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Journal of Chromatography A, 727 (1996) 275–289

JOURNAL OF CHROMATOGRAPHY A

Large-volume injection in residue analysis with capillary gas chromatography using a conventional autosampler and injection by programmed-temperature vaporization with solvent venting

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Received 15 May 1995; revised 10 October 1995; accepted 10 October 1995

Abstract

Large-volume injection in capillary gas chromatography may be used to compensate for the limited detection sensitivity of selective detectors when further evaporation of sample extracts is not feasible owing to increasing losses of volatile solutes or because the analytical method can no longer be performed automatically. Large-volume injection using programmed-temperature vaporization (PTV) with solvent venting has proved its worth as an on-line pre-chromatographic sample concentration technique that permits the reproducible analysis of pesticide residues over a wide volatility range.

Automated sampling and injection of a 12.5-μl volume of toluene extract using an HP 7673A autosampler with a 25-μl Hamilton syringe is described. The various parameters, including design of injector inlet, speed of injection, retention gap and initial column temperature, are discussed and the optimization procedure for split flow-rate, solvent evaporation temperature, solvent venting time and splitless or transfer time is reported. A test mixture containing C_{12}, C_{14} and C_{16} hydrocarbons, 1,7-dibromoheptane, 3,4-dichloroaniline, pentachlorobenzene, hexachlorobenzene and the pesticides heptenophos, propachlor, naled, lindane, heptachlor, aldrin, tetrachlorvinphos, dieldrin, *p,p'*-DDE and endrin in toluene was used, representing moderately volatile to high-boiling solutes and thermolabile compounds. Complete recovery of all compounds in the test mixture was achieved with the method developed. No degradation of the thermolabile pesticides naled and endrin was observed.

The precisions of quantitative determinations of 12.5-μl autosampler injections at different concentration levels were good to excellent down to concentration levels representing 1–2.5 ng per pesticide injected when applying atomic emission detection (AED). Data on standard deviations and the wide dynamic range of all test solutes are reported.

Keywords: Large-volume injection; Injection methods; Programmed-temperature vaporizer; Hydrocarbons; Pesticides

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0021-9673/96/$15.00
SSDI 0021-9673(95)01171-4

1. Introduction

Analyte detectability in trace analysis depends on the concentration factor of the extraction and clean-up procedure and the portion of the final extract that can be introduced into the gas chromatographic system. Pesticide residue analysis in food samples requires determination of the target compounds down to concentration levels of 0.01 ppm in order to be within the maximum residue tolerance levels. Screening analysis has been successfully performed for two decades in our laboratory by using the Deutsche Forschungsgemeinschaft (DFG) multi-residue method S 19 with the combination of GC with electron-capture detection (ECD) and nitrogen–phosphorus detection (NPD) with effluent splitting and more recently also with GC with atomic emission detection (AED). Both methods were conducted in parallel with more than 200 food samples and a comparison of the results has previously been reported together with a few chromatograms of food samples containing pesticide residues [1]. GC–AED was clearly more suitable for so-called "problem foodstuffs". On account of its higher selectivity and its wide linear dynamic range down to the lowest detectable concentrations, GC–AED produced more reliable results [2]. An increase in the sample extract volume to 200 μl (equivalent to 50 mg/μl of food sample) and a 2-μl injection volume using hot splitless injection makes sensitive detection possible [1,2].

At trace level concentrations between 0.01 and 0.05 ppm, many pesticides could, however, only be identified on one heteroelement trace, because the sensitivity of AED differs for different heteroelements. For this reason, an increase in detection sensitivity, especially for the nitrogen trace which is very important in pesticide analysis, was highly desirable in order to take advantage of complete information about the elemental composition of the analytes in the chromatogram. As a consequence of its high selectivity towards carbon, larger amounts of matrix compounds do not impair the detection of the various heteroelements in the specified AED traces when injecting large sample volumes using programmed-temperature vaporization (PTV) injection. This has been verified with the analysis of "problem foodstuffs" using classical hot splitless injection [1,2].

Several techniques for the introduction of larger volumes are available, of which on-column injection and large-volume injection with PTV with a "solvent split" are best developed. Recent contributions to this field have been frequent, variables affecting the sampling process have been extensively discussed [3–7].

The on-column injection technique is superior to other injection techniques with respect to reproducibility [8,9]. Moreover, this technique is the best for the introduction of thermolabile substances. However, frequent analysis of samples with higher contents of matrix compounds contaminates the column inlet and decreases the column efficiency, resulting in poor long-term stability [10–12]. Already in 1979 Vogt and coworkers [13,14] described a new injector that allowed the injection of up to 250 μl into a cold glass insert filled with glass-wool. During injection, the solvent was vented through the open split exit, then the split valve was closed and the injector flash heated at up to 30°C/s in order to transfer the analytes to the analytical column.

An additional advantage of this technique is that with cold sample introduction followed by temperature-programmed sample transfer, discrimination of high-boiling compounds is virtually absent and the quantitative performance is much better than that of hot splitless injection [15–17]. Low-volatile steroids, polycyclic aromatic hydrocarbons and polychlorinated biphenyls were reliably determined while losses of volatile compounds were observed [15,17–20]. These losses could be reduced by optimization of the injection parameters, the right choice of glass insert type and the use of adsorbents as trapping materials [21,22]. The precision of quantification with PTV injection in the solvent split mode is comparable to that with on-column injection [17,23,24].

Analysing organophosphorus insecticides, Stan and Müller [22] demonstrated that injection of thermolabile compounds could be achieved without significant losses when using the PTV injec-

tor. However, thermodegradation of thermolabile pesticides could only be reduced when glass inserts without a glass-wool filling or other packing materials were used [25]. Moreover, PTV compared with on-column injection retains more high-boiling matrix compounds, so that contamination of the analytical column is reduced [25–27].

