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Optimization and application of the PTV injector for the analysis of pesticide residues

The applicability of PTV splitless and solvent vent injection to the gas chromatographic analysis of 26 pesticides representing different chemical classes was evaluated. All parameters related to optimum injector performance in PTV splitless and PTV solvent vent (split vent) injection modes were tested. For PTV splitless injection of small sample volumes (1 μL), the inlet temperature program (initial inlet temperature, heating rate, final temperature), splitless time, and starting oven temperature were optimized. Parameters identified as optimal were then applied in PTV solvent vent injection. In the case of the PTV solvent vent technique, all injections were performed with pesticides dissolved in a binary mixture of cyclohexane-ethyl acetate (1 : 1, *v/v*). This solvent is used as a mobile phase in the HPGPC clean-up step involved in our multiresidue method. For the PTV solvent vent technique the following parameters were tested: maximum single injection volume, inlet temperature, vent flow, vent time, and vent period were determined for a single injection of 10 μL of sample. Thermodegradation and/or adsorption of some pesticides occurred as long as glass wool packed liner was used. To achieve lower detection limits, multiple injection concept was employed: 30 μL of standard solution were injected in three subsequent steps. Under optimized conditions even higher sample volumes could be injected. Good responses were obtained also for the compounds possessing relatively higher volatility compared to other tested analytes.

Key Words: Gas chromatography; PTV; Pesticide residues

Ms received: December 11, 2000; revised Ms received: February 26, 2001; accepted: February 28, 2001

1 Introduction

Due to its separation efficiency, sensitivity, and selectivity obtained by conventional detectors, capillary gas chromatography (GC) is nowadays the most popular technique in the analysis of pesticide residues (and other organic contaminants). However, the application range of GC is limited by the thermal stability of analytes, since relatively high temperatures have to be applied in conventional GC to volatilize sample components. Moreover, in the case of compounds containing polar groups (e. g. hydroxyl or amino groups) in their structure, adsorption at active sites in the injection port may occur.

With respect to these facts, GC operating conditions must be optimized in order to obtain satisfactory results also for compounds prone to thermodegradation and/or adsorption. The injection port is obviously the most critical part of GC and, consequently, great attention should be paid to the optimization of its performance parameters.

Among existing injection techniques, common splitless injection, which is the most popular in trace analysis, was found to cause highest losses of analytes due to the adverse effects mentioned above [1–3]. Their occurrence results in the phenomenon called matrix induced response enhancement (matrix effects): during analyses of real samples, analytes degradation and/or adsorption is reduced to some extent due to masking of active sites in the inlet by co-injected matrix components. Accordingly, the response of target compound is enhanced in their presence compared to standard prepared in the pure solvent. Apparent recoveries of some analytes approaching as much as hundreds of percent have been reported in several studies [4–7]. In other words, seriously overestimated analytical results are obtained during quantitation of trace amounts of analytes as long as matrix effects are not taken into account and appropriately compensated.

In those terms, some improvements of performance of classic splitless injection can be achieved using a carrier gas pressure pulse during injection – in so-called pulsed splitless injection [8–10]. It should be noted that an applied pulse may lead to significant decrease of matrix effects. However, at low concentration levels matrix effects still may remain very large for some analytes [9, 10]. In

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 1615-9306/2001/0505–0355$17.50+.50/0

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any case, on employing this technique, the performance of GC analysis can be improved since up to 5 μL of sample can be injected into a GC system without risk of inlet overflow with sample vapors. Consequently, lower detection limits are obtained.

With on-column injection, more accurate results for troublesome compounds may be expected compared to splitless injection as long as a clean GC system is used. Nevertheless, repeated injections of "dirty" samples result in quick deterioration of injector performance [3] due to contamination of the front part of column with non-volatile deposits.

Programmable temperature vaporization injector (PTV) was initially presented in 1979 by Vogt et al. [11, 12]. During the last decade, the number of applications using a PTV inlet for introduction of samples has increased significantly and many papers concerned either with optimization of PTV parameters or application in routine trace analysis have been published (see below). The advantages of PTV injection over other techniques consist in decreased analyte discrimination during injection step, better recoveries of thermodegradable compounds and less pronounced adverse effects of non-volatiles present in the sample on the injection process. However, the most important feature of this technique is the possibility to introduce large volumes of samples (up to hundreds μL) into GC system. Several alternative ways of operation of PTV injector have been employed in practice.

The first one is represented by a PTV splitless injection. The sample is introduced at a temperature below or close to the boiling point of solvent. Split exit is closed during sample evaporation and solvent vapors are vented via the analytical column. PTV splitless injection has been employed for both large volume injections of 20 μL of sample [13] and for conventional small volume injections [14]. The advantage of this technique is that no losses of volatile analytes occur (contrary to the solvent vent technique, see below). Operating parameters must be carefully optimized to avoid inlet overflow by sample vapors (and consequent losses of volatile compounds) as well as column flooding by excessive solvent (poor peak shapes of more volatile analytes).

The second method for introducing large volumes of samples is so-called solvent vent PTV injection. In this case, sample is injected at temperatures well below the boiling point of solvent, the temperature of inlet port is held at a low value while solvent vapor elimination occurs via the split exit. After the venting step, the inlet is rapidly heated and analytes are transferred onto the front part of the analytical column [15–21]. In that way, sample volumes up to several hundred μL can be injected [21–25]. For injection of large volumes the use of a packed inlet liner is recommended in order to prevent solvent from reaching bottom of inlet, which may lead to column flooding with liquid sample. Different packing materials for PTV injectors have been tested (glass wool, glass beads, PTFE, and Dexsil). However, for analyses of compounds sensitive to degradation or adsorption on active sites in packing, an empty liner is a preferred alternative [26, 27]. The need for thorough optimization of all parameters relevant to good performance of PTV injectors in solvent vent mode has been discussed in many studies for different analytes [12, 15, 18, 20, 22–25, 28–30]. It should be noted that although some general rules for PTV operation can be drawn from these experiments, experimental optimization of parameter settings is still needed in each particular case.

