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Performance of programmed temperature vaporizer, pulsed splitless and on-column injection techniques in analysis of pesticide residues in plant matrices

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Abstract

A programmed temperature vaporizer (PTV) injection technique has been recently implemented in our laboratory. In present paper its performance is compared with other GC injection techniques commonly used in trace analysis of organic contaminants. Twenty-six pesticides representing different chemical classes were selected for the study. This group comprised compounds typically subjected to discrimination in the injection port of the gas chromatograph, e.g., polar organophosphorus pesticides and thermolabile carbamates. In the first set of experiments standards in pure solvent were injected into GC systems employing different types of injection, i.e., (i) on-column, (ii) pulsed splitless, (iii) PTV solvent split, (iv) PTV splitless, and the responses of analytes were compared. Discrimination of troublesome compounds was significantly decreased with the application of PTV solvent split injection. In the second set of experiments repetitive injections of purified wheat samples were performed, with aims to evaluate the long-term stability of responses, as well as matrix effects in different stages of system contamination for each injection technique. The tolerance of the GC system to co-injected matrix components was increased in the order: on-column<pulsed splitless<PTV solvent split technique. As regards matrix effects, these were suppressed considerably with the PTV solvent split technique in comparison with pulsed splitless injection. With the latter technique after 66 injections of wheat samples relative responses (apparent recovery) reached as much as 450% for some compounds, while with the application of PTV matrix effects did not exceed 200% under the same conditions.

Keywords: Programmed temperature vaporizer; Pulsed splitless injection; Injection methods; Matrix effects; On-column injection; Response stability; Pesticides

1. Introduction

Gas chromatography (GC) is a powerful separation technique widely applied in trace level analysis. GC techniques have been developed for many years with aims to improve their performance characteristics, time and cost effectiveness and to broaden the spectrum of amenable compounds.

A great deal of attention has been paid to the injection port as a part of a gas chromatograph, which is very critical in terms of data accuracy. On-column injection is as a superior technique in terms of non-discriminative transfer of sample components into the GC system [1–5], however, it

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provides no separation of analytes from matrix coextracts [6,7]. For this reason in trace analysis on-column injection is applied mainly for simple matrices such as drinking water [8,9]. The use of a replaceable retention gap (deactivated precolumn without stationary phase) can be a good solution for more complex matrices, however it represents an additive source of active sites in the GC system (retention gap surface, connection to the separation column). For ''dirty'' samples, e.g., plant materials, vaporizing[1] injection techniques are more suitable. ''Classical'' hot splitless injection is the most frequently applied injection technique, however, some adverse effects such as discrimination of low volatiles, sorption and thermodegradation can occur [4,5]. Significant suppression of these effects in the injection port was achieved by the application of pressure pulse during splitless period (pulsed splitless injection) [11,12]. Another alternative to classical hot splitless injection is a programmable temperature vaporizer (PTV). This injection technique, first introduced by Vogt and co-workers in 1979 [13,14], comprises the injection into the cold liner (temperature held below or near the solvent boiling point) and subsequent rising of temperature and transfer of analytes. This technique was shown to avoid discrimination of low volatile compounds [15–18] and the degradation of thermally unstable analytes [4,5,19]. The main advantage of the PTV, however, consists in the possibility of large volume injection (LVI). In the solvent split mode, the PTV allows one to introduce up to 10^3 μl of sample into the GC system. Injection of large sample volumes not only enables significant improvement of overall sensitivity of the analytical method, but also makes the PTV injector applicable for the on-line coupling of GC with various clean-up and enrichment techniques [20].

The problem closely connected with the injection port is matrix-induced chromatographic response enhancement (''matrix effects''). Erney et al. [21], who for the first time thoroughly discussed this phenomenon in 1993, suggested, that matrix components present in the sample can block the active sites in the inlet and thus prevent degradation and adsorption of analytes at these spots. Consequently, the amount of analyte entering the column is higher in samples containing matrix than in pure solvent standards. This theory provided reasonable explanation for recoveries considerably exceeding 100%, which were reported in several studies for some compounds (mainly organophosphates containing polar P=O group). Matrix effects may represent a serious analytical problem, because of possible overestimation of analyte's concentration (as much as several-fold), if calibration solutions in pure solvent are employed. There are several options how to eliminate/compensate for matrix effects, some of them with limited applicability [22]. One of the most reliable approaches is the use of matrix-matched standards, i.e., standards with the same matrix composition as the analyzed sample [23–27]. This approach is quite time consuming, moreover appropriate blank material has to be available, which may not always be the case.

As the cause of matrix effects occurs in the injection port, the injection technique can significantly influence their extent. Modifications of the classical hot splitless injection, where adsorption/degradation is reduced, can bring significant suppression of matrix effects. This has been proven in case of pulsed splitless injection [28,29]. Regarding the PTV, matrix-induced response enhancement was reported by Mol et al. for several nitrogen and phosphorus pesticides in river water extracts [30]. However, no comparison with other injection techniques from this point of view is available in the literature.

In our previous paper we demonstrated the optimization of PTV parameters for the injection of multiple pesticide residues in pure solvent standards [31]. The study presented here aimed to evaluate the PTV performance in analysis of pesticide residues in real-life samples. PTV injection was compared with the two other commonly used GC injection techniques in terms of long-term stability of responses and the extent of matrix-induced response enhancement.

[1] In this paper, ''vaporizing'' techniques are defined as techniques which involve an oven-independently thermostatted chamber being permanently at a temperature above that of the oven or being temperature programmed (PTV) (definition by Grob and Biedermann [10]).

2. Experimental

2.1. Chemicals and materials

Certified pesticide standards were obtained from Dr. Ehrenstoffer, Germany (purity 95–99%). Pesticide residues grade solvents were obtained from Scharlau, Italy (ethyl acetate) and from Merck, Germany (cyclohexane, toluene). Anhydrous sodium sulfate (Penta Chrudim, Czech Republic) was activated for 5 h at 450°C. Wheat grains were obtained at a retail market.

