Journal of Chromatographic Science, Vol. 42, November/December 2004

Optimization of Conditions for PTV Large-Volume Injection Combined with Fast GC–MS

Eva Korenková[1,*], Eva Matisová[2], and **Jaroslav Slobodník[1]**

[1]Environmental Institute, Okruzná 784/42, 972 41 Kos, Slovak Republic and [2]Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology , Radlinského 9, Bratislava 81237, Slovak Republic

Abstract

Large-volume injection utilizing programmable temperature vaporizer in solvent vent mode is combined with fast capillary gas chromatography and mass spectrometric detection. Optimized injection and chromatographic conditions made possible manual injection of a 20-µL ethyl acetate extract containing 15 organochlorine pesticides and their separation on a short, 0.1-mm-i.d. column in less than 8 min.

Introduction

There is a revived interest in the development and implementation of methods of fast gas chromatography (GC). Recent papers (1–3) summarize the advantages of fast GC analysis, general approaches to fast GC method development, and practical aspects of fast GC utilizing open tubular capillary columns, with emphasis on trace analysis (2). Several approaches exist for increasing the speed of capillary GC analysis, and achieving analysis in a matter of minutes rather than tens of minutes. In trace analysis, methods of fast GC have been developed mostly with commercial GCs that are specifically designed for faster GC analysis, or conventional gas chromatographs (or both) with or without additional options for faster GC.

To avoid peak-width broadening, the injection system has to satisfy the required input band width. Any extra-column contribution to band broadening defeats the efficiency provided by fast GC (1). Splitting injection techniques ensure narrow input bands but result in poor limits of detection (LODs) because only very small sample quantities are introduced onto a column, and most of the sample is split to vent. Because of a low injection volume, the minimum detectable concentration is far too high for many practical applications in trace analysis. To improve the minimum detectable concentration, larger sample volumes have to be injected utilizing nonsplitting injection techniques (1).

Splitless and on-column injection, as well as the programmable temperature vaporizer (PTV), can readily be combined with fast GC, because of focusing effects (2). However, these techniques require optimization of various experimental parameters. Van Ysacker et al. (4) explored splitless injection in detail. Splitless injection is most often used in environmental analysis (5–7). Introduction of volumes up to 1 mL without any peak distortion was performed with a 0.1-mm i.d. column (6).

On-column injection is one of the most suitable injection modes for fast GC applications in trace analysis. Besides permitting injection of larger sample volumes, it also eliminates the discrimination of high-boiling analytes. For a narrow-bore column, a few microliters should be considered large volumes. Usual volumes for fast GC with narrow-bore analytical columns (e.g., 0.1-mm i.d., or less) are approximately 0.1 µL. Recent publications (8,9) presented a configuration that allows introduction of 40–80-fold larger sample volumes without any distortion of peak shapes. However, in analyzing very polar compounds, on-column injection has a limitation with regard to retention gap inertness (10). Analysis of real-life samples might lead to problems with the tolerance of the GC system to coinjected matrix components (11).

Introducing larger sample volumes than those permitted by on-column injection has two benefits: excellent LODs or simplified and faster sample preparation (or both). Several techniques have been developed to allow large volumes of organic solvent to be injected in conventional capillary GC (CGC). These were reviewed by Engewald et al. (12) and Korenkova et al. (13,14).

This paper describes the optimization of conditions for combining PTV (operated in solvent vent mode), fast CGC with narrow-bore columns (0.1-mm i.d.), and mass spectrometric (MS) detection. Large sample volumes of organochlorine pesticides (OCPs) extracted from water were injected in order to meet the sensitivity requirements of the European Union's new environmental legislation (15). The method was developed on a commercial GC equipped with additional options enabling fast GC.

*Author to whom correspondence should be addressed: email eva.korenkova@ene.gov.on.ca.

Experimental

Instrumentation

Chromatographic experiments were performed on an Hewlett-Packard HP 6890 Series GC (Waldbronn, Germany) equipped with a programmable temperature vaporization injector (PTV) with an upper pressure limit of 15 bar and an oven allowing fast oven temperature ramp rates (240-V power option). The GC was interfaced to an HP 5973 mass selective detector (MSD). The injector was fitted with a 71- x 2-mm i.d. multibaffled deactivated empty glass liner. An injection syringe of 25 µL was employed for sample injections.

