber [1]. Active sites are exhibited mainly by free silanol groups present in a glass liner and also by deposits originating from nonvolatile co-extracts in the injection port during preceding analyses. As a consequence, a larger amount of analyte is transferred to the GC column, resulting in enhanced response of analyte in real sample compared to that in neat solvent. Using neat solvent standards overestimated results may be thus achieved.

Matrix effects have been reported for certain compounds in many studies [1–9]. Among the pesticides, there are two basic groups subject to matrix induced response enhancement. The first group comprises organophosphates containing P=O bonds such as methamidophos, acephate, omethoate, dimethoate [1–4, 7, 9]. The other one comprises thermolabile compounds which are prone to degradation in the injection port. The carbamate pesticides (carbaryl), *N*-trihalogenmethylthio compounds (captan, dichlofluanid), and also some organohalogens (DDT, endrin, aldrin, chlorothalonil) may serve as typical examples [5, 7, 9–11].

Although various approaches aiming to reduce/eliminate matrix effects have been suggested [3, 5, 12, 13], their application to routine analyses proves rather complicated from a practical point of view. The first option is to perform an efficient clean-up to remove most of the matrix components present in a crude extract. However, it is usually neither practical nor possible to carry out such a thorough clean-up, especially when a broad spectrum of pesticides possessing different physico-chemical properties is to be analyzed. Many multi-residue methods for the analysis of pesticides in biotic matrices employ either gel permeation chromatography (GPC) or a liquid-liquid partition step as clean-up tools. Whenever sufficiently high recoveries are required for all analytes, the residual amount of co-extracts remaining in the analyte fraction is invariably relatively high because of overlap between respective elution zones [5, 6, 8, 9].

Under these circumstances the use of standards prepared from the matrix to be analyzed – *i.e.* "matrix matched" standards – represents a way of reliably correcting for response enhancement [1, 2, 5, 9, 12]. Unfortunately, this approach is much more laborious than conventional calibration using standards prepared in neat solvent, particularly when numerous samples of widely differing origin are to be analyzed. In addition, the stability of certain pesticides in matrix standards may be a limiting factor [13].

The addition of single compound additives in order to mask active sites in the injection port has been also studied [3]. However, this procedure did not prove to be generally effective in reducing matrix effects.

Recently, the use of pulsed splitless injection for the analysis of organic contaminants has been reported [10, 11, 14–19]. These techniques involve an increase of column head pressure for a short time period during sample injection (usually for 1 or 2 min). This set-up leads to a higher carrier gas flow rate (8–9 mL/min compared to 0.5–1 mL/min in conventional splitless injection) through the injector and thus to faster transport of sample vapors onto the GC column. Under these conditions the residence time of analytes in the injection chamber is much shorter than in normal splitless injection. As a result a significant suppression of analyte discrimination, adsorption, and/or degradation occurs in the inlet port. The responses of troublesome compounds (mentioned above) obtained with pulsed splitless injection are thus significantly higher than those obtained by conventional splitless injection [10, 14, 16, 19, 20]. In addition, due to the increased pressure higher volumes of sample can be injected (up to 5 µl) without the risk of backflash. Consequently lower detection limits can be achieved [11, 15–18].

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Table 1. Concentrations of pesticides in stock and working solutions (all in toluene).

Analyte	Stock solution (μg/mL)	Working solutions (μg/mL)		
		100 × diluted (level A)	400 × diluted (level B)	1000 × diluted (level C)
Acephate	47.1	0.471	0.118	0.047
Bromopropylate	20.4	0.204	0.051	0.020
Captan	56.0	0.560	0.140	0.056
Carbaryl	106.4	1.064	0.266	0.106
Chlorothalonil	15.2	0.152	0.038	0.015
Chlorpyrifos	55.9	0.559	0.140	0.056
Dichlofluanid	28.8	0.288	0.072	0.029
Dimethoate	37.7	0.377	0.094	0.038
Endosulfan-SO_4	10.7	0.107	0.027	0.011
Etrimfos	43.6	0.436	0.109	0.044
Iprodione	62.5	0.625	0.156	0.063
Lindane	6.0	0.060	0.015	0.006
Malathion	58.7	0.587	0.147	0.059
Methamidophos	40.8	0.408	0.102	0.041
Methidathion	63.8	0.638	0.160	0.064
Omethoate	47.0	0.470	0.118	0.047
Phosalone	72.6	0.726	0.182	0.073
Pirimiphos-methyl	38.4	0.384	0.096	0.038
Propham	153.3	1.533	0.383	0.153
Tolclofos-methyl	46.9	0.469	0.117	0.047
Tolylfluanid	29.2	0.292	0.073	0.029
Vinclozolin	13.6	0.136	0.034	0.014