PTV large-volume injection with solvent split was successfully applied to organophosphates in order to overcome the low detection sensitivity of flame photometric detection. Organophosphorus pesticides could be reproducibly determined in extracts from vegetables with manual injection of sample volumes as large as 50 μl into a PTV injector applying solvent venting. Surprisingly, losses of the most volatile organophosphate, dichlorvos, were hardly detectable although toluene was used as the solvent. A good GC resolution without any peak distortion was only achieved by using a retention gap between the injector and the analytical column [28].

For reproducible PTV injection in the solvent split mode of large sample volumes containing analytes with a wide range of volatility, the following parameters are of great importance: design of glass insert, choice of adsorbent material, choice of solvent, injection speed, solvent evaporation temperature (initial injector temperature), solvent venting time, split flow-rate, splitless time/transfer time and evaporation temperature for the solutes (final injector temperature).

The individual steps of a PTV process in the solvent split mode together with the typical temperature profile are shown in Fig. 1.

When designing a method for screening analysis, not all the parameters can be freely varied. In this paper, we report an automated injection of 12.5 μl into PTV injector operating in the solvent split mode using a conventional syringe autosampler with a 25-μl syringe.

2. Experimental

2.1. Gas chromatography

GC analyses were performed with a Hewlett-Packard (HP) Model 5890A Series II gas chromatograph equipped with an HP 7673A autosampler and HP 5921A atomic emission detector. The chromatograph was fitted with a 25 m × 0.32 mm I.D. fused-silica capillary column coated with a 0.17-μm film of HP-5 (SE-54). As precolumn, a 5 m × 0.32 mm I.D. retention gap

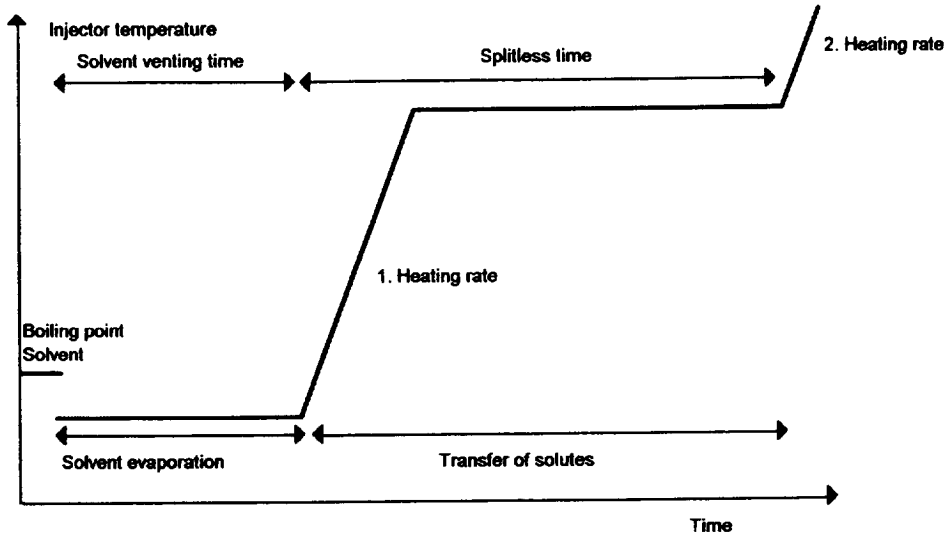

Fig. 1. Programmed-temperature vaporization.

deactivated with phenylsilicone was used. The column temperature was held at 50°C for 2 min after injection, then programmed at 25°C/min to 150°C, which was held for 2.5 min, then at 3°C/min to 205°C and finally at 10°C/min to 250°C, which was held for 10 min. Helium of 99.999% purity was used as the carrier gas.

2.2. Programmed-temperature vaporization

PTV injection was performed with as KAS 3 inlet (Gerstel, Mühlheim, Germany). The KAS 3 inlet was equipped with a 92 mm × 1.3 mm I.D. deactivated empty glass liner with baffles. Volumes of 12.5 μl were injected with an autosampler equipped with a 25-μl syringe. The injector starting temperature was 40°C. The column head pressure was adjusted to $6.2 \cdot 10^4$ Pa and the flow-rate through the split vent to 40 ml/min. After 40 s, the split valve was closed and the liner was flash heated at 12°C/s to 260°C, which was held for 1 min. The split valve was then opened and the liner was further heated at 12°C/s to 300°C, which was held for 1 min.

2.3. Atomic emission detection

The transfer line to the detector and the detector cavity were operated at 240 and 300°C, respectively. The spectrometer was purged with nitrogen at 2 l/min and the window with helium at 30 ml/min. Helium at 30 ml/min was used as make-up gas. The reagent gases were hydrogen at $2.1 \cdot 10^5$ Pa and oxygen at $1.4 \cdot 10^5$ Pa; for oxygen measurement, 10% CH_4 in N_2 at $3.5 \cdot 10^5$ Pa was used. The cooling water temperature was 63°C. Data were processed with a HP AED-Chemstation 5895A.

2.4. Materials

All solvents were Pestanal products from Riedel-de Haën (Seelze, Germany) and all analytical standards were supplied by Promochem (Wesel, Germany).