The third alternative of PTV injection is the "vapor overflow" technique, developed by Grob and co-workers [31, 32]. The sample is injected into PTV inlet held at temperature high above the solvent boiling point. The split vent is closed during evaporation of sample while the septum purge vent is wide opened so solvent vapors can be vented via this exit. Evaporating solvent causes the formation of a "cold spot" in the liner, where analytes are retained. After elimination of all solvent, the liner is heated again and analytes are transferred onto the GC column. The application range of this technique covers higher boiling compounds with volatility approximately similar or lower than alkane C_{25}. Losses of more volatile analytes occurring during vapor overflow can be hardly avoided.

The last-used PTV technique is referred to as Solid Phase Extraction-Thermal Desorption. The exceptional application field of this technique is in the determination of contaminants in water samples [33–35]. Analytes are trapped by an adsorptive material placed in the liner, through which water sample is passed. Residual water is then removed by the carrier gas flow. To prevent water from entering the GC column, carrier gas counterflow is applied. After water removal is complete, the packed liner is heated and desorbed analytes are transferred onto the GC column.

Recently, several applications using direct injection of water into GC via PTV injector have been published [36–39]. This approach permits large volumes of water samples to be directly injected onto GC without any sample preparation. A review concerned with different strategies in the analysis of micropollutants in water samples by PTV is presented in [39]. Several studies concerning on-line coupling of PTV-GC either with SPE automatic devices [40], or with LC [41–44] or SFE respectively [45] were reported.

In the presented study, we focused on the optimization of PTV injector parameters for the analysis of 25 pesticides in food samples extracts. Analytes with different physicochemical properties were selected including those known to be difficult to analyze by GC due to their degradation or

adsorption under common splitless injection conditions. Two alternative applications of PTV, (i) splitless and (ii) solvent vent technique were thoroughly tested.

2 Experimental

2.1 Instrumentation

A GC 6890 Plus (Hewlett-Packard, Palo Alto, USA) gas chromatograph equipped with an HP PTV injector (septumless head, CO_2 cooling) and automatic sampler ALS 7683 were used for the experiments. Acquired data were reprocessed by HP Chemstation A.06.03 software (Hewlett-Packard, Palo Alto, USA).

2.2 Injector accessories

Deactivated glass liners were used for analyses, multi-baffle empty liner (catalogue number HP 5183-2037) and glass wool packed single-baffle liner (catalogue number HP 5183-2038). Injection syringes of 25 and 100 μL were employed for sample injection.

2.3 Gas chromatography

Separations were performed on a DB-5 MS fused silica column (60 m × 0.25 mm × 0.25 μm) coated with 5% phenylmethylpolysiloxane stationary phase with column effluent split in parallel into electron capture (ECD) and nitrogen-phosphorus (NPD) detectors. Helium was used as a carrier gas (2 mL/min constant flow). Injection volumes and injection parameters were adjusted according to the aims of particular experiment and will be discussed later.

Oven temperature program is shown in detail later – see Table 3.

2.4 Materials

Certified pesticide standards were obtained from Dr. Ehrenstorfer, Germany (purity 95–99%). Pesticide residues grade solvents were obtained from Scharlau, Italy (ethyl acetate) and from Merck, Germany (cyclohexane, toluene).

Pesticide stock solutions were prepared by dilution in toluene, working solutions were then prepared by further diluting with the mixture ethyl acetate-cyclohexane 1 : 1, *v/v* (for PTV solvent vent optimization) or with toluene (for PTV splitless experiments), concentrations see **Table 1**.

Table 1. Concentrations of pesticides in stock and working solutions.

Pesticide	Stock solution (μg/mL) Toluene	Diluted working solutions (μg/mL) Toluene A1	Cyclohexane-ethyl acetate B1	Cyclohexane-ethyl acetate B2
Acephate	47.100	0.471	0.471	0.0118
Bromopropylate	20.400	0.204	0.204	0.0051
Captan	56.000	0.560	0.560	0.0140
Carbaryl	79.800	0.798	0.798	0.0200
Chlorothalonil	15.200	0.152	0.152	0.0038
Chlorpyrifos	58.800	0.588	0.588	0.0147
Cyhalothrin-lambda	35.600	0.356	0.356	0.0089
Cypermethrin	35.760	0.358	0.358	0.0089
Deltamethrin	144.000	1.440	1.440	0.0360
Dichlofluanid	28.800	0.288	0.288	0.0072
Dichlorvos	17.800	0.178	0.178	0.0045
Dimethoate	35.520	0.355	0.355	0.0089
Endosulfan-SO_4	10.700	0.107	0.107	0.0027
Etrimfos	43.584	0.436	0.436	0.0109
Lindane	6.040	0.060	0.060	0.0015
Malathion	58.700	0.587	0.587	0.0147
Methamidophos	40.800	0.408	0.408	0.0102
Methidathion	68.400	0.684	0.684	0.0171
Omethoate	47.000	0.470	0.470	0.0118
Permethrin	68.480	0.685	0.685	0.0171
Phosalone	39.600	0.396	0.396	0.0099
Pirimiphos-methyl	38.400	0.384	0.384	0.0096
Propham	153.300	1.533	1.533	0.0383
Tolclofos-methyl	48.960	0.490	0.490	0.0122
Tolylfluanid	29.200	0.292	0.292	0.0073
Vinclozolin	13.600	0.136	0.136	0.0034

3 Results and discussion

In spite of numerous published studies concerned with the optimization of PTV parameters, no general rules are available for the application of this technique in residue analysis and therefore each PTV system needs to be optimized individually with respect to the purpose of analysis, the character of analyzed samples, and target analytes. In the first part, our experiments concerning PTV splitless injection will be presented, in the second part, their implementation in the solvent vent technique is shown.