On-column and PTV injection represent other sample introduction alternatives which may be assumed to reduce and/or eliminate matrix effects. Direct injection of sample onto the analytical column in the former and slow evaporation of sample in the latter technique are the inherent features offering potential for elimination of discrimination, degradation, and other negative effects [21–23]. In spite of these assumptions, Mol *et al.* have reported matrix effects occurring in some extent during analyses of nitrogen and phosphorus containing pesticides by PTV-GC [24].

In our study, high resolution gas chromatography employing electron capture and nitrogen phosphorus detectors was used for analysis of wheat extracts spiked with 22 common modern pesticides representing different chemical classes. Pressure pulse parameters were optimized with the aim of minimizing the matrix induced response enhancement observed for some of these pesticides in conventional splitless injection. The possibility of improving the detectability of analytes by the increased volume of injected sample was also studied.

2 Experimental

2.1 Chemicals and Materials

Pesticide standards, all of 95% purity, were obtained from Dr. Ehrenstorfer (Germany). Stock and working solutions were prepared in toluene, see **Table 1**. All solvents used (ethyl acetate, cyclohexane, toluene) were analytical grade (Merck, Germany). Wheat grains were obtained at a retail market.

2.2 Apparatus

HPLC system HP 1090 equipped with PL gel (600 × 7.5 mm, 50 Å) high-performance column (PL Labs, UK) and with a Retriever II (Isco, USA) fraction collector was used for clean-up of extracts. All solvent evaporations were performed on a Büchi Rotary Evaporator.

For GC analyses, a HP 6890 gas chromatograph equipped with electronic pressure control (EPC), nitrogen-phosphorus detector (NPD), electron-capture detector (ECD) and autosampler HP 7673A was used. DB-5 MS column (60 m × 0.25 mm × 0.25 μm) connected via a Y-piece with both detectors was employed for separation of analytes. All data were stored and reprocessed with PC and HP Chemstation A.04.05.

2.3 Analytical Procedure

50 g of sample were homogenized with 50 g of anhydrous sodium sulfate (Na_2SO_4) and 200 mL of ethyl acetate for 2 min with a Turrax (10 000 rpm). The homogenate was filtered through the layer of 20 of sodium sulfate and the filter cake was rinsed 3 × with 25 mL of ethyl acetate. The combined filtrates were evaporated (38 °C, 250 mbar) down to 50 mL and the final volume of sample was then made up to 100 mL with cyclohexane in a volumetric flask.

2.4 Clean-up

A 2 mL aliquot of crude extract was injected onto a HPGPC (high performance gel permeation chromatography) column via

Table 2. The influence of pressure parameters during injection period upon responses (area count) of tested pesticides – ECD detected.

Analyte	Pulse duration 1 min Column head pressure (psi)				Pulse duration 2 min Column head pressure (psi)			
	20*	40	60	80	20*	40	60	80
Bromopropylate	54.79	63.35	64.87	66.71	52.34	65.44	65.85	58.17
Captan	132.50	181.14	197.36	192.07	108.93	189.92	196.09	179.24
Chlorothalonil	69.58	85.94	96.34	87.27	60.86	85.50	90.06	81.70
Chlorpyrifos	127.85	149.67	151.84	149.89	117.13	152.36	156.63	143.09
Dichlofluanid	66.19	77.74	81.00	78.45	59.63	78.34	82.45	77.52
Dimethoate	34.48	42.16	45.75	41.42	31.37	42.31	43.21	39.92
Endosulfan-SO_4	52.43	62.60	63.67	65.07	47.61	61.05	62.91	56.13
Lindane	36.04	44.10	48.30	44.17	33.00	43.28	45.45	41.85
Malathion	40.23	47.46	48.42	47.94	37.36	47.95	48.81	45.78
Methidathion	65.57	79.59	83.42	78.84	59.31	81.71	81.67	75.53
Phosalone	129.13	148.88	154.51	157.11	118.22	155.85	151.58	134.90
Tolclofos-methyl	58.12	67.27	70.73	69.33	53.43	66.92	71.08	65.80
Tolylfluanid	63.92	75.34	75.48	74.16	58.88	76.63	77.65	72.73
Vinclozolin	37.51	43.82	46.12	44.35	34.39	43.59	45.66	42.14

* – splitless injection column head pressure

a 2 mL sample loop. HPGPC conditions were as follows: mobile phase cyclohexane-ethyl acetate (1:1, *v/v*), flow rate 1 mL/min, collected fraction 15.5–31.0 mL. This "pesticide" fraction was concentrated in a rotary evaporator and remaining solvent was gently evaporated in a stream of nitrogen.