Pesticide stock solutions were prepared by dissolving neat standards in toluene, working solutions were prepared by their further diluting with ethyl acetate–cyclohexane (1:1, v/v) (standards for experiment 3) or with toluene (standards for experiments 1, 2 and 4). For concentrations of analytes see Table 1.

2.2. Apparatus and instrumentation

Wheat samples were processed with a Waring Blender homogenizer (Waring, USA) and a Turrax tissumizer (IKA, Werk, Germany). All solvent reductions were performed on a Büchi rotary evaporator (Büchi, Switzerland).

An automated high-performance gel permeation chromatography (HPGPC) system (Gilson, France) equipped with a PL gel (600×7.5 mm, 50 Å)

Table 1
Concentrations of pesticides in stock and working solutions

Pesticide	Stock solution (μg/ml) in toluene	Diluted working solutions (μg/ml)			
		In toluene		In cyclohexane–ethyl acetate (1:1)	
		STD1A	STD2A	STD1B	STD2B
Acephate	47.10	0.471	0.047	0.0118	0.0012
Bromopropylate	20.40	0.204	0.020	0.0051	0.0005
Captan	56.00	0.560	0.056	0.0140	0.0014
Carbaryl	79.80	0.798	0.080	0.0200	0.0020
Chlorothalonil	15.20	0.152	0.015	0.0038	0.0004
Chlorpyrifos	58.80	0.588	0.059	0.0147	0.0015
Cyhalothrin-lambda	35.60	0.356	0.036	0.0089	0.0009
Cypermethrin	35.76	0.358	0.036	0.0089	0.0009
Deltamethrin	144.00	1.440	0.144	0.0360	0.0036
Dichlofluanid	28.80	0.288	0.029	0.0072	0.0007
Dichlorvos	17.80	0.178	0.018	0.0045	0.0004
Dimethoate	35.52	0.355	0.036	0.0089	0.0009
Endosulfan-SO_4	10.70	0.107	0.011	0.0027	0.0003
Etrimfos	43.58	0.436	0.044	0.0109	0.0011
Iprodione	50.00	0.500	0.050	0.0125	0.0013
Lindane	6.04	0.060	0.006	0.0015	0.0002
Malathion	58.70	0.587	0.059	0.0147	0.0015
Methamidophos	40.80	0.408	0.041	0.0102	0.0010
Methidathion	68.40	0.684	0.068	0.0171	0.0017
Omethoate	47.00	0.470	0.047	0.0118	0.0012
Permethrin	68.48	0.685	0.068	0.0171	0.0017
Phosalone	39.60	0.396	0.040	0.0099	0.0010
Pirimiphos-methyl	38.40	0.384	0.038	0.0096	0.0010
Propham	153.30	1.533	0.153	0.0383	0.0038
Tolclofos-methyl	48.96	0.490	0.049	0.0122	0.0012
Tolylfluanid	29.20	0.292	0.029	0.0073	0.0007
Vinclozolin	13.60	0.136	0.014	0.0034	0.0003

column (Polymer Labs., UK) was used for clean-up of wheat extracts.

Two GC instruments characterized below were employed in our experiments:

Gas chromatograph 1: GC 6890 Plus equipped with a HP PTV injector (septumless head, CO_2 cooling) and an automatic sampler ALS 7683. Gas chromatograph 2: GC 6890 with a split/splitless injector, an on-column injector and an automatic sampler ALS 7683 (all Hewlett-Packard, Palo Alto, CA, USA). Acquired data were reprocessed by HP Chemstation A.06.03 software (Hewlett-Packard). Injector accessories: deactivated glass liners: (1) empty multi-baffle liner (catalog No. HP 5183-2037) for PTV injections, (2) empty double taper liner (catalog No. HP 5183-3315) for splitless injection.

All separations were performed on a fused-silica column coated with 5% phenyl–methylpolysiloxane DB-5 MS (60 m×0.25 mm, 0.25 μm) (J & W Scientific, Folsom, CA, USA) with column effluent split (outlet splitter – catalogue No. HP 0101-0594) in proportion 1:1 between electron-capture (ECD) and nitrogen–phosphorus detection (NPD) systems. Helium was used as a carrier gas.

A new injector liner and new column were installed before each long-term stability experiment.

2.3. Sample preparation

For our experiments matrix standards and blank samples of wheat were prepared. A 25-g amount of residue-free wheat was mixed with 125 ml ethyl acetate and 25 g sodium sulfate and homogenized for 2 min with the Turrax tissumizer. The suspension was filtered under vacuum, the volume of filtrate was reduced by evaporation to 25 ml and made-up with cyclohexane in a 50-ml volumetric flask. This crude extract was purified by HPGPC under the following conditions: mobile phase: cyclohexane–ethyl acetate (1:1, v/v), flow: 1 ml/min, injection volume: 1 ml, collected (''pesticide'') fraction: 14–28 ml, rinsing volume: 2 ml, i.e., total volume of the ''pesticide'' fraction was 16 ml. The collected fraction was evaporated by the rotary evaporator and the residue was dried by a mild stream of nitrogen. Evaporated purified extracts were further dissolved according to the scheme in Table 2.

In the case of the pulsed splitless and on-column injection (experiments 1 and 2), preparation of matrix samples was based on the standard operation procedure used in our laboratory, i.e., the residue after the evaporation of GPC ''pesticide'' fraction was dissolved in 1 ml of standard (matrix standards) or toluene (blank). However, the implementation of the PTV solvent split injection (experiment 3) presumes direct analysis of the collected GPC fraction. Hence, in PTV solvent split experiments, the residue was reconstituted to the original volume of the GPC fraction by dissolving it in 16 ml of the appropriate standard solution or cyclohexane–ethyl acetate mixture (see Table 2). Concentrations of standards were adjusted in order to achieve approximately the same injected amount of pesticides with all compared injection techniques (see Table 3).