3.1 PTV splitless injection

One of the most critical aspects in PTV splitless injection is the selection of the volume of injector liner. Internal diameters of the most commonly used types range from 1 to 3.5 mm. Liners with smaller i. d. provide benefits of better heat transfer and thus faster transfer of analytes onto GC column. Accordingly, less extensive degradation of thermolabile compounds was observed in the case of liners with higher ID [27]. On the other hand, larger ID of liner enables higher volumes of injection. Considering the above

Table 2. The overview of parameters optimized for the PTV splitless injection, printed in bold are variable parameters for the particular experiment – for details see the text.

	GC parameters				
	Initial inlet temperature (°C)	Inlet heating rate (°C/min)	Final inlet temperature (°C)	Splitless time (min)	Initial oven temperature (°C)
Experiment 1	**50**				**50, 70, 90**
	70	300	350	2	**50, 70, 90**
	90				**50, 70, 90**
Experiment 2			**250**		
	70	300	**300**	1	90
			350		
Experiment 3		**100**		3	
		200		1.5	
	70	**300**	350	1	
		400		1	90
		500		1	
Optimized parameters	70	**400**	350	1	90

facts, liners with ID 2 mm and a volume of 180 μL were used in our study.

Generally, the volume of vapors resulting from injection of 1 μL of organic solvent into hot inlet is typically from 200 to 500 μL. Vapors of such volumes can easily overflow out of the liner, which results not only in a loss of sensitivity but also in a successive contamination of the GC system. In the experiments with PTV splitless injector, toluene was used as injection solvent since in our current multi-residue method sample is transferred to it prior to GC analysis. In addition, among all conceivable solvents toluene forms the lowest volume of vapors and has the highest boiling point (113°C).

3.1.1 Experiment 1

As a first step, optimal starting oven and inlet temperatures were searched for. For this purpose, 1 μL of working solution A1 was injected into PTV injector at three different temperatures – see **Table 2**. In **Figure 1** there are shown peak heights[1)] of selected analytes obtained by injections of testing mixture that were carried out under different combinations of inlet and oven temperatures. It is evident that with higher starting oven temperature (70 and 90°C) peaks are relatively higher (due to narrower peaks).

At the lowest oven temperature setting (50°C), the distortion of peaks eluted in the middle part of the chromatogram (analytes of medium volatility) was observed for all examined inlet temperatures. This phenomenon can be attributed to the recondensation of injected solvent at the front part of the GC column and it was possible to eliminate it by setting higher oven temperatures. At 90°C no peak deformation was observed due to a lower portion of recondensed solvent. Described effects were in agreement with the observations reported by Grob et al [12], who recommended to increase starting oven temperature during PTV splitless injection so that only small portion of injected solvent recondenses but solvent focusing effects are still achieved.

On the other hand, some temperature dependence of peak heights could be observed for different inlet temperatures. Slight decrease of responses of early eluting compounds (methamidophos, dichlorvos, acephate) occurred at higher starting injector temperatures. This was obviously due to a higher volume of solvent vapours formed after sample injection and their losses via septum purge vent. It can be concluded that starting inlet temperature should be set at rather lower values (e. g. 50°C) while oven temperature is held at optimal value with respect to the physico-chemical properties of used solvent. For further experiments oven temperature 90°C and inlet temperature 70°C were employed. Although 70°C was not the best value with regards to attainable peak heights, it was accepted as a compromise allowing elimination of cooling of the inlet with liquid CO_2.

3.1.2 Experiment 2

The next parameter tuned was the final inlet temperature: 1 μL of test solution A1 was injected under conditions shown in Table 2. No significant differences in responses of analytes were observed at all tested settings. Moreover, the latest eluting analyte deltamethrin was transferred quantitatively onto column even at lowest temperature (250°C). However, during injection of real samples co-injected matrix components may negatively influence the transfer of analytes. To bake out most of the non-vola-

[1)] From a practical point of view peak heights are used for evaluation of injector performance in this part due to their better relevance to the characterization of sensitivity (or signal/noise ratio respectively).