GC

Separations were performed on an HP-1MS fused silica column (15 m × 0.1 mm × 0.4 mm). The PTV injector was operated in a solvent vent mode. A sample volume of 2 × 10 µL was injected manually into a cold injector (50°C, held 1 min). The second injection was made after 0.4 min. The solvent elimination was achieved by a vent flow of 100 mL/min of the carrier gas applied for 0.9 min. At the end of solvent venting, the split vent was closed and the sample was transferred to the capillary column by heating the inlet at a rate of 720°C/min to 300°C and holding the final temperature for 5 min. The splitless time was 2 min to ensure a complete sample transfer. After a completion of the sample transfer, the inlet was heated to 310°C, purged, and returned back to the initial temperature. The initial oven temperature was maintained constant at 45°C during the solvent evaporation and sample transfer steps (3.4 min). It was then increased to 280°C at the rate of 120°C/min and held at final temperature for 6 min. Helium (0.5 mL/min) and hydrogen (1.1 mL/min) in a constant flow mode were used as carrier gases.

Table I. Target and Qualifier Ions Used in SIM MS Detection

ID	Organochlorine pesticides	Target ion* (*m/z*)	Qualifier ions† (*m/z*)
1	1,3,5-Trichlorobenzene	180	145
2	1,2,4-Trichlorobenzene	180	145
3	1,2,3-Trichlorobenzene	180	145
4	Pentachlorobenzene	250	215,108
5	α-HCH	219	181
IS‡	Propazine	214	–
6	Hexachlorobenzene	284	249
7	γ-HCH	219	181
8	Pentachloronitrobenzene	265	295
9	Aldrin	66	263,91
10	Isodrin	193	66,263
11	cis-Heptachloroepoxide	353	263
12	*o,p*'-DDE	246	318,176
13	*p,p*'-DDE	246	318,176
14	Dieldrin	79	263
15	Methoxychlor	227	274

* The most abundant ion in the mass spectrum of the analyte used for quantitation in the full-scan, as well as SIM, modes.
† Characteristic ions used for confirmation of compound identitiy in the SIM mode.
‡ Internal standard.

Temperatures of the transfer line, ion source, and quadrupole were 305°C, 230°C, and 150°C, respectively.

In the full-scan data acquisition mode the MSD was scanned in the range from 30 to 400 amu at the scan rate of 3.89 scan/s. Ions monitored in the selected ion monitoring (SIM) acquisition are listed in Table I. A target ion and one or two additional ions (qualifier ions) of each pesticide were selected from the electron impact ionization mass spectrum. The target ion (the most abundant ion in the mass spectrum) was used for compound quantitation in both full-scan and SIM modes (16). The qualifier ions were used to increase the reliability of compound identification in the SIM mode. Their presence and intensities relative to that of the target ion give an additional evidence of correct target compound identification. The MSD was tuned to *m/z* 69, 219, and 502, corresponding to perfluorobutylamine (PFTBA).

Materials and sample preparation

The organochlorine pesticide standards and internal standard propazine were obtained from various sources (16). All standards were at least 95% pure. Pesticide stock solutions of 200 mg/L were prepared in isooctane and stored in a refrigerator. Two 20 mg/L composite standard solutions were prepared in isooctane. The composite solutions were used to prepare dilute solutions and to spike water samples to the required concentrations. A solution of internal standard propazine (99% purity) of 10 mg/L was prepared in ethyl acetate and stored in the refrigerator.

Helium and hydrogen, both 99.999% quality, were supplied by Messer Tatragas (Bratislava, Slovak Republic) and Air Products (Bratislava, Slovak Republic), respectively. SDB-XC membrane extraction disks (styrene–divinylbenzene, 47 mm, 3M Empore) (Varian, Zug, Switzerland) were used for the sample enrichment.

The sample preparation procedure utilizing solid-phase extraction (SPE) on membrane extraction disks was described elsewhere (16). Ethyl acetate was used for the elution of pesticide residues from filters after their preconcentration from water. For calibration purposes 500 mL of tap water were spiked with a composite solution of 15 pesticides (Table I) at a concentration of 0.05, 0.1, 0.2, 0.5 µg/L each, and extracted. The internal standard propazine at a concentration of 0.2 µg/L was added before the SPE extraction. The extraction procedure provides a 500-fold enhancement in the concentration of target compounds relative to the original sample.