3. Results and discussion

3.1. Selection of injection parameters held constant during the optimization process

3.1.1. Design of glass insert, choice of solvent and possible packing

The design of the injector glass insert is of major importance in capillary GC and was extensively discussed by Grob [29]. Inserts consist of glass tubes of various internal diameters to be used empty or packed with glass-wool or other adsorbent materials. Glass-wool is not suitable for analytes prone to degradation owing to its large adsorptive surface [21,25,30,31]. Adsorbents used in thermotrapping, such as Tenax TA and Thermotrap TA, need careful checking with the analytes with respect to degradation [5]. In pesticide residue analysis, a glass insert with baffles is popular with hot splitless injection. This type of insert has also been successfully employed with manual injection of sample volumes as large as 50 μl into a PTV injector applying solvent venting [28]. Organophosphorus pesticides could be reproducibly determined in extracts from vegetables by means of flame photometric detection with recoveries and reproducibilities comparable to those with on-column injection and losses of the most volatile organophosphate dichlorvos were hardly detectable although toluene was used as solvent.

Our group has used toluene successfully as the final solvent in multi-residue analysis for about two decades. It has also proved suitable for manual large-volume injection. There was no reason, therefore, to experiment with different solvents. Recent systematic studies on injector design and the use of adsorbents as insert packings reported reduced losses of volatile solutes when using Tenax TA with rapid large-volume injection [5]. In preliminary experiments, the suitability of Tenax TA (25–35 mesh) as an injector packing was studied. The desorption of low-boiling pesticides such as tetrachlorvinphos, dieldrin, *p,p'*-DDE and endrin required desorption temperatures of more than 260°C to achieve complete solute transfer in an acceptable time. Under these conditions naled, an organophos-

phate of moderate thermostability, was found to be almost quantitatively degraded to dichlorvos by loss of two bromines. Endrin, frequently used as an indicator for polar sites in the GC system, was transformed partially into endrin aldehyde and endrin ketone. Since a variety of active ingredients representing many different chemical classes have to be analysed in multi-residue methods, it must be considered that losses of other pesticides may also occur with the use of adsorbents as packings. This is in accordance with earlier observations with thermolabile pesticides [25].

As a consequence of the preliminary results, no further investigation of packing materials was undertaken and naled and endrin were included in the test mixture formulated for the optimization process as indicators of thermal burden and the possible formation of adsorptive sites.

3.1.2. Injection volume and speed of injection

Since the use of a commercial autosampler was a main prerequisite for automated operation of the whole system (PTV injection, and GC–AED), all PTV parameters were optimized with regard to the autosampler (HP 7673A), which usually operates with a 10-μl syringe (Hamilton). The largest volume that can be injected is given by the largest syringe that can be mounted, which is a 25-μl volume syringe with a maximum injection volume of 12.5 μl. With the autosampler used here, the injection speed is very high and not adjustable. Therefore, the injection was a so-called "at-once injection" typical of manual operation and especially with the HP autosampler which was designed for extremely rapid injection.

Optimization was carried out throughout a study to obtain the best results for the injection of 12.5-μl sample volumes. The chosen column head pressure was not varied during sample introduction.

3.2. Initial column temperature and retention gap

The column temperature during transfer of the solutes is of great importance for the peak shapes over the whole GC elution range. Injection distinctly below the solvent boiling point leads to solvent recondensation at the beginning of the column, so that migration of the starting band can be avoided ("solvent trapping"). However, recondensation of large solvent volumes causes peak distortion which Grob called "peaks splitting in space" [32]. This peak distortion can be observed with analytes of moderate and low volatility when they reach the capillary column together with the solvent. A good GC resolution without any peak distortion was achieved with manual large-volume injection up to 50 μl, but only by using a retention gap in front of the analytical column. Without using the retention gap, peak distortion was unavoidable. An increase in the initial temperature of the analytical column reduced "peak splitting in space" with the low-boiling pesticides whereas a decrease in the initial temperature resulted in increased "peak splitting in time" with the volatile organophosphates. Peak distortion was found to be completely eliminated after the installation of the retention gap [28]. "Peak splitting in space" can be prevented by using a retention gap that is long enough to take up the recondensed solvent. In this study, a 5 m $\times$ 0.32 mm I.D. retention gap deactivated with a thin film of phenylsilicone phase was used in front of the analytical column. The length of the retention gap was certainly oversize according to our experience with manual large-volume injection [28] and was not subject to variation because of the good peak shape obtained throughout the study.

An initial column temperature of 50°C during solute transfer was found to give good peak shapes for all peaks in preliminary studies; the value differs from that used in the earlier manual large-volume injection, where 100°C was found to be optimum with the same solvent but larger injection volumes and a different instrumental set-up [28]. In multi-residue analysis with classical hot-splitless injection, 100°C is also applied as the initial column temperature using toluene and the same type of column, resulting in a narrow initial band width and consequently a good peak shape with all pesticides without having to use a retention gap [1,2].

3.3. Parameters to be optimized

Since the injection procedure described in this paper is based on good results obtained with an earlier manual large-volume injection method, it was known that the application of a baffled glass insert together with a retention gap and toluene as solvent had already proved itself, giving reliable results even with the most volatile pesticides under investigation.

The most important parameter to be considered is certainly the evaporation temperature of the solvent (venting temperature). Large-volume injection with solvent venting is a procedure for preconcentration of the sample solution in the injector. The solvent should be evaporated without loss of analytes. All the evaporating solvent can be vented before entering the analytical column as long as no analytes are mixed with the solvent vapour. The success of the separation of the solvent from the most volatile solutes in the injector depends on solvent evaporation temperature, solvent evaporation time (venting time) and the split flow-rate. This critical point must be experimentally adjusted by means of a mixture of analytes representing the whole range of volatility, varying the parameter settings one after another. The recovery of the analytes can easily be monitored using the peak areas of the various analytes. The composition of the mixture of the test analytes is given in Table 1. The mixture was composed of volatile stable hydrocarbons, volatile pesticides and environmental pollutants and less volatile pesticides. Endrin and naled were included as indicator substances for active sites in the GC system and thermodegradation, respectively.