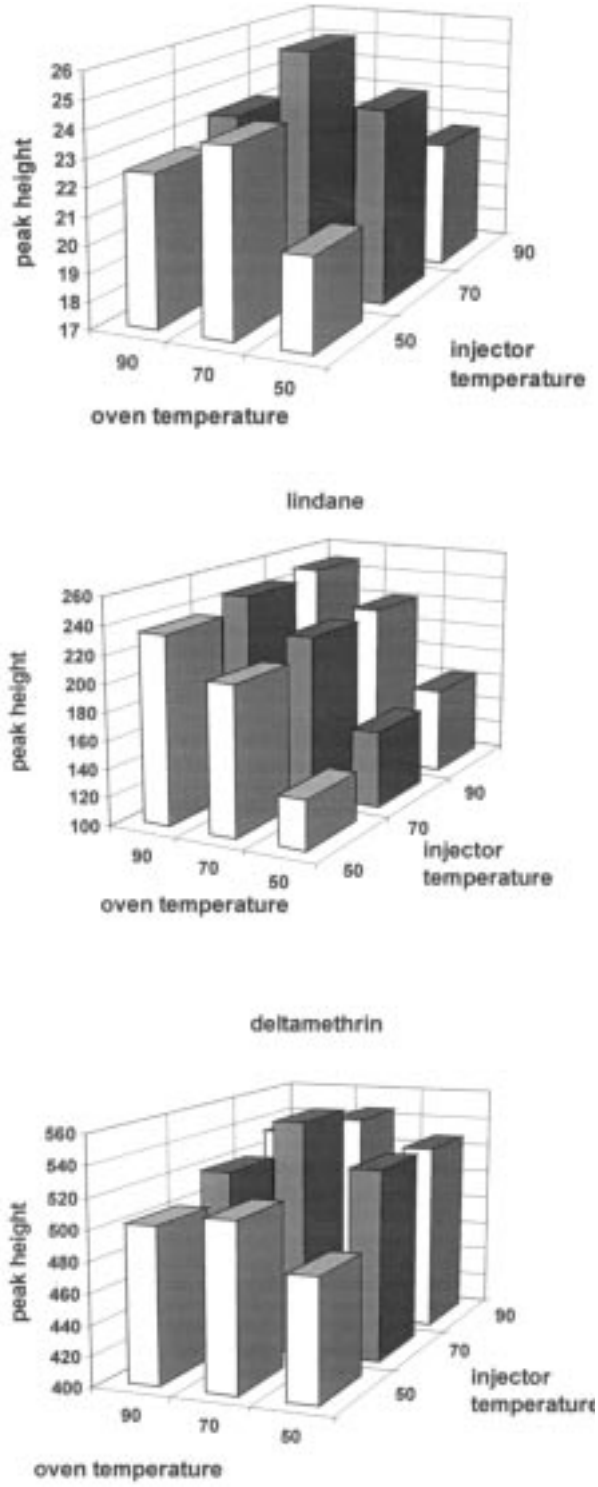

Figure 1. Peak heights obtained under different inlet and oven temperature settings, 1 μL of standard mixture A1 injected – conditions see Table 2 – Experiment 1.

tile deposits, final inlet temperature was set to 350°C in following experiments.

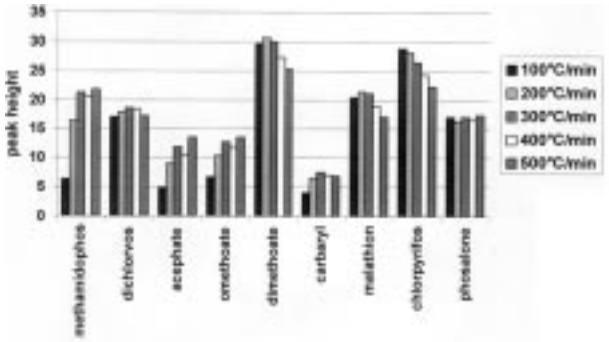

Figure 2. Peak heights of selected NPD detected pesticides obtained with different PTV heating rates, 1 μL of A1 solution injected – conditions see Table 2 – Experiment 3.

3.1.3 Experiment 3

As the last parameter, inlet heating rate was optimized. Again, 1 μL of testing solution A1 was injected under conditions shown in Table 2. The splitless time (measured from the injection of sample) was adjusted according to applied heating rate so that final inlet temperature was achieved before the end of this period. In **Figure 2** there are summarized peak heights obtained with different heating rates of inlet. It can be seen that the lowest heating rate, representing the "mildest" conditions did not result in highest responses for troublesome compounds. For example, the best responses for methamidophos and acephate were obtained with heating rates equal or higher than 300 K/min. This was probably caused by the fact that compounds tending to adsorption in the liner were better released from active sites by "shock" heating. Also slower heating rate caused longer residence time of analytes in the inlet and higher thermal stress posed upon them. On the other hand, a slight decrease in responses could be seen at the highest heating rate, 500 K/min, probably because of thermal degradation of analytes (conditions in the inlet at this rate practically corresponded to common splitless injection). In addition, too rapid heating might lead to liner overflow with sample vapors, resulting in potential losses of the more volatile compounds.

Optimized operation parameters of PTV injector in splitless mode are shown in Table 2.

As stated above, PTV splitless injection is not the preferred application mode of PTV in trace analysis. Therefore our effort was further focused on the PTV solvent vent technique, which seems to be more promising with respect to the possibility of injection of large sample volumes and thus obtaining low detection limits.

3.2 PTV solvent vent injection

3.2.1 Single injection

As mentioned in the introduction, during large volume PTV injection liquid sample must not reach the bottom of the in-

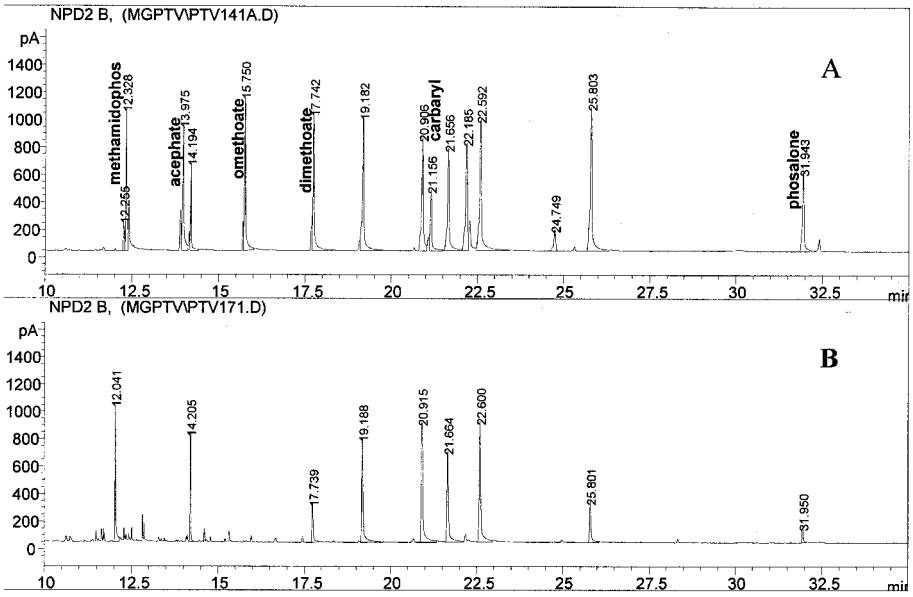

Figure 3. Chromatograms obtained by PTV injection into (A) empty liner and (B) liner packed with glass wool plug, 10 μL of standard mixture B1 injected.