2.4. GC conditions

Parameters of four different injection techniques examined in this study are described below (experiments 1–4). Parameters of the PTV injector are

Table 2
Preparation of wheat matrix-matched standards for long-term stability experiments

Sample code	Description of sample	Residue of GPC ''pesticide'' fraction dissolved in[a]
MS1A	Matrix standard, level 1, in toluene	1 ml of STD1A (see Table 1)
MS2A	Matrix standard, level 2, in toluene	1 ml of STD2A (see Table 1)
BLA	Blank in toluene	1 ml of toluene
MS1B	Matrix standard, level 1, in cyclohexane–ethyl acetate (1:1)	16 ml of STD1B (see Table 1)
MS2B	Matrix standards, level 2, in cyclohexane–ethyl acetate (1:1)	16 ml of STD2B (see Table 1)
BLB	Blank in cyclohexane–ethyl acetate (1:1)	16 ml of cyclohexane–ethyl acetate (1:1, v/v)

Table 3
Injected amount of analytes and sample using different injection techniques

Injection technique	Sample code/amount of analyte injected (ng)		Injected volume (μl)	Amount of injected sample (mg)
	Concentration level 1	Concentration level 2		
Experiment 1 – on-column	MS1A 0.06–1.53	MS2A 0.006–0.153	1	1.0
Experiment 2 – pulsed splitless				
Experiment 3 – PTV solvent split	MS1B 0.045–1.15	MS2B 0.0045–0.115	30	1.9

based on the optimization procedure described in our recent paper [31]. Separation conditions were the same for all experiments except for the starting oven temperature:

2.4.1. Experiment 1

On column injection: injection volume: 1 μl; injection temperature: oven track; starting oven temperature: 100°C for 0.1 min.

2.4.2. Experiment 2

Pulsed splitless injection: injection volume: 1 μl; injection temperature: 250°C; pressure pulse: 60 p.s.i. for 2 min; splitless period: 1.9 min; starting oven temperature: 90°C for 2 min (1 p.s.i.=6894.76 Pa).

2.4.3. Experiment 3

PTV solvent split injection: injection volume: 3× 10 μl; injection temperature: 0°C for 4.8 min, 400°C/min to 350°C; vent flow: 70 ml/min; vent pressure: 5 p.s.i.; vent period: 4.8 min; splitless period: 1 min; starting oven temperature: 60°C for 5.8 min

2.4.4. Experiment 4

PTV splitless injection: injection volume: 1 μl; injection temperature: 70°C for 0 min, 400°C/min to 350°C; splitless period: 1 min; starting oven temperature: 90°C for 1 min

Separation conditions: carrier gas flow: constant 2 ml/min (34 cm/s); column temperature: starting oven temperature×min (specific for each injection technique – see above), 25°C/min to 190°C, 2.5°C/min to 225°C, 15°C/min to 280°C for 17 min.

3. Results and discussion

In analysis of real-life samples for each examined technique following questions can be formulated:

(1) How many samples can be injected until contamination of GC system results in unacceptable performance of analysis, in other words system maintenance is necessary (e.g., replacing of liner, cutting the front part of analytical column/replacing retention gap)?

(2) Is it essential to use matrix matched standards for calibration? What is the difference in the extent of matrix effects at the beginning of the sequence and after a number of injections?

Although advantages and applicability of PTV injection technique have been discussed by many authors, data documenting the performance of PTV injector in relation to matrix ''dirt'' present in samples are rather limited. In order to answer the above mentioned questions, we focused on the influence of matrix components both in short-term (matrix-induced response enhancement) and long-term (stability of responses) time scale. PTV performance characteristics were compared with two other injection techniques: (i) on-column injection, as a ''reference'' technique and (ii) pulsed splitless injection, as a technique routinely used in our laboratory for pesticide residue analysis [29]. Twenty-six pesticides possessing wide range of physico–chemical properties were used in our experiments.

In the first part of presented study the performance of four injection techniques: (i) on-column, (ii) pulsed splitless, (iii) PTV solvent split and (iv) PTV splitless (experiments 1–4), was compared for injections of neat solvent standards into ''clean'' GC systems (new separation column, new liner). In the

second part of our experiments the tolerance of three above mentioned injection systems (i–iii, experiments 1–3) to successive contamination by matrix components was compared. For this purpose the sequence comprising repetitive injections of wheat samples was analyzed by each GC system (new injection liner and new separation column were installed prior to each long-term stability sequence).

3.1. Analysis of pesticides in pure solvent standards – "clean" GC systems

Repeated injections ($n=3$) of pesticide standard mix (STD1A in experiments 1–3, STD1B in experiment 4 – see Table 1) were carried out.

To be able to compare data obtained by two different GC instruments, responses of analytes were related to the compound not sensitive to the discrimination[2] in the injection chamber. For ECD detected pesticides the reference compound was lindane, which is a very stable non-polar and non-adsorptive compound. For NPD detected pesticides chlorpyrifos was selected as a reference analyte. This compound is stable, relatively non-polar and, according to our previous experience, it shows negligible discrimination [29].

In Fig. 1 peak areas related to the reference compound together with standard deviations calculated from three repetitive injections are plotted. It is obvious, that responses of some analytes strongly depend on the injection technique used. Thermo labile compounds carbaryl and captan show significant loss with the use of any of vaporizing techniques. Both PTV techniques, however, provide better results for these analytes, when compared to the pulsed splitless injection. Similar observations for thermally unstable pesticides (carbamates, trichlorfon) have been reported by Müller and Stan [4,5]. This fact might be attributed to lower temperature (contrary to isothermal injection), at which transfer of analytes onto GC column occurs. Depending on their volatility, not all analytes are necessarily exposed to the highest injection temperature. Lower volume of PTV liner, resulting in higher linear velocity of carrier gas, as well as fewer active sites on its surface, probably also contribute to better recoveries of troublesome analytes.

PTV injection is generally regarded as a technique avoiding discrimination of high boiling compounds [15,17,32]. Needle distillation, considered as the main cause of low volatiles loss, is in PTV prevented by the injection into the cold inlet. However, in our experiments deltamethrin and other late eluting pesticides, such as cyhalothrin-lambda, were significantly discriminated with all three vaporizing techniques; no improvement of recoveries was recorded with the application of PTV – see Fig. 1. This observation leads us to the conclusion, that low volatile analytes may also be lost by mechanisms other than needle distillation, for example by their retention at imperfectly heated spots in the inlet.