The following parameters were finally varied to find the optimum conditions: split flow-rate, solvent evaporation temperature, solvent venting time and splitless time/transfer time.

3.4. Optimization of the split flow-rate

The split flow-rate was varied with a fixed evaporation temperature of 40°C and solvent venting time of 40 s; all parameters are given in Table 2.

Table 1
Composition of the test mixture used for the optimization process

Substance	Formula	Element trace
Dodecane	$C_{12}H_{26}$	C
Tetradecane	$C_{14}H_{30}$	C
1,7-Dibromoheptane	$C_7H_{14}Br_2$	Br
3,4-Dichloroaniline	$C_6H_5Cl_2N$	Cl
Pentachlorobenzene	C_6HCl_5	Cl
Heptenophos	$C_9H_{12}ClO_4P$	Cl
Hexadecane	$C_{16}H_{34}$	C
Propachlor	$C_{11}H_{14}ClNO$	Cl
Naled	$C_4H_7Br_2Cl_2O_4P$	Br
Hexachlorobenzene	C_6Cl_6	Cl
Lindane	$C_6H_6Cl_6$	Cl
Heptachlor	$C_{10}H_5C_{17}$	Cl
Aldrin	$C_{12}H_8Cl_6$	Cl
Tetrachlorvinphos	$C_{10}H_9Cl_4O_4P$	Cl
Dieldrin	$C_{12}H_8Cl_6O$	Cl
p,p'-DDE	$C_{14}H_8Cl_4$	Cl
Endrin	$C_{12}H_8Cl_6O$	Cl

The dependence of the recoveries of the analytes on the split flow-rate is presented in Fig. 2 as graphs of peak area against split flow-rate. Fig. 2 is presented in two parts to make the graphs clear. As can be seen, the split flow-rate can be increased up to 100 ml/min without losses of analytes being observed, with the exception of the most volatile analyte dodecane. At 40 ml/min even this relative volatile analyte was found to be completely recovered.

3.5. Optimization of the solvent evaporation temperature

A very critical parameter is the evaporation temperature. To simplify the method, the injector was operated without external cooling. The peak areas measured at various initial injector temperatures are reported in Fig. 3, which is also in two parts for clarity. With a fixed solvent venting time of 40 s and a flow-rate of 40 ml/min, considerable losses of solutes were observed at 50°C and higher temperatures but an almost complete recovery of all test compounds was found at 40°C. This holds true even for dodecane and the relatively volatile pesticide lindane, a pesticide known to be prone to losses

Table 2
Parameter settings for the optimization of the split flow-rate

PTV	*Temperature programme of analytical column*
Solvent evaporation temperature: 40°C	50°C hold for 2.0 min, 30°C/min to
Solvent venting time: 40 s	150°C holdm for 2 min, 3°C/min to 205°C,
Splitless time: 80 s	10°C/min to 250°C, hold for 10 min
1st heating rate: 12°C/s	
1st plateau temperature: 260°C	Injection volume: 12.5 μl
1st hold time: 60 s	Split flow-rate: variable
2nd heating rate 12°C/s	
2nd plateau temperature: 300°C	
2nd holding time: 60 s	

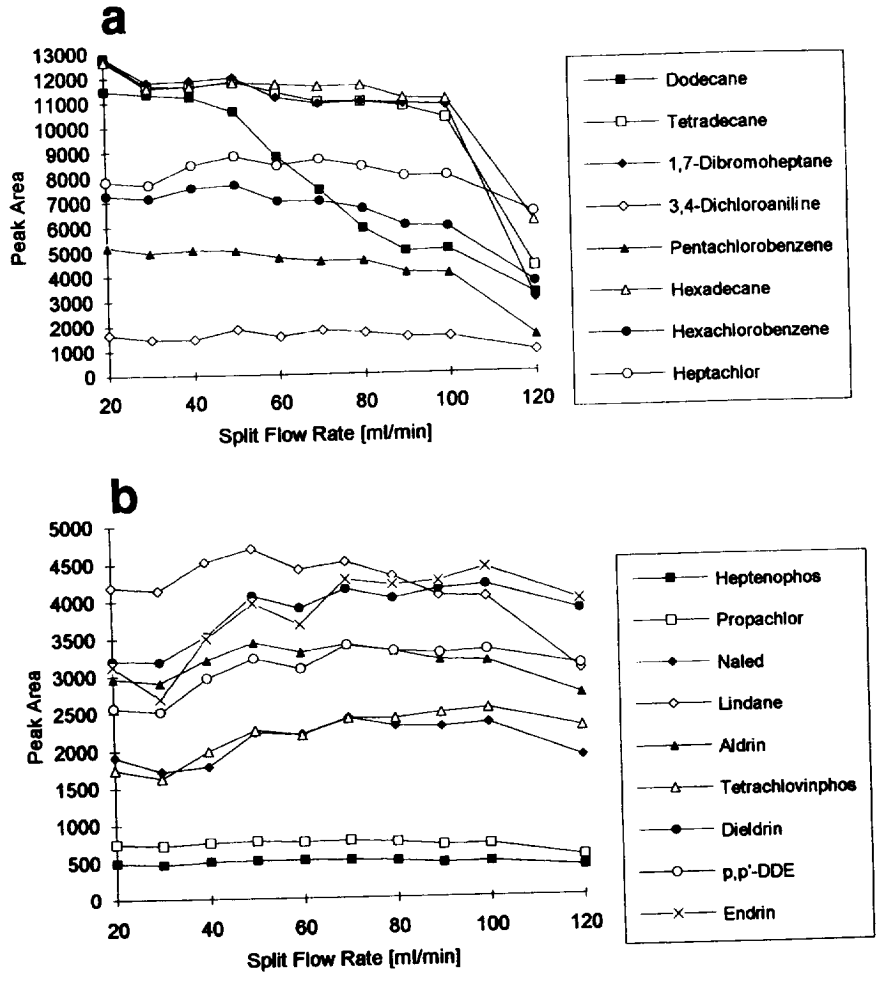

Fig. 2. Dependence of analyte recovery on the split flow-rate.