let, otherwise column flooding and the loss of sample via split exit can occur. As a consequence, peak shape deterioration and contamination of the GC system occurs. Because of these facts, as a first step of this set of experiments the maximum injection volume was determined. The GC column was disconnected from the injection port while carrier gas flow through the inlet was still maintained. Successively increasing volumes of solvent were manually injected until the presence of the liquid at the bottom of inlet was observed. It was necessary to perform this test as quickly as possible because after a few minutes carrier gas pressure was automatically shut off due to the detected leak in the inlet. Up to 10 μL of ethyl acetate-cyclohexane mixture (1 : 1, *v/v*)[2] was injected into a multi-baffle liner without visible overflow with the liquid. However, to attain sufficiently low detection limits for target analytes, higher volume of injected sample was needed.

Considering promising results reported in studies employing packed liners [24, 26, 27] we decided to test the possibility of their use with our PTV inlet. Unfortunately, liners packed with PTFE or Dexsil which were identified by other authors [27] as the most suitable for injections of troublesome compounds are not commercially available. To avoid problems related to laborious in-house preparation of such liners and taking into account rather uncertain repeatability of liner performance we tested the use of the single baffle liner packed with silanized glass wool which is commercially available for the Hewlett-Packard PTV injector. **Figure 3** shows the differences in responses of sensitive analytes when injections were carried out into empty multi-baffle liner and into single-baffle liner packed with glass wool. Unfortunately, in the latter case dramatic peak losses were observed due to the thermal degradation or adsorption of these analytes at active sites that are unavoidably present on the glass wool surface. The benefits of this type of packing consisted in improved peak shapes of inert compounds since accelerated evaporation of solvent from large packing surface occurred. It should be emphasized that during injections of 10 μL of solvent into empty liner, it was more advantageous to use the "fast plunger" autosampler injection setting to achieve nebulization of sample in the injection port. Also, with slow plunger injection higher risk of solvent penetration to the bottom of liner existed [27]. With respect to the above-mentioned facts, an alternative concept of large volume introduction based on repeated injections of smaller amounts of sample into multi-baffle empty glass liner was employed. In next steps of the presented study, following operation parameters of the injector were optimized for 10 μL injection volume: (i) vent flow, (ii) vent pressure, (iii) inlet temperature during solvent elimination and (iv) duration of the whole process.

During PTV injector optimization in solvent vent mode, the most important aspect was the amount of solvent remaining in the liner after the venting period was finished (this is then transferred onto analytical column). If the residual volume of solvent was too high (more than 3–4 μL according to our previous observations), too long flooded zone formed in the front part of column resulted in "peak band broadening in space" (the term defined by Grob et al.

[2] Cyclohexane-ethyl acetate mixture is used as mobile phase in HPGPC clean-up and therefore it was used for testing of large volume PTV injection.

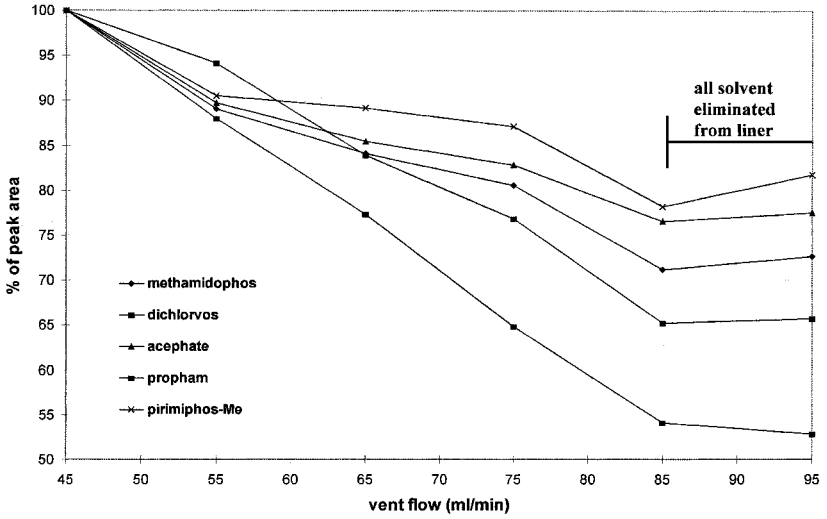

Figure 4. The relationship between peak areas and increasing vent flow values, 10 μL of standard mixture B1 injected, injection temperature 0°C, vent pressure 5 psi, vent time 1 min.

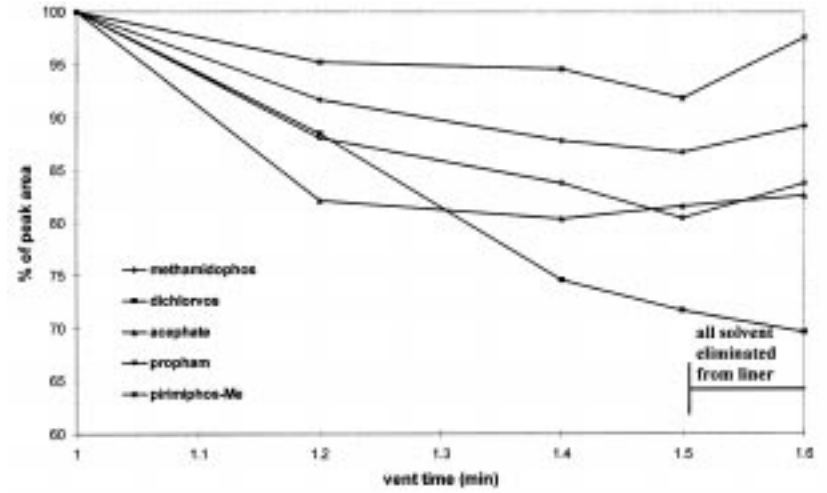

Figure 5. The influence of increased vent time on responses of analytes, 10 μL of standard mixture B1 injected, injection temperature 0°C, vent pressure 5 psi, vent flow 55 mL/min.