2.5 Preparation of Spiked Samples

The purified sample obtained after HPGPC clean-up (see Section 2.4) and evaporation of solvent was re-dissolved in 1 mL of standard solution (working solutions A, B, C, see Table 1) to obtain the "matrix standard" with known concentrations of analytes. "Blank" samples were prepared similarly by diluting the residue after evaporation in 1 mL of toluene.

2.6 GC Identification and Quantification

Matrix standards and corresponding standards in neat solvent were analyzed by GC under the following conditions:

Inlet temperature: 220 °C

Carrier gas: helium

Ramped flow: 1 mL/min (hold 40 min), 0.5 mL/min^2 to 3 mL/min (hold till the end of analysis)

Oven temperature program: 90 °C (2 min), 10 °/min to 190 °C, 2.5 °/min to 225 °C, 15 °/min to 280 °C (hold 10 min), 20 °/min to 300 °C (hold 16 min)

ECD

Temperature: 300 °C

Gases: anode (nitrogen) 6 mL/min; make-up (nitrogen) 60 mL/min

NPD

Temperature*:* 300 °C

Gases*:* air 60 mL/min; hydrogen 3 mL/min; make-up (nitrogen) 10 mL/min

Matrix standards were analyzed at three different concentration levels (A, B, C). Three injections of each standard were made together with bracketing clean standards and the responses were compared.

3 Results and Discussion

Pulsed splitless injection is undoubtedly one of the challenging techniques which may significantly improve the performance of classic split/splitless injection. Unfortunately, the information on its application to the analysis of pesticide residues belonging to different groups and occurring at various concentration levels is rather limited. One of the most thorough studies concerned with this topic [16] documented significant reduction of matrix effects by pulsed injection for 6 organophosphorus pesticides. However, only compounds representing a single pesticide class and, moreover, at rather high concentrations (approx. 0.4 µg/mL in sample prior to GC analysis) were tested.

Based on available literature data it is evident that no general rules exist for deriving optimal parameters for pulsed splitless injection which would result in most efficient suppression of matrix effects. Accordingly, in our experiments various injector settings were tested to acquire a better knowledge on their influence on the performance of quantitation process.

The pesticides peak areas (detected by two detectors in parallel operation) in solvent standards obtained with different pressures and durations are shown in **Table 2** and **Table 3**. Generally higher responses were obtained for shorter pulse time (1 min) at higher column head pressures (60–80 psi). A decline in the responses of several troublesome compounds is observed at high column head pressures (80 psi), especially with longer pulse time (2 min); however, lower responses are also evident with 1 min pulse time for high flow rates. This phenomenon is probably due to the sweeping of the analytes from the solvent film which is formed during cold focusing at the front part of chromatographic column [25]. The loss of some analytes (methamidophos, acephate, omethoate, *etc.*) becomes more distinct with

Table 3. The influence of pressure parameters during injection period upon responses (area count) of tested pesticides – NPD detected.

Analyte	Pulse duration 1 min Column head pressure (psi)				Pulse duration 2 min Column head pressure (psi)			
	20*	40	60	80	20*	40	60	80
Acephate	0.43	1.83	2.07	1.49	0.45	1.50	1.80	1.12
Carbaryl	6.21	9.12	10.09	9.12	6.00	9.76	10.01	9.25
Chlorpyrifos	18.75	22.89	22.79	21.59	16.95	21.96	22.45	22.54
Dimethoate	10.40	14.17	15.53	12.95	9.32	13.70	13.67	13.76
Etrimfos	15.58	19.20	20.86	18.43	14.21	18.57	19.25	19.59
Malathion	14.92	18.98	19.15	18.07	13.46	17.92	17.96	18.54
Methamidophos	1.80	4.22	4.50	3.75	1.64	3.04	2.84	2.81
Methidathion	22.21	29.24	30.53	27.38	19.43	27.84	27.37	28.06
Omethoate	3.19	6.46	7.42	5.82	2.65	5.26	6.21	5.68
Phosalone	14.16	16.34	16.90	16.79	12.30	15.81	15.07	14.94
Pirimiphos-methyl	14.70	17.84	18.39	17.27	13.00	16.88	17.73	18.31
Propham	12.55	15.61	16.44	15.57	11.69	15.66	15.06	14.91
Tolclofos-methyl	11.71	14.33	14.79	13.83	10.69	13.46	14.00	14.65