Organophosphates methamidophos, acephate and omethoate represent another group of troublesome analytes. Due to their physico–chemical properties, namely the presence of a polar P=O group in their molecule, they are easily absorbed on the active sites of the GC inlet. As can be seen in Fig. 1, with both pulsed splitless and PTV splitless injection strong discrimination of these three organophosphates occurs. On the other hand, with the PTV solvent split injection loss of the above mentioned analytes is significantly reduced. The superiority of PTV solvent split over PTV splitless injection mode may be explained by substantially higher volume injected with the former technique (30 vs. 1 μl, total amount of injected pesticides is the same). Similar difference in responses of polar/labile pesticides between both PTV modes, were reported by Mol et al. [30], who suggested, that with higher sample volume the injected liquid penetrates lower in the liner packing, which results in faster transfer of analytes onto the analytical column and shortened time for adsorption/degradation processes. Although liner packing was eliminated in our experiments, because of the potential risk of degradation/sorption, and the empty "multi-baffle" liner was employed, the same mechanism may be valid.

[2]Within this paper "discrimination" is defined as a selective loss of sample components in the injector chamber (definition by Ref. [32]). For the purpose of Section 3.1, discrimination is regarded as a response of analyte relatively to its response when using on-column injection.

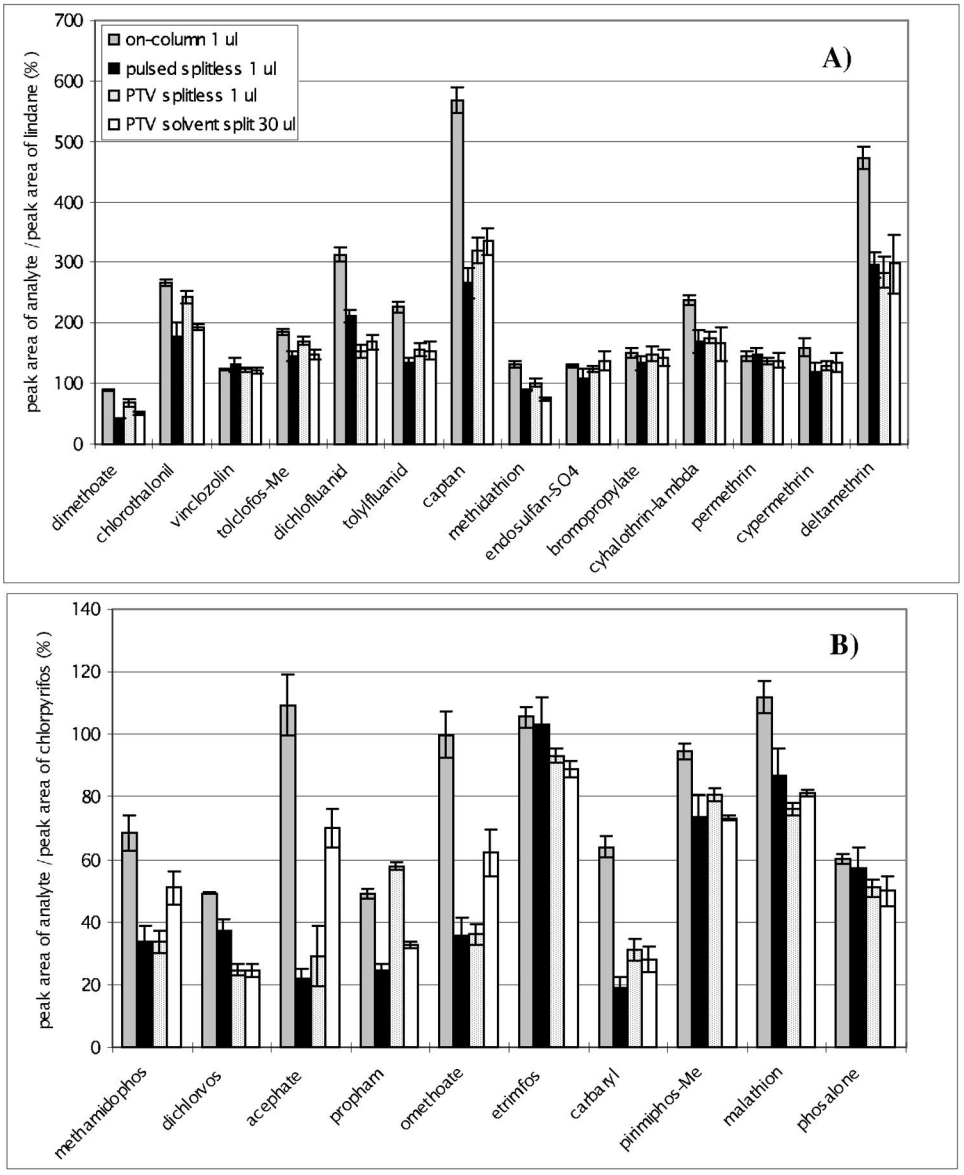

Fig. 1. Mean responses of analytes ($n=3$) obtained in ''clean'' GC systems with different types of injection. Peak areas expressed relatively to the reference compound: (A) ECD detected pesticides – lindane as a reference compound; (B) NPD detected pesticides – chlorpyrifos as a reference compound. Concentration level 1 (STD1A, STD1B).

3.2. Analysis of wheat samples – long-term stability of responses

The long-term stability sequence consisted of repeated sets of 11 samples (see Table 4), each set comprised both matrix standards and pure solvent standards. In this way, we were able to calculate matrix effects in particular stages of system contami-

Table 4
Sequence arrangement for the testing of long-term stability and matrix effects

Sample code		Number of injections
Experiments 1, 2 (on-column, pulsed splitless)	Experiment 3 (PTV solvent split)	
STD1A	STD1B	1
BLA	BLB	1
MS1A	MS1B	3
STD1A	STD1B	1
STD2A	STD2B	1
MS2A	MS2B	3
STD2A	STD2B	1

nation by matrix components. Injections into each GC system were performed until significant deterioration of responses was encountered.