[44]). As a result of this phenomenon, distortion of peaks of intermediately volatile compounds was observed in the gas chromatographic run. As long as solvent volume remaining in the liner was too low or there was not even any solvent left, losses of more volatile analytes occurred. In addition, solvent liquid film formed at the front part of column before oven temperature program was started helped to focus especially early eluting analytes (i.e. methamidophos, dichlorvos). Generally, the amount of solvent condensed in the front part of GC column could be regulated either by means of inlet parameters setting (vent flow, vent pressure, inlet temperature, and vent time) or by the changes of column temperature program. In any case, all of these parameters were mutually dependent. Thus for instance the decrease of vent flow caused the same effect as the vent pressure decrease. Similarly, increased vent flow exhibited the same effect as decreased vent pressure. To obtain good accuracy of analytical data, careful optimization of all parameters had to be carried out.

Figure 4 documents the relationship between peak areas and applied vent flow, and **Figure 5** shows the relationship for peak areas and applied vent time. The higher the vent flow or the longer the vent time, the lower the peak areas of the more volatile compounds; these effects were most pronounced in the case of dichlorvos, the most volatile among all the tested analytes. Although not shown here in detail, similar relationships could also be demonstrated for other parameters like vent pressure.

Table 3. The summary of optimized conditions for all tested modes of PTV injection.

GC method step	GC parameter	PTV splitless injection	PTV solvent vent injection	
			Single injection	Multiple injections
Solvent venting period	Injection volume (μL)	1	10	3 × 10
	Inlet temperature (°C)	70	0	0
	Vent flow (mL/min)	–	45	45
	Vent pressure (psi)	–	5	5
	Vent time (min)	–	1	3 × 1.5
Transfer of analytes	Inlet heating rate (°C/min)	400	400	400
	Final inlet temperature (°C)	350	350	350
	Splitless time (min)*	1	1	1
	Initial oven temperature (°C)	90	60	60
Separation of analytes	Carrier gas flow (mL/min)	2	2	2
	Oven temperature program	25 K/min to 190°C 2.5 K/min to 225°C 15°C/min do 280°C (hold 17 min)	25 K/min to 190°C 2.5 K/min to 225°C 15°K/min to 280°C (hold 17 min)	25 K/min to 190°C 2.5 K/min to 225°C 15 K/min do 280°C (hold 17 min)
	GC run time	39.7	40.7	45.2

* In PTV splitless injection splitless time is counted from the time of injection. In PTV solvent vent mode it is counted since the end of solvent elimination period.

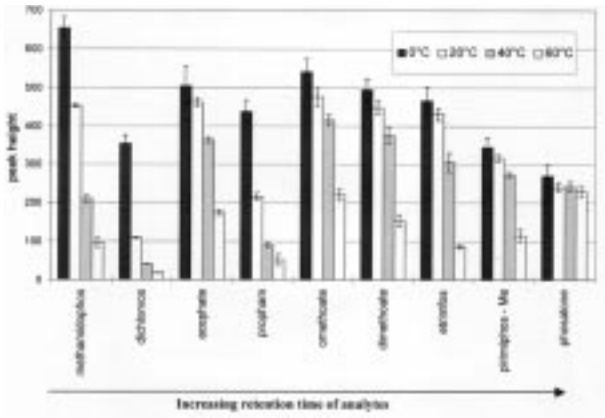

Figure 6. Peak areas of analytes obtained at different injection temperatures, 10 μL of standard mixture B1 injected.

In accordance with other studies concerned with the PTV optimization [13, 18], inlet temperature setting during solvent elimination process was identified as the most important parameter affecting efficient transfer of analytes onto analytical column in a narrow band. Better recoveries of the more volatile analytes at lower inlet temperatures reported in the above cited papers could be attributed rather to larger volumes of solvent remaining in the liner at low inlet temperatures [18] than to the injection port temperature itself. In our approach we compared the responses of analytes under such conditions, when the volume of solvent remaining in the inlet at all injection temperatures was approximately the same (the amount of transferred solvent was estimated on the basis of the solvent peak area). In **Figure 6** there are shown mean responses of selected analytes under different inlet temperature settings. It is documented here that at lower inlet temperatures peak areas were significantly higher. This trend was more pronounced for more volatile compounds while in the case of the least volatile pesticide phosalone the differences are negligible. In general, cold trapping of analytes in the liner played an important role in the solvent venting process. Accordingly, inlet temperature should be held at lowest practical value.

As regards initial oven temperature, its value should be set up so that the condensation of the injection solvent is optimal. In our experiments, temperatures set at 40, 60, and 80°C were tested. With starting oven temperature held at 40°C, peaks of the late eluted compounds were distorted due to a large amount of solvent condensed in the GC column and consequent band broadening in space. At 80°C significant decrease in responses of early eluting compounds (methamidophos, dichlorvos, acephate) was observed. Probably no solvent recondensed at this temperature and, consequently, no solvent focusing effect occurred. As a compromise, a temperature of 60°C was used for the next set of experiments since both the peak shapes and analytes responses were satisfactory. Optimized parameters for PTV solvent vent injection are summarized in **Table 3**.