* – splitless injection column head pressure

increasing volatility and shorter retention times, respectively. Contrary to these observations, Wylie and Uchyiama [16], employing the same type of HP injector, did not experience any losses of volatile organophosphorus pesticides when a 70 psi pressure pulse was applied. As a compromise, in routine practice the pressure pulse should not be set above 60–70 psi whenever volatile analytes are to be analyzed, otherwise compounds losses due to sweeping by carrier gas stream will occur. Further suppression of analyte evaporation might be achieved by injection of solvent with different polarity and/or lowering the starting column temperature. However, these options have not been tested in our experiments.

The next parameter which it is necessary to set-up in a splitless injection method is the splitless time period, *i.e.* the time for which the split vent is closed during injection. We have tested different splitless time periods; however, no significant differences in the peak areas were observed when split vent was opened either before or after the end of the pulse time. On setting the splitless time shorter than that for the pulse, the residual sample vapors are rapidly swept out of the injection port preventing any residues from remaining in the injector liner and/or in split capillaries.

In the next set of experiments, pesticide standards prepared both in matrix and in neat solvent were subsequently injected into the GC at two column head pressures (40 and 80 psi) to compare the extent of matrix effects (expressed as relative detector response of analyte in matrix standard and in neat solvent). In accordance with theoretical assumptions reviewed in the introduction, higher pressure pulse resulted in certain reduction of matrix effects for compounds tending to adsorb/thermodegrade in the injection port under conventional conditions, see **Table 4** and **Figure 1**. No significant differences between responses at lower and higher column head pressures occurred for other analytes. Statistical assessment of the results (two-factor analysis of variance – ANOVA, see **Table 5**) has demonstrated that for all troublesome compounds at concentration levels A and C there existed a statistically significant difference between relative responses at both tested pressures. At concentration level B some slight decrease in relative responses can also be seen; nevertheless, it was not proved statistically significant by ANOVA. As already mentioned [16], almost complete elimination of matrix effects can be achieved with pulsed splitless injection. However, this assumption is no longer valid when very low concentrations of pesticides are to be determined. Although at concentration level A (the highest one) matrix effects were relatively small on using a 80 psi pressure pulse, the lower the concentration the more distinct the matrix effects became, independently of the pulse pressure used. At concentration level C the relative responses of troublesome compounds were in the range 190–623 %. It should be noted that such high values were obtained because the responses of particular compounds in solvent standards were close to or below limits of detection. Therefore their response was assigned to 0.1 area counts for the calculation of relative responses.

In order to test also other parameters of the pulsed splitless injection, the pulse duration was altered – see **Table 6**. Lower matrix effects were obtained with longer pulse times at concentration levels A and B; however, at the lowest concentration level C this relationship was not so distinct. Statistical differences were found at concentration levels A and B using two-factor analysis of variance (ANOVA) between relative responses obtained with tested pulse times, see Table 5. No difference was found between both pulse times at concentration level C. Similarly, as in the case of testing the pulse pressures, absolute responses of some compounds were very low resulting in high relative responses at low concentration levels. As mentioned in previous paragraphs, absolute responses of some compounds are lower at higher pulse time, presumably due to the losses caused by partial evaporation of analyte molecules from the solvent layer by high flow rate of carrier gas. Accordingly, as a compromise we set the pulse time at 1 min to get higher peak response.

Based on the observations discussed above, optimized pressure pulse parameters were applied in further experiments. Considering the almost constant number of active sites present in the injection port, further reduction of matrix effects by injection of larger amount of sample could be presumed. Using common splitless injection, typically only a small volume of sample can

Table 4. Comparison of relative responses of tested pesticides obtained with two pulse pressure intensities, RSD (%), *n* =3.