Samples containing target pesticides at two different concentration levels were used in our experiments. The lower level was approximately twofold higher than estimated limits of detection (LODs), while higher level was close to maximum residue limits (MRLs) set in European Union (EU) legislation (see Table 3). Wheat was used as a matrix, since it represents an important crop in terms of food control and its main coextracts – waxes – are important matrix components also in other plant materials. From this aspect, data obtained in this experiment can be generalized to some extent.

3.2.1. Long-term stability of responses

In Figs. 2–4 typical chromatograms, obtained by NPD, in different stages of long-term stability sequence are shown. Responses of three compounds, representing (i) polar organophosphorus pesticides – methamidophos, (ii) thermolabile compounds – car-

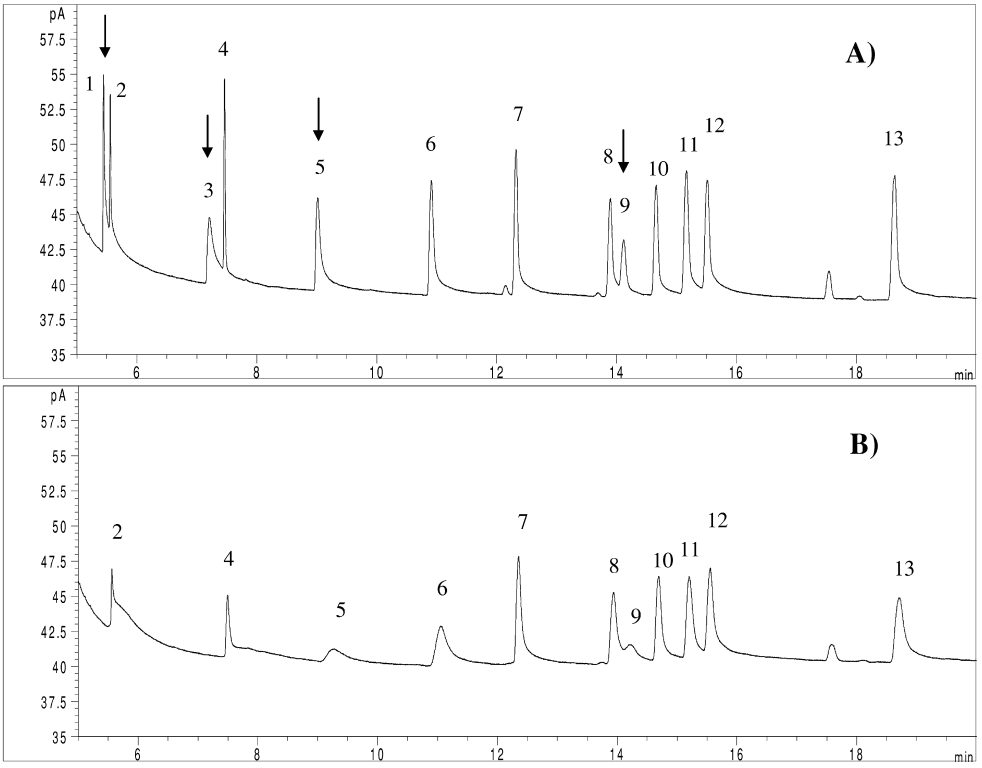

Fig. 2. Long-term stability of the GC system using the on-column injection – the chromatograms (NPD signal) of 1 μl injection of standard in toluene at concentration level 1 (STD1A) after: (A) 10 injections of wheat samples; (B) 31 injections of wheat samples. Peaks of troublesome analytes are marked by arrows. Analytes: 1=methamidophos, 2=dichlorvos, 3=acephate, 4=propham, 5=omethoate, 6=dimethoate, 7=etrimfos, 8=tolclofos-Me, 9=carbaryl, 10=pirimiphos-Me, 11=malathion, 12=chlorpyrifos, 13=methidathion.

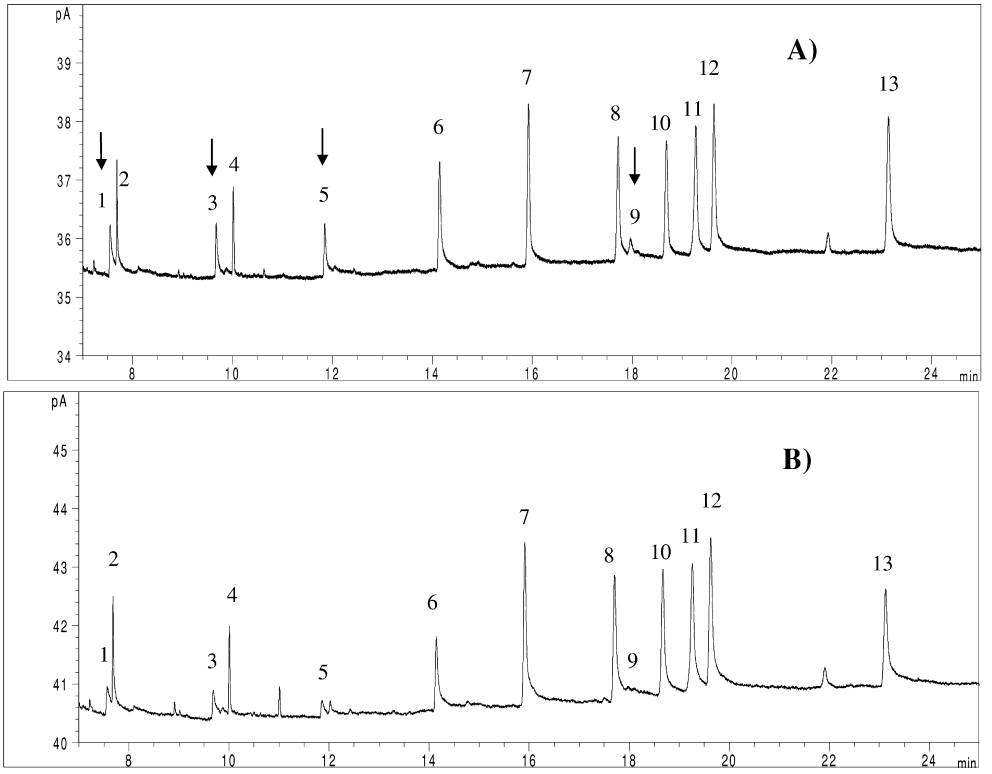

Fig. 3. Long-term stability of the GC system using the pulsed splitless injection – chromatograms (NPD signal) of 1 μl injection of standard in toluene at concentration level 2 (STD2A) after: (A) 10 injections of wheat samples; (B) 87 injections of wheat samples. Peaks of troublesome analytes are marked by arrows. Analytes: see Fig. 2.