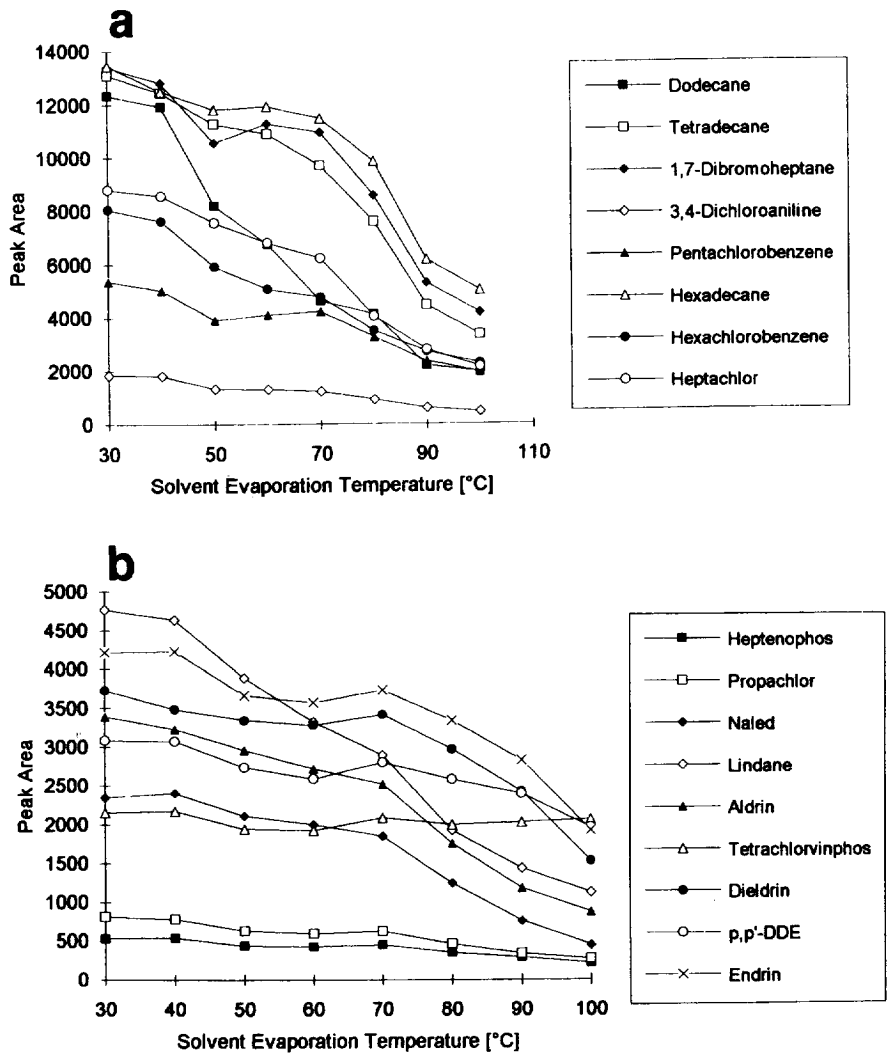

Fig. 3. Dependence of analyte recovery on the solvent evaporation temperature.

even with common evaporation procedures used for concentrating sample extracts.

3.6. Optimization of the solvent venting time

The solvent venting time was set to 40 s for the first two optimization steps, and in the next step this parameter was varied. The probability of loss of analytes increases with decreasing solvent concentration in the injector owing to their increasing partial gas pressures. The venting time was raised in equal steps from 10 to 90 s with the other parameter left constant at its established optimum value: a solvent evaporation temperature of 40°C and a split flow-rate of 40 ml/min. Fig. 4 shows that all the volatile analytes were completely recovered for venting times up to 40 s and only dodecane was lost starting with the split vent open for 50 s. All other analytes were found to be recovered with only small losses up to 70 s, demonstrating a remarkable ruggedness of the

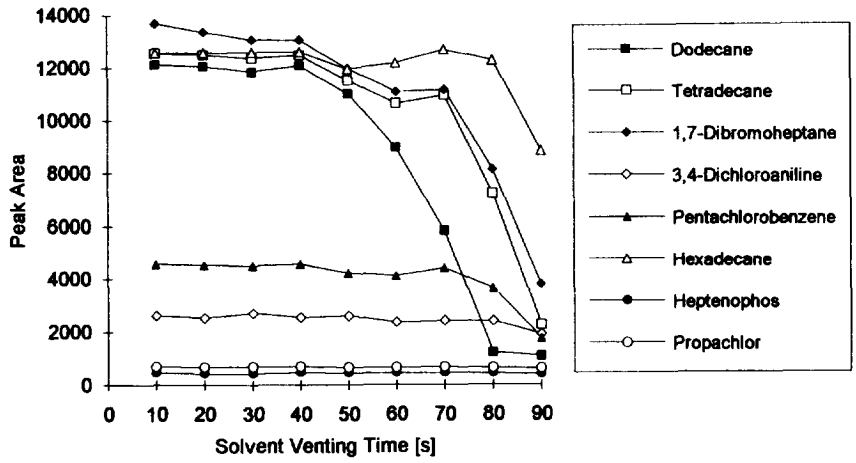

Fig. 4. Dependence of analyte recovery on the solvent venting time.

method which is favourable for automated routine analysis.