Results and Discussion

The MS has an advantage of unambiguous compound identification. In order to reach the sensitivity of electron capture detection (ECD) (17), sample extract volumes as large as 20 µL have to be analyzed. Because of a limitation imposed by a sample capacity of the liner (cf. below), this volume was introduced into the PTV injector by repeated injection of 10 µL.

The amount of solvent left in the liner, prior to the sample transfer from the liner onto an analytical column, is a critical parameter (18). A high solvent residue results in a wide initial band and, consequently, peak distortion. On the other hand, elimination of too much solvent through the split vent results in

a loss of more volatile analytes. At a given injector temperature, the extent of the solvent elimination can be controlled by three parameters: (*i*) the time the split vent is open (vent end time), (*ii*) the inlet pressure during solvent elimination (vent pressure), and (*iii*) the flow through the split vent (vent flow). Injector initial temperature was maintained at a temperature below the pressure corrected solvent boiling point in order to ensure presence of the liquid solvent. In the manual injection set-up used, a minimum vent end time was limited by the time needed for introduction of the second portion of the sample (~ 0.3 min). In order to increase the injection reproducibility, the second injection was always made at 0.4 ± 0.03 min. The effect of vent pressure on the speed of solvent elimination opposes that of vent flow. The higher the vent pressure (or the lower the vent flow) the slower solvent elimination. To simplify optimization of solvent elimination from the liner, the vent pressure was set to 0 bar. Thus, the vent flow was the only parameter that needed to be optimized to eliminate most of the solvent with minimum analyte losses. It was found in preliminary experiments that for a 10-µL volume injected into the PTV at 50°C, a vent flow of 100 mL/min was sufficient to eliminate most of ethyl acetate in 0.4 min before sample losses occured.

Sample capacity of a liner

For a cold splitless injection, the initial injector temperature is below a pressure corrected solvent boiling point. The sample amount that can be injected under such conditions is limited by the volume of liquid solvent that can be retained in the liner, rather than by the volume of solvent vapor. In order to avoid losses of sample by overflow of the liner, it was important to find out the maximum sample capacity (i.e., maximum injection volume) of the used empty multibaffled liner. This was achieved by single injections of increasing sample volumes of ethyl acetate spiked with a mixture of investigated analytes into a cold injector (initial temperature 50°C), followed by a rapid heating of injector and analysis. For an illustration, the response of an early eluting 1,2,3-trichlorobenzene (*m/z* 180) and more retained hexachlorobenzene (*m/z* 284) as a function of the injected volume are plotted in Figure 1. Peak areas of the OCPs increased linearly up to a sample volume of approximately 10 µL. For sample volumes larger than 10 µL, the solvent flooded zone in the liner was so long that the liquid reached the bottom of the liner and some of it, together with analytes, was lost via the split exit. Therefore, in all further experiments the maximum volume of 10 µL was injected at a time. This value was in agreement with the maximum injection volume that was determined visually for this type of liner (18). Volume of liquid solvent retained by the liner could be increased by the use of packed liners. Typical packing materials are Tenax or silanized glass wool, however, problems with an incomplete desorption or degradation may arise (19).

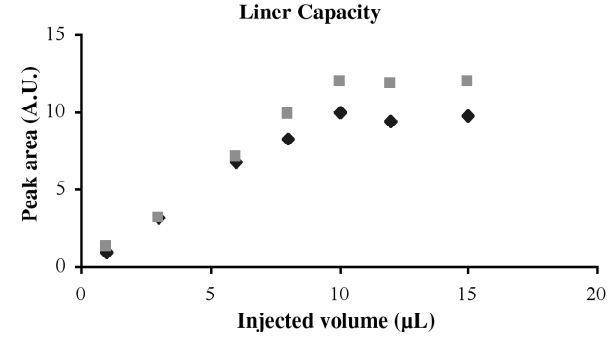

Figure 1. Evaluation of maximum sample capacity of empty multibaffled liner (71- × 2-mm i.d.). PTV operated in a cold splitless mode. (♦) 1,2,3-Trichlorobenzene (*m/z* 180) and (■) hexachlorobenzene (*m/z* 284); concentration in ethyl acetate, 0.1 mg/L per component. See text for other conditions.