baryl, (iii) stable, non polar pesticides – propham, within the analyzed sequence are plotted in Fig. 5. In this experiment, the peak heights were used for data reprocessing, because this parameter is crucial in terms of analyte detectability. As in previous experiment, different instruments were used and responses were therefore expressed relatively to the reference compound (chlorpyrifos). The *x*-axis in the graphs represents the number of matrix samples injected to the GC system prior to the current sample.

Using the on-column technique, peaks of sensitive analytes (polar organophosphates, carbaryl) are extremely distorted or even not detectable after 14 injections of wheat samples (see Fig. 2). The considerable drop of analyte responses after a few injections of matrix samples, when using on-column injection, is also obvious from Fig. 5. These observations confirm the fact that superiority of on-column injection holds exclusively for pure solvent standards and this technique cannot be reliably used for the analysis of larger series of complex samples.

On the other hand, GC systems employing both pulsed splitless and PTV solvent split injection showed very good tolerance to matrix components load. With the pulsed splitless injection approximately 90 matrix samples could be injected, before peak of carbaryl became undetectable (see Fig. 3). However, using the PTV solvent split injection, carbaryl could be reliably quantified even after 136 injections (see Fig. 4). The excellence of PTV was also proved by the stability of peak shape of methamidophos, acephate and omethoate. Peaks of these pesticides are prone to tailing and the severe deterioration of their shape is a good indication of the need for the system maintenance (replacing the liner, cutting the front part of separation column). With PTV their reliable quantitation was possible even after 136 injections of wheat samples, while these analytes

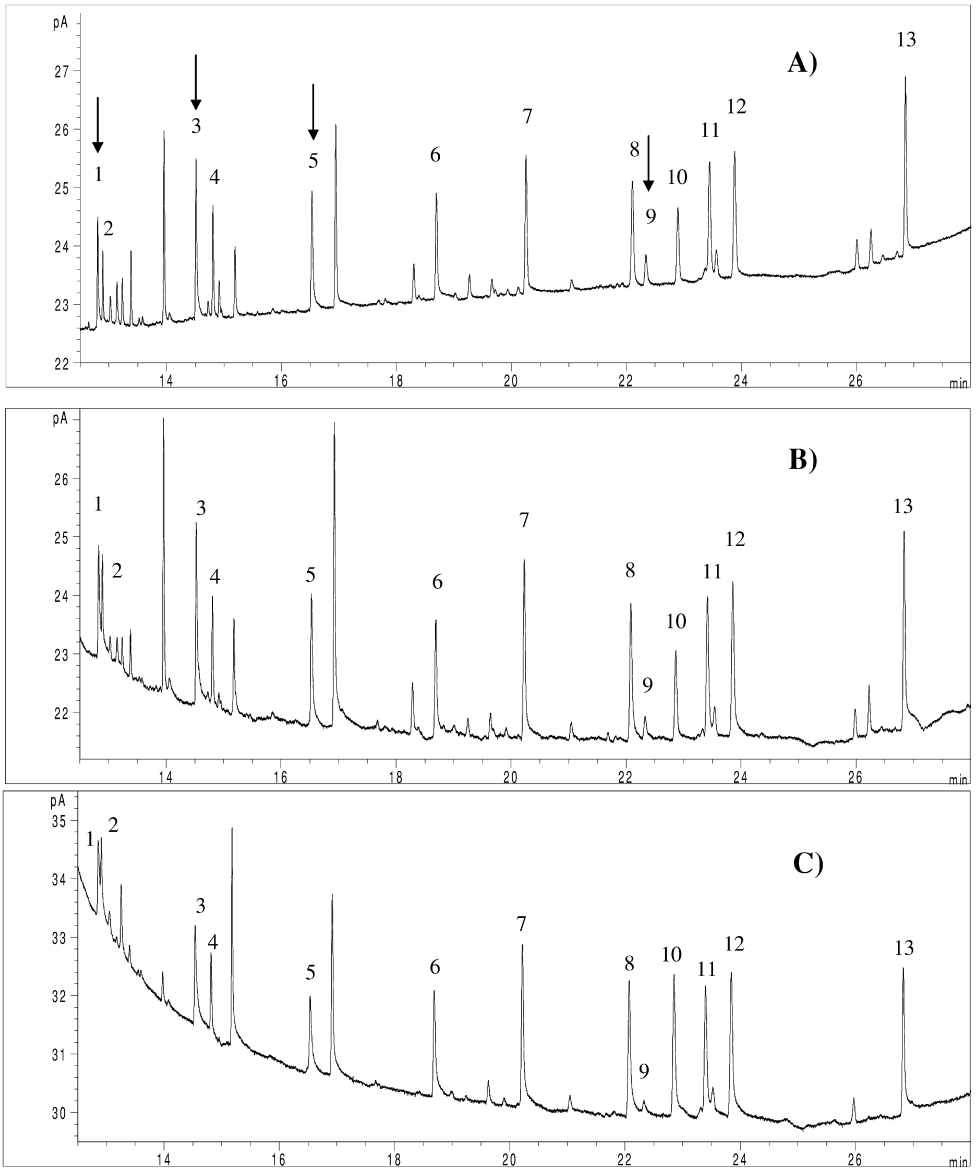

Fig. 4. Long-term stability of the GC system using the PTV solvent split injection – chromatograms (NPD signal) of 30 μl of standard in cyclohexane–ethyl acetate at concentration level 2 (STD2B) after: (A) 10 injections of wheat samples; (B) 87 injections of wheat samples; (C) 136 injections of wheat samples. Peaks of troublesome analytes are marked by arrows. Analytes: see Fig. 2.

were hardly detected after 87 pulsed splitless injections. The excellence of the PTV solvent split system is even more convincing if we consider the fact, that the amount of matrix per injection was almost twofold compared to the pulsed splitless system (see Table 3).