3.7. Optimization of the splitless time or transfer time

The last parameter to check was the time necessary for complete transfer of the analytes from the heated injector together with the remaining solvent via the retention gap to the column head. The transfer time is simply the time the split valve is closed after initiating the flash heating of the injector which evaporates the solutes and transfers them to the analytical column as shown in Fig. 1. This time was found not to be critical; the transfer was complete after 30 s with the injector heating programme applied. However, the splitless time was set at 80 s throughout the method in order to ensure the recovery of all highly volatile analytes even in the presence of any food matrix that might contribute to retardation of the evaporation process in the injector.

As a result of the optimization experiments for the injection procedure with solvent venting, a solvent evaporation temperature of 40°C, together with a split flow-rate of 40 ml/min and a solvent venting time of 40 s, was found to operate well with toluene. It is crucial that each of these three interactive parameters be carefully adjusted with respect to the other two; the interdependence of evaporation temperature and split flow-rate is of greatest importance. These two parameters must be mutually balanced after changing the instrumental design and solvent. All other settings of the optimized large volume injection procedure are given in Table 3.

The large-volume injection procedure as described has been used in routine pesticide residue analysis with AED for more than 2 years, and a comparison with a traditional GC–ECD/NPD method has already been published [1]. Therefore, in this paper only one chromatogram is shown to demonstrate the large-volume injection with the test mixture used in the optimization procedure. In Fig. 5, only three element traces are shown. Note the excellent peak shape of all analytes throughout the chromatogram. This is the result of band concentration at the starting area in the analytical column by a good operating solvent effect.

As mentioned above, the test mixture contained volatile compounds in addition to some prone to degradation due to thermolability (naled) and to polar sites in the chromatographic system (endrin). This mixture has also been used to investigate the reliability of quantitative determination with this method.

Table 3
Optimum settings for PTV injection with solvent venting

PTV	*Temperature programme of analytical column*
Solvent evaporation temperature: 40°C	50°C hold for 2.0 min, 30°C/min to
Solvent venting time: 40 s	150°C hold, for 2 min, 3°C/min to 205°C,
Splitless time: 80 s	10°C/min to 250°C, hold for 10 min
Split flow-rate: 40 ml/min	
1st heating rate: 12°C/s	Injection volume: 12.5 μl
1st plateau temperature: 260°C	
1st hold time: 60 s	
2nd heating rate: 12°C/s	
2nd plateau temperature: 300°C	
2nd holding time: 60 s	

3.8. Precision of the 12.5-μl autosampler injection

A series of test mixtures with various concentrations between 9 pg/μl and 14 ng/μl produced by dilution of a stock solution were analysed. Five replicates were analysed at each concentration level and the relative standard deviation was calculated. The results are given in Table 4. The quantitative determinations were found to exhibit good to excellent precision at all concentration levels down to the concentration level representing 1–2.5 ng per pesticide injected. Such precision was also found in our laboratory for samples analysed for pesticide residues in food. The determination in any heteroelement trace was found definitely not to be affected by matrix compounds, thus resulting in excellent performance also with samples in routine analysis. Only at the lowest concentration level representing injected amounts of 100–250 pg were the relative standard deviations found to be higher for most of the analytes. This is to be expected with values close to the limit of determination.

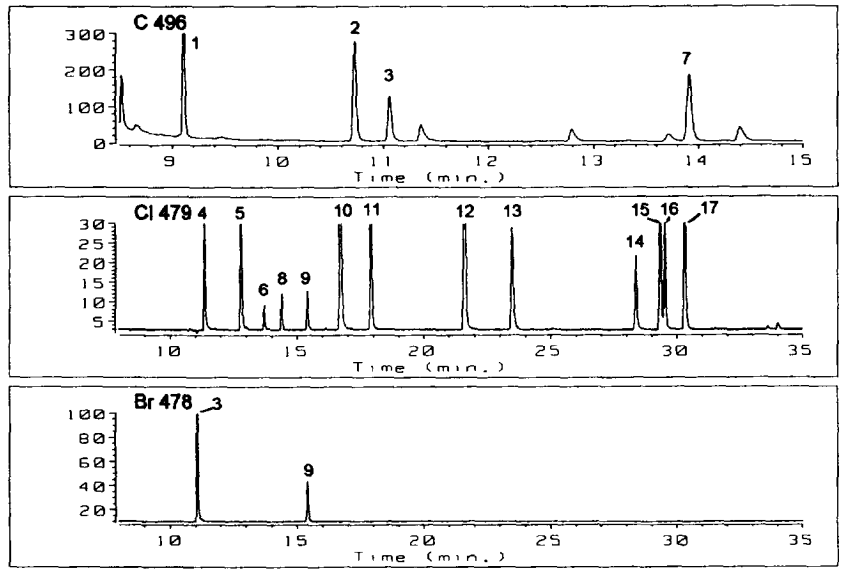

Fig. 5. Chromatograms obtained with AED showing the carbon, chlorine and bromine traces of the test mixture with the stock solution diluted 1:2000 (see Table 4).