Analyte	Concentration level A				Concentration level B				Concentration level C			
	40 psi	RSD	80 psi	RSD	40 psi	RSD	80 psi	RSD	40 psi	RSD	80 psi	RSD
Acephate	260	10.3	164	5.1	452	13.7	367	7.7	724	7.5	623	13.0
Bromopropylate	106	1.6	96	1.1	106	2.2	102	5.5	114	0.9	101	2.5
Captan	149	9.4	113	2.2	157	9.9	142	8.4	197	7.6	190	9.4
Carbaryl	228	7.5	145	2.7	287	8.3	211	5.2	318	12.2	294	1.3
Chlorothalonil	121	3.4	107	2.9	132	3.8	131	6.1	164	3.4	144	3.8
Chlorpyrifos	106	3.5	99	2.4	106	2.7	106	5.7	124	2.7	103	6.0
Dichlofluanid	105	2.5	100	2.0	109	3.2	106	4.9	129	3.4	108	2.9
Dimethoate	116	2.5	108	4.5	124	6.9	153	7.6	176	5.0	149	4.8
Endosulfan-SO_4	112	2.5	98	1.4	111	2.8	107	5.3	119	1.4	110	2.2
Etrimfos	106	1.3	101	3.0	108	2.1	108	4.8	110	2.3	87	0.6
Lindane	103	2.7	95	2.0	103	1.0	96	4.5	109	2.9	112	2.8
Malathion	109	2.1	101	2.5	113	3.8	110	4.8	126	2.5	123	2.1
Methamidophos	185	5.0	144	3.2	214	6.4	187	5.9	527	15.0	381	2.5
Methidathion	111	2.7	93	1.6	120	6.0	109	4.9	131	14.2	113	3.4
Omethoate	186	5.9	131	3.4	488	6.0	226	7.1	654	13.9	300	8.5
Phosalone	119	2.6	103	1.6	121	4.5	118	4.7	141	1.3	138	5.7
Pirimiphos-methyl	104	2.3	99	2.4	107	2.4	102	6.1	107	5.6	111	4.4
Propham	105	2.3	103	2.9	107	2.7	108	3.5	116	2.3	127	3.7
Tolclofos-methyl	99	1.9	99	2.1	101	3.4	106	5.4	122	7.2	109	1.6
Tolylfluanid	108	2.5	102	2.0	111	3.6	111	6.0	136	5.0	113	2.9
Vinclozolin	100	2.2	98	1.6	103	2.6	112	4.9	113	3.6	104	2.4

Notice: lower detectable area was set to 0.1 area units

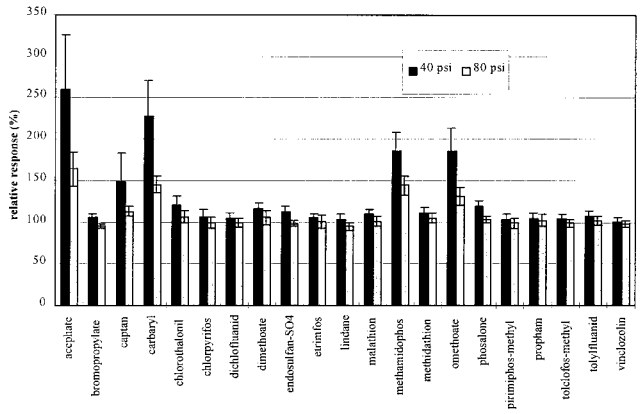

Figure 1. The effect of pulse pressure intensity on relative responses of analytes, pulse time 1 min, injection 1 µl, concentration level A, RSD *n* = 3.

be injected (up to 1 µL) employing conventional types of liners (glass deactivated liners, volumes 800–990 µL). A pressure pulse applied to the column head during injection permits injection of larger sample volumes (up to 5 µL) [11, 15–18]. A higher amount of sample introduced under these conditions is assumed to swamp out active sites in the liner, thus allowing a larger portion of analytes to pass to the column. These considerations prompted us to undertake subsequent experiments in which the injection of larger volumes of samples and its relationship to the matrix effects was tested.