As mentioned above, only few studies focusing on the same problem have been published. Müller and Stan [6] realized long-term stability study for four organophosphorus and carbamate pesticides in spinach samples, comparing the performance of PTV splitless, hot-splitless and on-column injection. Grob et al. [33] examined the long-term stability of the PTV splitless and hot splitless injection systems,

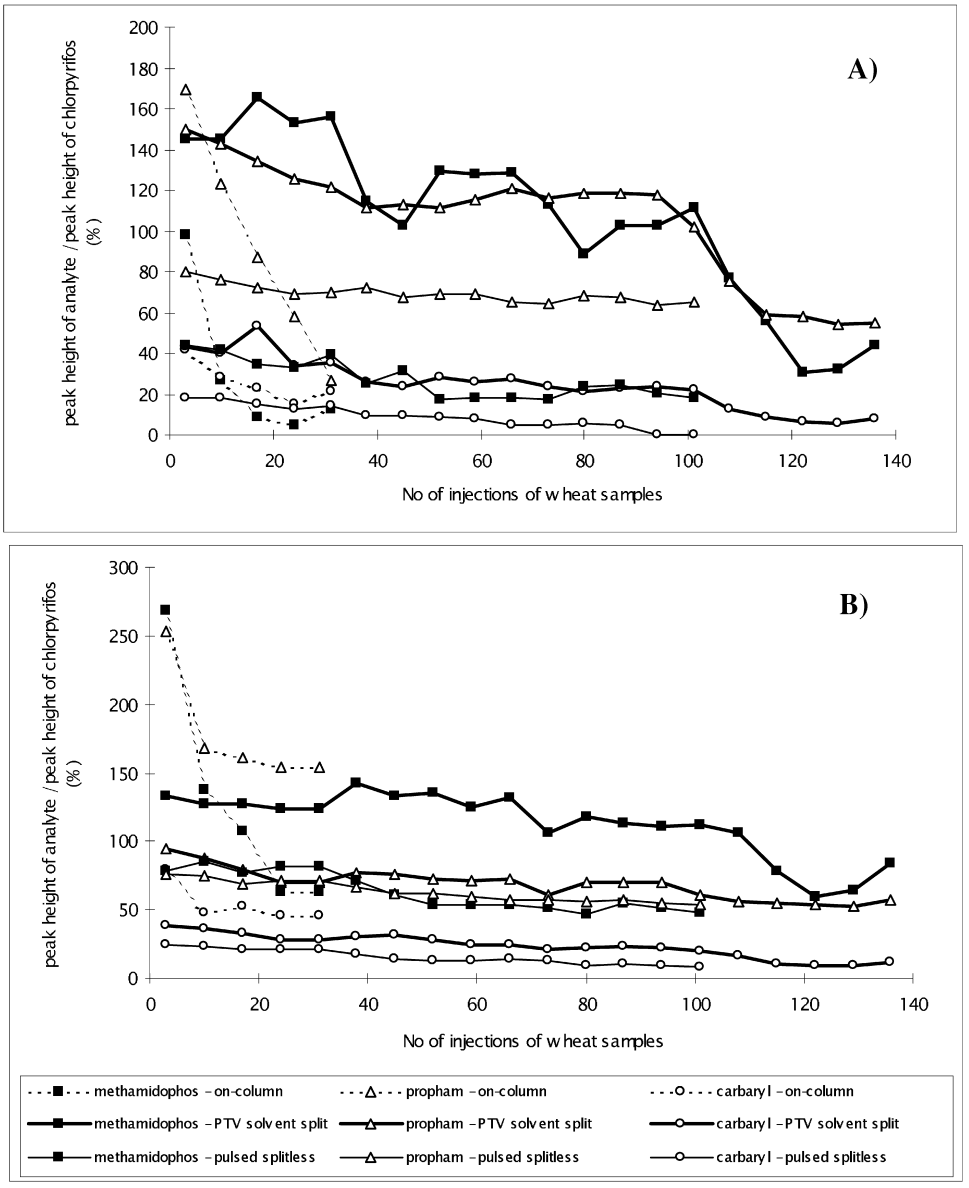

Fig. 5. Long-term stability of GC–NPD systems with different types of injection. Peak heights related to chlorpyrifos. Concentration level 2. (A) Standards in pure solvent (STD2A, STD2B); (B) matrix standards (MS2A, MS2B).

employing *n*-alkanes as analytes and methyl polysiloxane (DC-200) or olive oil as the ''dirt''. Although none of the above mentioned studies employed the same experimental set-up as presented here (different injection modes, broader spectrum of compounds involved in our study), our results are in good agreement with results of these authors. Both PTV splitless and solvent split injection techniques provide superior stability and protection of GC column from matrix components.

The reason for this is, that using PTV, non-volatile matrix components are more effectively retained in the inlet, i.e., the extent of the contamination of the GC column is lower than with isothermal (hot)

splitless injectors. This can be attributed to the way of sample evaporation with both techniques. While in isothermal injectors, evaporation occurs in the gas phase (sample components are evaporated from the droplets of aerosol formed at the tip of the syringe needle), in PTV injectors sample is first deposited at the liner wall and subsequently sample components are evaporated according to their boiling points. In this way, the risk, that high boiling ''dirt'' is swept onto the separation column, is lower.