Thermal decomposition of analytes

When working with organochlorine pesticides, a critical test for the instrument performance is the analysis of endrin and DDT. An active liner will result in the decomposition of endrin into endrin aldehyde and endrin ketone and in the decomposition of DDT into DDD and DDE. It was found that the extent of endrin decomposition depends on the rate of PTV inlet heating. When the inlet was heated at a rate of 60°C/min, partial thermal decomposition of endrin into endrin aldehyde and endrin ketone was observed. The decomposition increased with increasing inlet heating rate, and endrin degradation products could be observed only at a rate of 360°C/min. At a rate of 720°C/min, neither endrin aldehyde nor endrin ketone were detected, probably because of their further decomposition. These results showed that the heating rate of the PTV might have been a parameter to optimize when analyzing thermolabile compounds. Because of its degradation, endrin was excluded from further experiments. The degradation of other organochlorine pesticides was not observed.

Fast GC separation

Widely used conventional methods for analysis of organochlorine pesticides recommend 30-m × 0.25-mm (or 0.53 mm) i.d. columns, which result in chromatographic runs of approximately 20 min or more (20–22). The analysis time could be significantly reduced by the use of a smaller inside diamter,

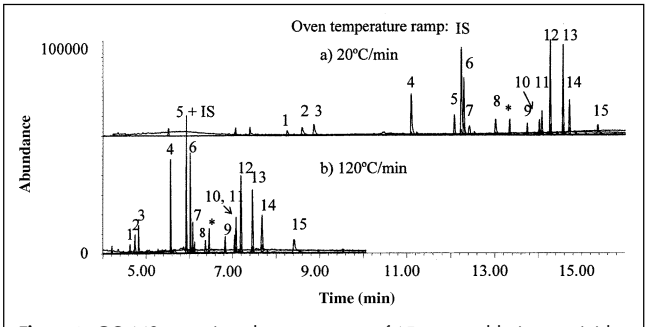

Figure 2. GC–MS target ion chromatograms of 15 organochlorine pesticides extracted from tap water obtained at oven temperature rate (A) 20°C/min and (B) 120°C/min. Preconcentrated volume, 500 mL; concentration, 0.5 µg/L per component; concentration of internal standard (IS) propazine, 0.2 µg/L; and injected volume of ethyl acetate extract, 10 µL. PTV temperature program: 50°C/min (0.5 min), 720°C/min to 300°C (hold 4 min). Solvent vent: 100 mL/min until 0.4 min. Oven temperature program: 45°C (hold 2.4 min), 20°C/min (or 120°C/min) to 300°C (hold 5 min). Carrier gas: He, 0.5 mL/min, constant flow. See Table I for peak identification. Peaks (*) are impurities from the solvent.

analytical columns (e.g., 0.1 mm), hydrogen as a carrier gas, and fast oven temperature ramps (1,2).

Oven temperature ramp

A typical effect of the "slow" and fast oven temperature ramp on the separation of 15 pesticides on a 10-m × 0.1-mm column is shown in Figure 2. Despite a significant shortening of the analysis time, all analytes are still resolved except for coelution of α-HCH with the internal standard propazine.

Carrier gas

Further time savings can be achieved by using hydrogen as a carrier gas at higher flow rates without significant increase in the plate height equivalent (1,2). A separation of the organochlorine pesticide mixture using helium and hydrogen carrier gas while keeping all other chromatographic conditions identical is compared in Figure 3. In addition to a further decrease in analysis time the previously unresolved α-HCH and internal standard were then separated with baseline resolution.