Injection of sample volumes exceeding 1 µL onto the GC column resulted in significant peak distortion, although cold solvent focusing had been realized, see **Figure 2.a**. This effect is probably caused by an excessively long and non-homogenous flooded zone of condensed solvent formed at the front part of the capillary column during solvent focusing. Therefore, we installed a 5 m retention gap (of the same internal diameter as the column), which we expected to assist in focusing zones by a "stationary phase focusing mechanism" [25]. In this manner better peak shapes were obtained and up to 4 µL of sample could be injected without pronounced loss of resolution, see **Figure 2.b**. Volumes exceeding 4 µL lead to some peak broadening. Since the volume of liner used was 900 µL (splitless single-taper liner) while the calculated volume of vapor of toluene at 250 °C and 60 psi pressure was only approximately 319 µL, liner overflow was improbable. Consequently, we attributed the peak broadening and distortion rather to an inappropriate retention gap length unable to accommodate more than 4 µL of solvent. The situation might be improved by use of a longer retention gap (*e.g.* 10 m) or one of the same length but with a larger internal diameter. Another alternative is to use a different solvent, showing better compatibility with the stationary phase of the analytical column, for injection into the GC. However, since the highest injection volume feasi-

Table 5. Statistical evaluation of the differences in relative responses of tested compounds with two pulse intensities (two-factor ANOVA, $\alpha = 0.05$) and two pulse times.

Concentration level	Pressure		Pulse time	
	F_{stat}	F_{crit}	F_{stat}	F_{crit}
A	12.780	4.351	31.788	4.351
B	2.761	4.351	13.530	4.351
C	4.900	4.351	1.076	4.351

Table 6. Relative responses of tested pesticides obtained under two different pulse durations (1 and 2 min), pulse 60 psi, injection volume 1 µL, RSD (%), $n = 3$.

Pesticide	Concentration level A				Concentration level B				Concentration level C			
	1 min	RSD	2 min	RSD	1 min	RSD	2 min	RSD	1 min	RSD	2 min	RSD
Acephate	206	4.6	164	5.1	366	15.0	367	8	589	25.0	623	13.0
Bromopropylate	112	1.9	96	1.1	112	1.2	102	5	110	1.4	101	2.5
Captan	125	4.6	113	2.2	158	2.7	142	8	165	2.7	190	9.4
Carbaryl	193	1.3	145	2.7	238	11.8	211	5	149	11.8	294	1.3
Chlorothalonil	108	0.7	107	2.9	115	1.1	131	6	106	0.8	144	3.8
Chlorpyrifos	111	2.0	100	2.0	121	2.8	114	5	128	0.6	107	2.9
Dichlofluanid	114	1.3	100	2.0	112	3.4	106	5	123	1.2	108	2.9
Dimethoate	112	3.4	105	3.2	124	9.9	124	6	150	7.4	100	3.6
Endosulfan-SO_4	118	2.1	98	1.4	112	1.4	107	5	109	1.8	110	2.2
Etrimfos	108	3.0	101	3.0	112	2.2	108	5	121	2.0	87	0.6
Lindane	110	0.7	95	2.0	113	2.7	96	4	113	1.6	112	2.8
Malathion	113	1.4	101	2.5	121	6.0	110	5	120	1.8	123	2.1
Methamidophos	198	14.1	144	3.2	215	9.0	187	6	365	14.0	381	2.5
Methidathion	110	0.8	93	1.6	126	3.4	109	5	108	5.1	113	3.4
Omethoate	140	0.3	131	3.4	214	3.5	226	7	285	15.3	300	8.5
Phosalone	110	2.6	103	1.6	124	5.0	118	5	111	10.8	138	5.7
Pirimiphos-methyl	111	1.4	99	2.4	114	2.0	102	6	104	4.8	111	4.4
Propham	112	0.9	103	2.9	120	4.1	108	3	115	6.4	127	3.7
Tolclofos-methyl	114	0.7	99	2.1	121	2.1	106	5	117	3.3	109	1.6
Tolylfluanid	117	3.7	102	2.0	122	2.5	111	6	118	0.7	113	2.9
Vinclozolin	111	0.2	98	1.6	116	6.9	112	5	109	1.6	104	2.4