Table 4
Precision of the 12.5-μl PTV injection in solvent vent mode at different concentration levels

Substance	Element trace	Concn. of standard soln. (μg/μl)	1:200 dilution		1:1000 dilution		1:2000 dilution		1:10 000 dilution		1:100 000 dilution	
			Av. area[a]	R.S.D. (%)	Av. area[a]	R.S.D. (%)	Av. area[a]	R.S.D. (%)	Av. area[a]	R.S.D. (%)	Av. area[a]	R.S.D. (%)
Dodecane	C	2.0	57686	0.6	11784	1.5	5959	2.0	1490	2.0	185	5.9
Tetradecane	C	2.0	64616	0.0	12365	0.9	6185	2.0	1482	1.9	166	5.4
1,7-Dibromoheptane	Br	2.8	67438	1.1	13798	2.1	6802	1.4	1683	3.1	537	2.9
3,4-Dichloroaniline	Cl	1.3	12578	1.0	2748	1.9	1328	2.4	248	3.6		
Pentachlorobenzene	Cl	1.3	23422	1.1	4706	1.8	2303	1.4	570	2.7	65	7.1
Heptenophos	Cl	0.9	2551	1.8	483	1.1	234	3.0	54	6.3		
Hexadecane	C	2.0	60679	0.1	12364	1.8	6085	2.1	1451	3.4	172	10.7
Propachlor	Cl	1.1	3635	0.7	738	1.4	358	1.7	90	2.5		
Naled	Br	1.3	17394	1.4	3122	2.4	1430	2.5	318	3.4		
Hexachlorobenzene	Cl	1.9	36922	1.2	7496	1.4	3651	0.7	902	2.5	105	10.3
Lindane	Cl	1.2	22228	1.1	4471	1.6	2161	0.9	534	3.3	63	26
Heptachlor	Cl	2.4	40350	1.7	8214	1.2	4000	0.9	985	2.5	107	12
Aldrin	Cl	1.1	16249	1.1	3266	0.9	1588	0.5	393	3.8	47	17
Tetrachlorvinphos	Cl	1.3	11323	1.2	2182	2.9	1036	2.6	232	2.2	21	42
Dieldrin	Cl	1.1	17442	1.2	3639	1.6	1779	1.3	450	3.1	62	16
p,p'-DDE	Cl	1.3	14605	1.5	2949	2.5	1436	1.8	351	3.6	54	37
Endrin	Cl	1.6	22540	1.5	4502	1.7	2166	1.5	525	2.7	61	20

[a] Average peak area for five injections.

Table 5
Dynamic range of quantitative determination with 12.5-μl PTV injection with solvent venting

Substance	Element trace	Peak No.	Range of concentration calibration graph (ng per 12.5 μl)	Straight-line equation		Correlation coefficient, r
				Slope, b	y-Intercept a	
Dodecane	C	1	0.25–25.00	5796	180	0.9998
Tetradecane	C	2	0.25–25.00	6108	132	0.9998
1,7-Dibromoheptane	Br	3	0.35–35.00	4777	320	0.9997
3,4-Dichloroaniline	Cl	4	1.63–81.25	1923	99	0.9999
Pentachlorobenzene	Cl	5	0.16–16.25	3567	44	0.9997
Heptenophos	Cl	6	1.13–56.25	569	−15	0.9999
Hexadecane	C	7	0.25–25.00	6105	102	0.9998
Propachlor	Cl	8	1.38–68.75	659	9	0.9999
Naled	Br	9	1.63–81.25	2707	−242	0.9998
Hexachlorobenzene	Cl	10	0.24–23.75	3887	65	0.9996
Lindan	Cl	11	0.15–15.00	3670	34	0.9996
Heptachlor	Cl	12	0.30–30.00	3375	66	0.9996
Aldrin	Cl	13	0.14–13.75	2924	29	0.9996
Tetrachloirvinphos	Cl	14	1.63–81.25	1666	−4	0.9995
Dieldrin	Cl	15	0.14–13.75	3248	44	0.9996
p,p'-DDE	Cl	16	0.16–16.25	2230	30	0.9997
Endrin	Cl	17	0.20–20.00	2775	26	0.9996

Nevertheless, the quantitative results fit in the dynamic range of the calibration graphs for all the analytes investigated in this study, as can be seen from Table 5, where the linear regression data for each analyte calculated from the four lowest detectable concentration levels are given. Even the critical analyte endrin shows a good linear response with respect to concentration over three orders of magnitude starting at the limit of determination, so that quantification can be carried out down to lowest concentration levels. This wide dynamic range is another remarkable feature of AED.

4. General discussion

4.1. Initial column temperature

The temperature of the column is held below the boiling point of the solvent during solute transfer from the PTV injector to the analytical column. Owing to recondensation of the solvent vapour, the initial part of the column is flooded and a small initial band of the solutes is produced by the solvent effect. However, excessive recondensation of solvent vapour may lead to peak distortion, in particular peak broadening in space. This was observed in earlier work with the injection of 50-μl volumes [28]. Since solvent venting could not be extended owing to losses of more volatile solutes, the column temperature was adjusted to only a few degrees below the solvent boiling point, namely to 100°C, as also applied with hot splitless injection of 2 μl. In this case only a small volume of solvent recondenses, just enough for reconcentrating volatile analytes by the solvent effect [27]. In this study, applying a maximum injection volume of 12.5 μl with solvent venting, excessive solvent vapour recondensation did not need to be considered. In preliminary studies, an initial column temperature of 50°C was found to be optimum with respect to peak shape and initial band width for all solutes.

The excellent results obtained with the injection procedure described are based on the combination of several beneficial effects. The sample liquid is expelled extremely rapidly from the syringe and leaves the syringe needle nebulized into the baffled insert liner. This combination prevents droplets falling directly on to the

retention gap entrance. The temperature inside the liner drops immediately with the evaporation of solvent, as recently measured for various solvents by Mol et al. [7] using thermocouples placed inside the liner. Cooling occurs because the heat capacity of the liner is low. This supports the deposition of the sample solution by recondensation of solvent.