Initial oven temperature

Another parameter relevant to the reduction of analysis time is the choice of initial oven temperature. First analytes elute long after the solvent front, which creates an empty gap in time. However, attempts to increase the initial oven temperature were unsuccessful, resulting in a deterioration of peak shape at the initial oven temperature of 70°C and eventual coelution of trichlorobenzene isomers at the initial oven temperature of 100°C. The optimum initial oven temperature of 45°C was finally selected. At lower oven temperature (34°C), the focusing and therefore the separation of trichlorobenzenes did not improve significantly, although longer temperature equilibration time or cryogenic cooling was needed between the two successive runs. To test that the coelution of trichlorobenzenes at 100°C was not caused by an overload of solvent, the vent flow of the PTV was increased from 100 to 600 mL/min. Their separation did not improve, and a 15% loss of pentachlorobenzene was observed.

Using optimized conditions, (45°C initial oven temperature, 120°C/min oven ramp, H_2 carrier gas) relative standard deviations of retention times and peak areas (n = 3, concentration of each analyte 0.2 µg/L) were in the range of 0.6–1.4% and 1.04–11.5%, respectively (16). Responses in the full-scan acquisition mode of the MS were studied in the range between 0.05 and 0.5 µg/L. Except for aldrin, good linearity was obtained for most compounds with correlation coefficients (r^2) between 0.945 and 0.994. Detection limits under full-scan acquisition were comparable with those typically obtained under single ion monitoring (SIM) acquisition, ranging between 0.01 and 0.1 µg/L. Target analyses in the SIM mode gave detection limits lower by a factor of 2–5. For comparison, the United States Environmental Protection Agency Method 508 limits of detection for GC–ECD, after enrichment of 1-L water sample, are in the range of 0.02–0.5 µg/L (21).

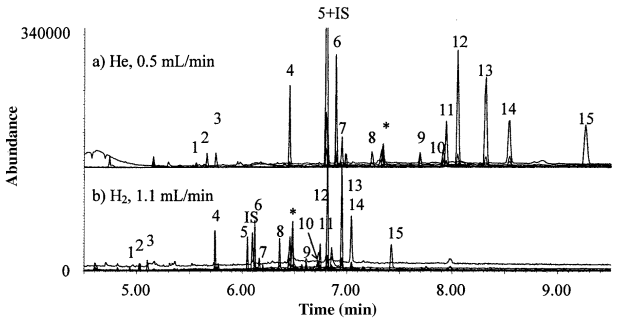

Figure 3. GC–MS target ion chromatograms of 15 organochlorine pesticides extracted from tap water obtained at optimized conditions using (A) helium and (B) hydrogen as a carrier gas. Preconcentrated volume, 500 mL; concentration, 0.5 µg/L per component; concentration of internal standard (IS) propazine, 0.2 µg/L; and injected volume of ethyl acetate extract, 2 x 10 µL. PTV temperature program: 50°C (1 min), 720°C/min to 300°C (5 min). Solvent vent: 100 mL/min until 0.9 min. Oven temperature program: 45°C (3.4 min), 120°C/min to 280°C (6 min). See Table I for peak identification. Peaks (*) are impurities from solvent.

Conclusion

Successful combination of large volume PTV injection with fast GC required harmonization of three injector parameters. With the vent end time set to 0.4 min and the vent pressure set to zero bar, the solvent vent flow was adjusted to maximize solvent elimination with minimum analyte losses. With these parameters, 20 µL of water sample extract in ethyl acetate was successfully introduced without any peak distortions, using repeat injection of 10 µL. As indicated earlier, the PTV inlet heating rate is a parameter affecting decomposition of thermolabile compounds in the injector. Several chromatographic conditions were also optimized to separate 15 OCPs in less than 8 min, with detection limits satisfying legislative requirements. These included the carrier gas type, initial oven temperature, and oven temperature gradient, in combination with the use of a narrow bore capillary column (0.1-mm i.d.).

Acknowledgments

The authors gratefully acknowledge support from Slovak Grant Agency (VEGA, project No. 1/9126/02) for part of this research.