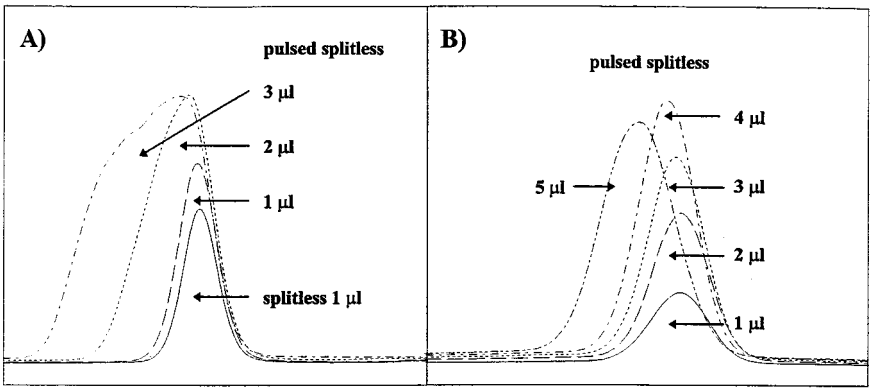

Figure 2. Peak shapes obtained by pulsed splitless injections of different volumes of sample onto GC column without retention gap (A) and with installed retention gap (B).

ble with a HP 7673 Autosampler equipped with 10 µL syringe is 5 µL, we have not tested these options. In all subsequent experiments we therefore evaluated the effect of injection volume upon matrix effects of tested pesticides in the range of 1–4 µl of the sample.

Table 7 summarizes relative responses of all the tested compounds obtained for different injection volumes. It is apparent that the matrix effects diminish with increasing injection volume. When as much as 4 µL of sample was injected, matrix effects were almost completely eliminated and at concentration level C (1000 × diluted solution) the uppermost range of relative responses of "troublesome" compounds was within 110–122%. In addition, increased absolute responses of all compounds provided improved detection limits and better repeatability, see **Figure 3**.

It should be noted that the data for iprodione are not reported here since this compound gave either very small responses or none at all. Problems reported with the determination of iprodione by GC arise from degradation during the run [14]. Hence this compound is difficult to determine by GC. Alternative methods of determination (*e.g.* HPLC) should therefore be employed to obtain accurate analytical results for this compound.

4 Conclusions

Matrix effects occurring in the GC analysis of some pesticides in extracts from real samples have a negative impact on the accuracy of generated results. To compensate for detector response enhancement, calibration should be carried out using matrix

Table 7. Relative responses of tested pesticides obtained with different injection volumes.

Injection	Pulsed splitless 60 psi, 1 min												Splitless		
Dilution of sample	100 ×				400 ×				1000 ×				100 ×	100 ×	1000 ×
Volume of injection	1 µl	2 µl	3 µl	4 µl	1 µl	2 µl	3 µl	4 µl	1 µl	2 µl	3 µl	4 µl	1 µl	1 µl	1 µl
Acephate	**133**	**116**	97	96	**218**	**164**	**144**	**112**	**n.d.**	**224**	**147**	103	**206**	**270**	**n.d.**
Bromopropylate	98	102	100	99	110	105	100	101	**112**	107	93	103	**130**	**114**	98
Captan	**111**	110	102	103	110	110	106	100	**125**	**120**	101	104	**145**	**152**	**178**
Carbaryl	**149**	**133**	**111**	**111**	**199**	**183**	**137**	**116**	**275**	**203**	**158**	**122**	**174**	**229**	**286**
Chlorothalonil	**112**	**115**	100	96	**115**	**129**	**121**	**112**	**140**	**141**	**123**	100	**119**	**135**	**155**
Chlorpyrifos	102	110	97	102	103	109	109	106	**116**	109	95	103	**111**	**114**	**121**
Dichlofluanid	105	100	100	105	105	97	92	98	100	99	87	103	**114**	**111**	91
Dimethoate	107	108	96	98	**125**	**128**	**117**	101	**170**	**153**	**120**	102	**112**	**150**	**189**
Endosulfan-SO_4	101	104	102	104	102	100	105	100	106	111	95	98	**129**	**115**	93
Etrimfos	101	105	104	107	96	105	110	98	**116**	**128**	106	106	106	**125**	**140**
Lindane	99	105	103	100	105	110	106	102	**110**	**118**	106	105	103	**121**	108
Malathion	103	108	105	103	108	104	99	96	**126**	**123**	109	102	**112**	**116**	**132**
Methamidophos	**145**	107	**112**	97	**189**	**160**	**157**	105	**n.d.**	**247**	**126**	105	**191**	**237**	**n.d.**
Methidathion	106	109	102	108	109	**125**	**111**	103	**134**	**136**	109	99	**126**	**141**	**159**
Omethoate	**123**	110	108	99	**185**	**169**	**134**	106	**n.d.**	**202**	**139**	98	**157**	**231**	**289**
Phosalone	109	110	98	97	100	105	108	100	**121**	**116**	104	104	**161**	**138**	**152**
Pirimiphos-methyl	103	106	103	104	107	109	109	106	101	**121**	108	96	107	108	109
Propham	100	106	103	104	**111**	**118**	109	103	**225**	**139**	**112**	107	102	**115**	**262**
Tolclofos-methyl	99	107	103	101	101	109	108	103	110	**111**	101	98	106	105	**122**
Tolylfluanid	102	108	105	102	**112**	**111**	103	108	**111**	**112**	97	105	**123**	**114**	99
Vinclozolin	97	106	104	105	**115**	107	102	103	110	110	96	104	106	**119**	105