The evaporation rate of the injected liquid is proportional to the mole fraction of solvent vapour in the purge gas and to the total gas flow leaving the injector. When the solvent has been completely evaporated, the temperature returns to the initial PTV temperature and the evaporation of the most volatile solutes increases. Therefore, the split should be closed before the solvent has been completely eliminated, taking advantage of the solvent trapping effect. Probably the simultaneous evaporation of the solutes in the concentrated solution with the solvent during flash heating is beneficial to labile solutes. It reduces the thermal burden and also obviously degradation due to adsorption. This can be seen from the high recoveries of naled and endrin without the observation of any degradation products. The effect of dirt in the sample, however, was not studied systematically with respect to degradation of labile solutes. The assumption is supported by observations in pesticide residue analysis with food samples over a long period of time [1,2]. The transfer of the preconcentrated sample via the retention gap resembles on-column injection and results in excellent peak shapes and small initial bands.

4.2. Large-volume injection: where is the optimum in pesticide multi-residue analysis?

The drawback when using classical injection techniques in GC is that only a small proportion of the sample extract obtained after sometimes laborious preparation procedures can be introduced into the GC system. With common autosamplers operating with 2-ml sample vials, usually a 1-ml final extract volume is prepared of which 1 or 2 μl are injected in hot splitless injection. This means that more than 99% of the sample is discarded even when both confirmatory analyses and also quantitative determinations are carried out with the same sample. One way to increase the concentration factor and correspondingly effectively the detection sensitivity is further evaporation of solvent down to a final extract volume of 200 or even 100 μl. These volumes can be handled with some technical skill; the addition of surrogate standards and internal standards allows the control of losses during the concentration steps and compensation for variations in the final volumes of the extracts. The common autosamplers can be used with small modifications. In our laboratory, these methods have been applied in both pesticide residue analysis in food samples and the analysis of environmental contaminants in water using AED or MS detection in the selected-ion monitoring (SIM) mode [33,34]. The reproducibility of the methods was found to be satisfactory. With these procedures up to 2% of the final extract of a sample can be applied to one analysis.

A further increase in detection sensitivity in automated analysis using common equipment can only be achieved with solvent evaporation after injection. PTV injection with solvent venting is the method of choice for pesticide residue analysis in food and for many types of environmental sample analysis. The method described in this paper approaches the optimum detection sensitivity that can be reached in automated screening analysis for the wide variety of pesticides and environmental contaminants with the equipment available. In this context, only MS and AED are discussed because concentrated samples with a high matrix content require the application of highly selective detectors.

With GC–MS analysis, the maximum detection sensitivity in screening analysis is reached on concentrating the sample extract down to 100 μl. Target compound screening analysis requires 12.5 μl for one group of target compounds using SIM with time window programming. About six injections for more screening analyses on other target compounds and for confirmatory and quantitative analyses can be carried out automatically with the remainder of a sample's extract left for further manual experiments.

With GC–AED analysis, the sample extract

can be reduced to 100 or only 150 μl. Depending on the target compounds to be analysed, between one and five sets of element traces have to be recorded in screening analysis. One or two injections are required for confirmatory analysis with GC–MS in either the full-scan or SIM mode and one to five for quantitative analysis when using selected element traces. We emphasize that this kind of evaluation is worth considering with respect to application to real samples when developing methods using large-volume injection.

In a recent study on the residues of nitro musks in human tissue, GC–AED was applied, recording the element traces of nitrogen and oxygen which exhibit relatively low sensitivity [35]. Limits of determinations between 1 and 2 ng were achieved for the five target compounds. Samples of 1 g of human adipose tissue were extracted and cleaned by gel permeation chromatography and subsequent silica gel adsorption chromatography. The resulting eluates were evaporated to a final volume of 100 μl by means of a gentle stream of nitrogen. With the injection of 12.5 μl according to the method described in this paper, musk xylene was easily detected both in the nitrogen and oxygen traces and quantified as 70 ng/g in human fat using the nitrogen trace. In another fraction from silica gel chromatography of the same sample, 20 ng/g of musk ketone were detected, close to the minimum detectable level. In the same samples hexachlorobenzene, β-HCH, *p,p'*-DDE and a number of polychlorinated biphenyl congeners could be easily detected by means of the chlorine trace.

The automated introduction of larger volumes, up to 1 ml, however, is a technique that should be further elaborated and investigated, especially for applications with samples containing volatile solutes where concentration by evaporation is not feasible. Another field is samples resulting from micro liquid–liquid extraction or solid-phase extraction, where the evaporation of the final extract could be omitted, saving preparation time and gaining in reproducibility. In general, it is our experience that large-volume injection with solvent venting is an on-line pre-chromatographic concentration process that can be better controlled than the usual evaporation method using a stream of nitrogen at room temperature or at a controlled elevated temperature. As demonstrated in this and previous studies [28,35], large-volume injection with solvent venting allows precise optimization and reproducible performance.

5. Conclusion

Automated injection of sample volumes of 12.5 μl using PTV injection with solvent venting has proved to be a reliable technique in capillary GC that can be performed with conventional equipment for automated GC analysis. The method has been applied with toluene as the solvent in pesticide residue analysis of food samples. It is of special importance with respect to this type of trace analysis because it overcomes the inherent lower sensitivity of AED to certain very important heteroelements such as nitrogen. By means of this injection technique, it was possible to carry out screening analysis for pesticide residues at the low concentration levels necessary to control the maximum tolerance level of 10 μg/kg with minimum modification of the established clean-up procedures. It should be emphasized that the method shows excellent precision in quantitative analysis and a wide linear dynamic range down to the limit of determination.

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