3.2.2 Multiple injections

Considering the need to attain low detection limits of target analytes, approximately 2 mg of sample matrix had to be injected into the GC system. With regard to the procedure commonly used for sample processing prior to GC analysis[3)], a 30 μL aliquot of GPC "pesticide fraction"

[3)] 2 mL of crude extract contaning 1 g of original sample are injected onto HPGPC column, 15–30 mL fraction is collected and 30 μL aliquot is injected into PTV.

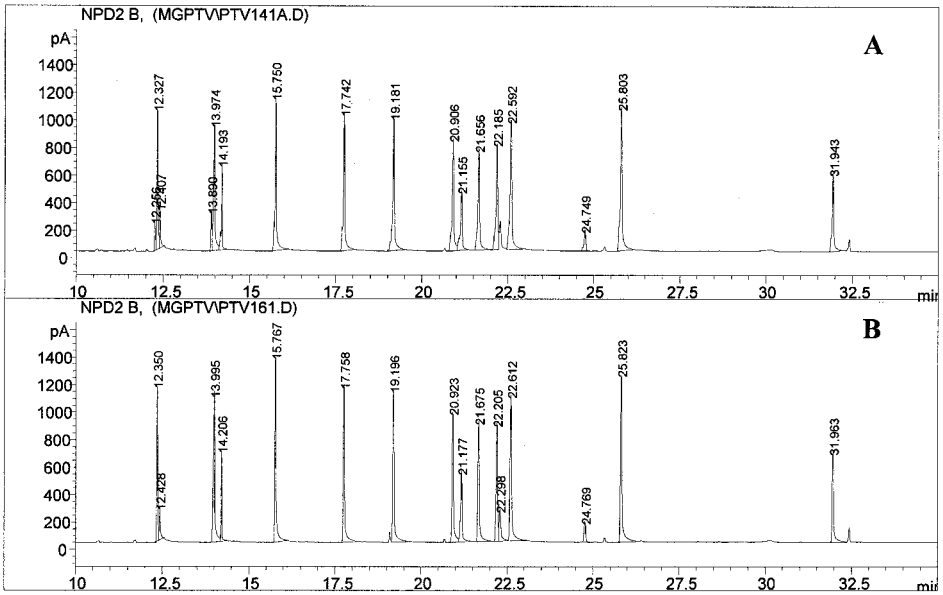

Figure 7. Chromatograms of pesticide standard mixture B1 injections, 3×10 μL injected. (A) Injection under conditions optimized for single 10 μL injection. (B) Injection under conditions optimized for triple 3×10 μL injection.

should be transferred to the GC column by PTV injection. To meet this requirement, 3 replicate 10 μL injections of purified "pesticide fraction" had to be performed. In theory, practically unlimited amount of solvent can be injected by repeated injections which are realized always into "empty" liner as soon as solvent from previous injection is removed. The only limiting factors in this concept are the potential losses of more volatile analytes.

Practically, inlet parameters tuned for single injection of 10 μL portions of samples (see Section 3.3) were applied, with the exception of the solvent venting time which was extended to 3 min.

Unfortunately, during repeated injections the volume of solvent accumulated in the inlet after solvent venting was too high. This resulted in peak splitting during chromatographic separation, see **Figure 7.A**. Further optimization of conditions was therefore necessary for fine-tuning of solvent volume left in the liner. In the only study [18] concerned with application of the same type of HP injector with a similar spectrum of compounds, very intensive solvent elimination conditions were applied (low vent pressure 0 psi and very high vent flow 300 mL/min for shorter time). Unfortunately, authors did not report recoveries of target analytes under these conditions. Nevertheless, during our experiments it was recognized that with respect to recoveries of more volatile compounds it was more advantageous to apply longer venting time period instead of decreased vent pressure or increased vent flow values. This can be documented by comparison of peak areas obtained under different vent flows and vent times (Figure 4 and Figure 5). With increasing vent flow value, the loss of volatile analytes was more pronounced compared to increasing vent time value. Better recoveries of tested analytes (higher by 10–20%) were obtained with longer vent time (1.5 min) rather than with higher vent flow (85 mL/min).

The final optimized PTV solvent vent conditions were as shown in Table 3. The chromatogram obtained under these conditions is shown in **Figure 7.B**.

As the drawback of a longer vent time (1.6 min) lower peak heights of early eluting compounds were observed compared to those obtained with single 10 μL injection and shorter vent time (1 min). Due to longer venting time, in the first two injections almost all the solvent was removed from the inlet. Thus insufficient solvent was present to "keep" the analytes in the GC and they were consequently lost via the split line. This can be documented graphically: whilst good linearity of repeated injections was obtained for the less volatile compounds, the curve for methamidophos or dichlorvos is obviously nonlinear, see **Figure 8**. Repeatability of peak areas obtained in three successive injections of 2×10 μL and 3×10 μL of B1 standard solution ranged from 0.5% to 3%.

As mentioned above, the purpose of the present study was to incorporate PTV injection into the GC step of a routinely used multi-residue method. As a part of the validation protocol, the repeatability of PTV injection under optimized conditions was tested. For this purpose, 10 repeated injections of standard solution B2 were performed and RSD values for each particular pesticide were calculated. RSD values ranged from 0.7% to 7%, higher values

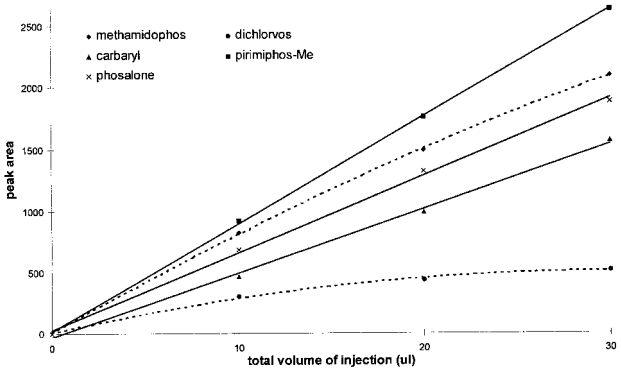

Figure 8. The dependence of peak area on the injected amount of sample (pesticide standard mixture B1), final optimized PTV conditions used.

were obtained for analytes eluted at higher retention times (e.g. deltamethrin).