n.d. – not detected

Bold faced type indicates results exceeding upper limit for "acceptable" recovery (110%) according to Council Directive 94/43/EC, Off. J. Eur.Com. L227, 1994.

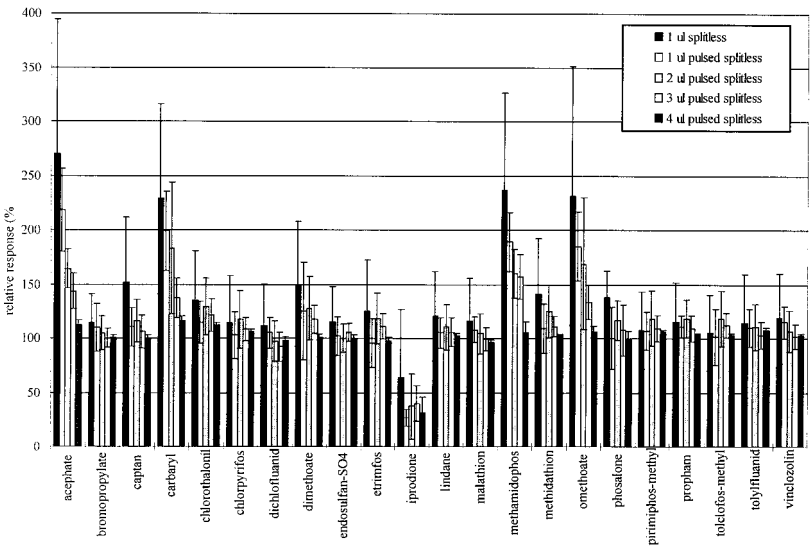

Figure 3. Relative responses of tested pesticides obtained with different injection volumes (concentration level B, RSD *n*=3, pressure pulse 60 psi, 1 min).

matched standards. However, the preparation of these solutions renders the analytical process more laborious. Moreover, additional uncertainty is always introduced when low volumes of standards and blanks are measured during the preparation of matrix standards. The use of pulsed splitless injection thus appears to be a reliable way of overcoming matrix effects, permitting routine use of standards in neat solvent. Some generalizations related to the operating of pulsed splitless injection are summarized in the following points:

i) To obtain good responses for all analytes, including early eluting species, the pulse pressure time should not exceed 1 min with the intensity not exceeding 60 psi, otherwise losses of volatile compounds occur.

ii) Higher pulse intensity (80 psi compared to 40 psi) gives lower relative responses (ratio of analyte response in matrix standard to response of the standard in neat solvent) for all groups of analyzed compounds

iii) Longer pulse time (2 min compared to 1 min) results in lower relative responses. However, a pulse time exceeding 1 min leads to significant losses of volatile compounds causing poorer detectability.

iv) Injection of sample volumes exceeding 1 µL causes peak distortion unless a retention gap is used. Its attachment enables sample volumes up to 4 µL to be injected without significant loss of resolution and overall separation quality.

v) Injection of larger sample volumes (up to 4 µL) results in lower relative responses, with 4 µL injection providing almost complete suppression of matrix effects.

In conclusion, pulsed splitless injection represents an effective tool whenever problems caused by matrix effects are to be solved. Benefits accruing from lower detection limits and higher repeatability of the analytical results can be anticipated. However, it should be noted that the geometry of the injection port, the type of liner, and the analytical column used can play an important role when using this injection technique and, consequently, differences may be experienced between results obtained by different instruments. In addition, the history of the GC system, *i.e.* the contamination of the inlet port and the front part of analytical column, should also be considered.

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