3.2.2. Matrix-induced response enhancement

Fig. 6 shows relative responses of selected analytes, calculated as average response of three con-

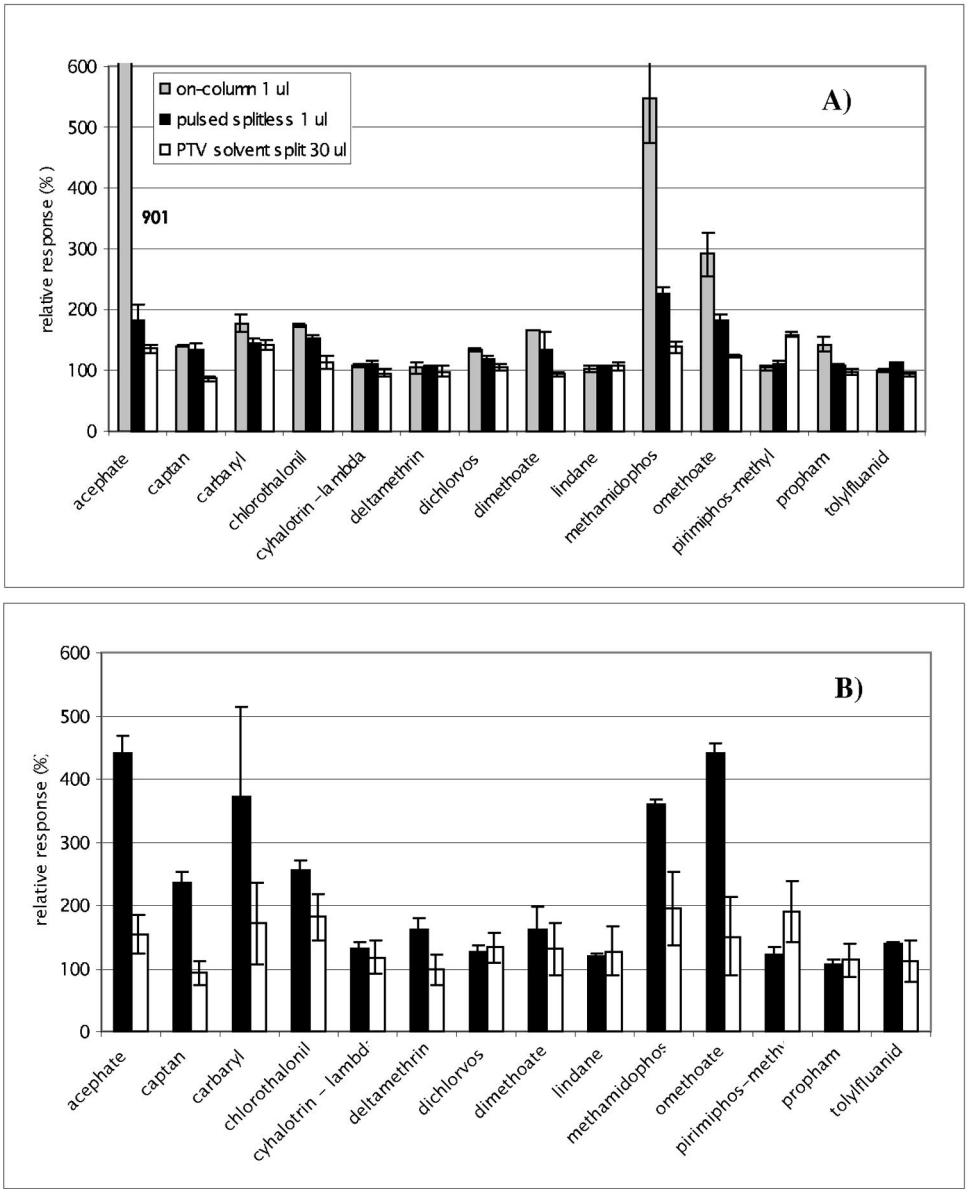

Fig. 6. Relative responses of analytes in GC systems employing different injection techniques. Concentration level 2 (STD2A, STD2B). (A) Injection into ''clean'' GC system (after 10 injections of wheat samples); (B) injection into ''dirty'' GC system (after 66 injections of wheat samples).

sequent matrix standards divided by average response of two surrounding solvent standards×100% – see Table 4. Matrix effects are illustrated for the injection into (A) ''clean'' GC system (after 10 matrix samples) and (B) ''dirty'' system (after 66 injections). Results are presented for the lower concentration level (level 2), where matrix effect (according to expectation) were more distinct.

Critical assessment of generated data can be summarized as follows:

(1) The extent of matrix effects is increased considerably with successive contamination of GC system.

As shown in Fig. 6, matrix effects are more intensive in the contaminated GC systems in comparison with ''clean'' systems with all injection techniques employed. As matrix components build-up in the injector, new active sites are formed. Accordingly, adsorption and/or degradation of analytes increases. At the same time, matrix components present in the injected sample are able to fully protect analytes from these spots. This phenomenon is also documented in Fig. 5, where responses of matrix samples are more stable than those of neat standards with all injection techniques involved (this is particularly pronounced for the on-column injection).

Generally, the above mentioned effects are most significant for troublesome compounds, already discussed in the previous sections (methamidophos, acephate, omethoate, carbaryl, captan).

(2) Matrix effects can be encountered even with on-column injection.

It has been demonstrated, that matrix effects are not exclusively connected with the injection chamber. From Fig. 6 it can be seen, that strong matrix-induced chromatographic response enhancement occurs using the on-column injection. It has been observed, that in contaminated GC system relative responses can exceed 1000% (e.g., 1500% for methamidophos after 17 wheat samples).

(3) Application of PTV results in suppression of matrix effects.

With the PTV solvent split injection technique matrix effects were reduced in comparison with the pulsed splitless injection. With the latter technique relative responses reached 450% after 66 injections of wheat samples, whereas using PTV, matrix effects did not exceed 200% in any case.

4. Conclusions

The use of the PTV injection technique was proven to be a suitable way of increasing the performance of the GC system. With PTV in the solvent split mode, the injection volume can be increased by a factor of 30 in comparison with common splitless techniques. Analytical process can be thus simplified by removing the evaporation step after clean-up procedure and limits of detection of current method can be decreased. Moreover, important characteristics of GC system influencing accuracy of generated data, such as matrix-induced chromatographic response enhancement or long-term stability of analytes responses on repetitive injections of real-life samples, can be improved dramatically. This is especially desirable, if troublesome analytes such as polar organophosphates or unstable compounds are to be determined. In this study, the superiority of the PTV solvent split technique over isothermal injection was demonstrated for a very broad spectrum of compounds of different physico–chemical properties.

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