References

1. P. Korytár, H.-G. Janssen, E. Matisová, and U.A.Th. Brinkman. Practical fast gas chromatography: methods, instrumentation and applications. *Trends Anal. Chem.* **21:** 558–72 (2002).
2. E. Matisová and M. Dömötörová. Fast gas chromatography and its use in trace analysis. *J. Chromatogr. A* **1000:** 199–221 (2003).
3. F. David, D.R. Gere. F. Scanlan, and P. Sandra. Instrumentation and applications of fast high-resolution capillary gas chromatography. *J. Chromatogr. A.* **842:** 309–19 (1999).
4. P.G. van Ysacker, H.M. Snijders, H.-G.M. Janssen, and C.A. Cramers. The use of non-splitting injection techniques for trace analysis in narrow-bore capillary gas chromatography. *J. High Resol. Chromatogr.* **21:** 491–97 (1998).
5. S. Hrouzková, M. Simeková, E. Matisová, and P. Korytár. Present

trends in analysis of mixtures of organic compounds by fast gas chromatography. *Chem. Listy.* **96:** 673–83 (2002).
6. P. Sandra and F. David. High-throughput capillary gas chromatography for the determination of polychlorinated biphenyls and fatty acid methyl esters in food samples. *J. Chromatogr. Sci.* **40:** 248–53 (2002).
7. J.W. Cochran. Fast gas chromatography time-of-flight mass spectrometry of polychlorinated biphenyls and other environmental contaminants. *J. Chromatogr. Sci.* **40:** 254–68 (2002).
8. P. Korytár, E. Matisová, H. Lefflerová, and J. Slobodník. Large volume injection in fast gas chromatography with on-column injector. *J. High Resol. Chromatogr.* **23:** 149–55 (2000).
9. E. Matisová, M. Simeková, S. Hrouzková, P. Korytár, and M. Dömötörová. Factors influencing chromatographic data in fast gas chromatography with on-column injection. *J. Sep. Sci.* **25:** 1325–31 (2002).
10. M. Dömötörová. Fast capillary gas chromatography and its use in pesticides analysis. Diploma Thesis, Department of Analytical Chemistry, Faculty of Chemical and food Technology, STU, Bratislava, 2001.
11. J. Zrostlíková, J. Hajslová. M. Godula, and K. Mastovská. Performance of programmed temperature vaporizer, pulsed splitless and on-column injection techniques in analysis of pesticide residues in plant matrices. *J. Chromatogr. A* **937:** 73–87 (2001).
12. W. Engewald, J. Teske, and J. Efer. Programmed temperature vaporisers-based large volume injection in capillary gas chromatography. *J. Chromatogr. A* **842:** 143–61 (1999).
13. E. Korenková, E. Matisová, and J. Slobodník. Large volume injection in capillary gas chromatography. *Chem. Listy.* **95:** 528–39 (2001).
14. E. Korenková, E. Matisová, and J. Slobodník. Large volume injection in capillary gas chromatography using programmable temperature vaporizer injector. *Chem. Listy.* **96:** 82–89 (2002).
15. European Union Water Framework Directive, Amended proposal COM, 2001, p. 17.
16. E. Korenková, E. Matisová, and J. Slobodník. Study on the feasibility of coupling large-volume injection to fast gas chromatography with mass spectrometric detection for analysis of organochlorine pesticides. *J. Sep. Sci.* **26:** 1193–97 (2003).
17. C. Aguilar, F. Borrull, and R.M. Marcé. Determination of pesticides in environmental waters by solid-phase extraction and gas chromatography with electron-capture and mass spectrometry detection. *J. Chromatogr. A* **771:** 221–31 (1997).
18. M. Godula, J. Hajslová, K. Mastovská, and J. Ksivánková. Optimization and application of the PTV injector for the analysis of pesticide residues. *J. Sep. Sci.* **24:** 355–66 (2001).
19. H.G.J. Mol, P.J.M. Hendriks, H.-G. Janssen, C.A. Cramers, and U.A.Th. Brinkman. Large volume injection in capillary GC using PTV injectors: comparison of inertness of packing materials. *J. High Resol. Chromatogr.* **18:** 124–28 (1995).
20. U.S. EPA. Method 608.1: Organochlorine Pesticides in Waste Water. 821/R-93-010-A, National Technical Information Service, Springfield, VA, 1993.
21. U.S. EPA. Method 508: Determination of Chlorinated Pesticides in Ground Water by GC-ECD. EA-600/4-88-039, National Technical Information Service, Springfield, VA, 1991.
22. U.S. EPA. Method 505: Organohalide Pesticides and PCB products in water. 821/R-93-010-B, National Technical Information Service, Springfield, VA, 1989.

Manuscript accepted September 16, 2004.