Since during automated HPGPC clean-up respective pesticide fractions are collected into opened flasks, some evaporation of HPGPC mobile phase (cyclohexane-ethyl acetate, 1:1, *v/v*) may occur resulting in changed ratio of solvents composing the elution mixture. To recognize potential impact on PTV performance, standard solutions in different compositions of mobile phase (cyclohexane-ethyl acetate 75:25, 60:40, and 25:75, *v/v*) were prepared at concentration levels corresponding to the solution B1, see Table 1. 30 μL of standards prepared in this way were then injected into the PTV injector. No degradation of peak shape was observed with the composition of solvent mixture up to 60% of cyclohexane. However, with 75% of cyclohexane peak splitting occurred. Due to higher amount and higher boiling point of injected cyclohexane (compared to ethyl acetate), an excessive amount of solvent condensed at the front part of the column leading to peak splitting. In practice, this change in ratio of both solvents is not probable. Hence, good robustness of the injection process was confirmed with regards to slight variations in solvent composition.

4 Conclusions

The results presented in this study refute the generally accepted assumption that GC employing PTV injection is applicable preferably for less volatile analytes. It was demonstrated that a good accuracy of generated data could be obtained for pesticides with wide range of physico-chemical properties including also relatively volatile compounds.

PTV splitless injection can be successfully used to effectively substitute "classic" splitless injection, offering the advantages of lower thermal degradation and/or adsorption of target analytes. Suppression of those effects will in the end lead to lower matrix effects during analyses of real samples and better reliability of acquired analytical results.

Whenever lower detection limits are required, PTV solvent vent is an option, which combines both the advantages of PTV splitless injection and also the possibility of injection of large volumes of sample. However, thorough tuning of all injector parameters is necessary for good performance of this injection mode.

Regarding the strategy of PTV optimization, experience obtained for each particular mode of this injection technique in this study is summarized below. It should be noted that slightly different experiencies can be encountered using different type of PTV injector and that presented results are fully valid only for the tested type of HP PTV injector.

PTV splitless injection

(Parameters were optimized for injection of 1 μL of toluene mixture)

- low starting inlet temperature (70°C) helped to avoid solvent evaporation and consequent overflow of low volume PTV injector liner with solvent vapors
- optimized inlet heating rate (400 K/min) assured good transfer of analytes from inlet and eliminated the risk of liner overflow with solvent vapors
- final inlet temperature (350°C) had to be set high enough to keep inlet clean even in the case when relatively "dirty" samples are injected
- splitless time 1 min was shown to be long enough to quantitatively transfer analytes onto analytical column
- better responses of troublesome compounds prone to matrix effects were obtained with PTV splitless injection compared to classic splitless injection mode

PTV solvent vent injection

- maximum applicable single injected volume of standard solution into multi-baffle liner using PTV solvent vent technique was 10 μL in case of cyclohexane-ethyl acetate (1:1, *v/v*) mixture or 20 μL of toluene. **Viscosity and surface tension** were probably the most important factors influencing the retention of respective solvent in the liner. **Glass wool packing** allowed to inject higher volume of sample but led to excessive degradation of sensitive analytes.
- The strategy of inlet parameters settings to obtain optimal **single** injection PTV performance is as follows:

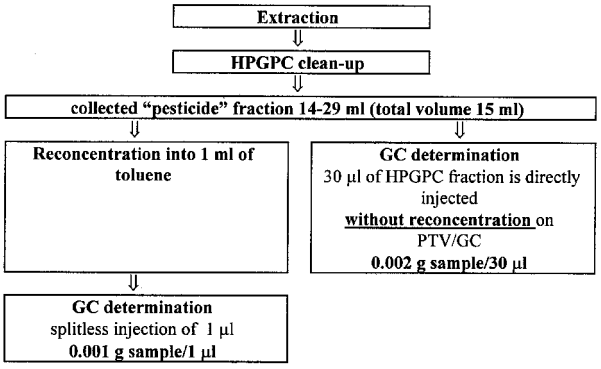

Figure 9. The implementation of PTV injection into used analytical method.

  - inlet temperature should be held at as low as practicable value to decrease evaporation of analytes during venting period, 0°C sufficiently suppressed solvent evaporation
  - vent flow, vent pressure and vent time had to be mutually optimized, increased vent time had to be applied if higher sample volumes were injected, increased vent flow or decreased vent pressure caused higher losses of more volatile compounds

- 30 µL of sample could be injected into empty liner using **multiple** injection PTV technique, for this purpose further tuning of inlet conditions was necessary
  - longer vent time and higher vent flow had to be applied to avoid solvent accumulation in the liner
  - during repeated injection some losses of early eluted compounds could not be avoided, however good repeatability of generated data was proved
  - injection of binary mixture of solvents (cyclohexane-ethyl acetate) was successfully employed; the changes in mixture composition did not significantly influence the performance of the injector

In the future experiments, developed PTV solvent vent injection step will be incorporated in the currently used multi-residue method. The intended mode of implementation of the injection step is presented in **Figure 9**. In that way, laborious sample reconcentration following HPGPC clean-up can be omitted and the overall lab sample throughput is significantly increased.

Acknowledgement

This study was conducted within the activities related to the project MSM 223300004. Authors wish to thank to the Ministry of Education and Youth of the Czech Republic for providing additional funds to support research in this field (project OK 342).

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