ELSEVIER

Journal of Chromatography A, 737 (1996) 9–14

JOURNAL OF CHROMATOGRAPHY A

Gel permeation chromatography–high-performance liquid chromatography combination as an automated clean-up technique for the multiresidue analysis of fats

Gerhard G. Rimkus*, Marcus Rummler, Ingo Nausch

Food and Veterinary Institute Schleswig-Holstein, Department of Residue and Contamination Analysis, P.O. Box 2743, D-24517 Neumünster, Germany

Abstract

The well-known and almost universally utilizable clean-up technique of gel permeation chromatography (GPC) and subsequent conventional silica-gel column chromatography was automated by an on-line solvent evaporation of the GPC fraction, followed by normal-phase HPLC separation. The ternary solvent system *n*-hexane–toluene–acetone (88:10:2, v/v/v) was used as the mobile phase which resulted in only one HPLC fraction for all relevant analytes. The HPLC column was cleaned automatically after each sample by backflushing with polar solvents. The recoveries and reproducibilities of 35 analytes (mainly organochlorine compounds) were in the range of 77–90% and 3–7%, respectively; the high efficiency of the HPLC separation provided very clean extracts for the GC analysis. This automated clean-up technique is routinely used for the multiresidue analysis of various fat-containing food and biota samples.

Keywords: Fats; Sample handling; Environmental analysis; Multiresidue methods; Pesticides; Polychlorinated biphenyls

1. Introduction

The residue analysis of organochlorine pesticides and other environmental pollutants in complex biological matrices, such as fats, oils and fat-containing foods, needs extensive analyte purification and concentration. The analysis by highly sensitive and specific chromatographic and detection techniques (e.g., GC–electron-capture detection (ECD) and GC–MS) is especially only practicable with very clean and almost matrix-free extracts. Furthermore, a thorough clean-up minimizes matrix effects on column behaviour and detector response, permits more consistent and reproducible injections and extends the lifetime of the capillary columns. Gel permeation chromatography (GPC), also called size-exclusion chromatography, is an excellent technique for the practically quantitative separation of compounds up to a molecular mass of 400 u (e.g., organochlorine compounds) from macromolecular compounds, such as lipids (600–1500 u). However, small amounts of these macromolecular substances reach the GPC eluate and have to be removed because they would interfere with the subsequent analysis. These complex samples therefore very often require a two-step clean-up which combines different chromatographic techniques in series. In most cases, in the second step the extracts are separated on various adsorbents by polarity. The combination of these two techniques results in a powerful two-dimensional clean-up by molecular size and polarity. Many laboratories of food control in Germany successfully use the combi-

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00020-9

nation of GPC and subsequent conventional silica-gel column chromatography [1,2]. In this study we report the automation of this clean-up method by an off-line GPC–HPLC combination for the routine analysis of fat-containing food and biota matrices.

2. Experimental

2.1. Chemicals

The analytical standards were supplied by Promochem (Wesel, Germany); the solvents specified for residue analysis were purchased from E. Merck (Darmstadt, Germany) and Promochem.

2.2. Sample preparation

Up to 2 g of pure or extracted fat was dissolved in 10 ml ethyl acetate–cyclohexane (1:1, v/v) and the solution was filtered by a disposable PTFE filter into a 15 ml tube which was screwed to the sample input module of the GPC system. After the automated GPC–HPLC clean-up, the HPLC fraction was carefully evaporated to about 1 ml in a vacuum rotary evaporator with a water bath temperature of 60°C and a vacuum of 20 kPa. The fraction was filled up to 1 or 2 ml and transferred to an autosampler vial ready for the GC analysis.

2.3. Gel permeation chromatography (GPC) and high-performance liquid chromatography (HPLC)

For the GPC including the automated on-line solvent evaporation the model 601 GPC/Autovap System from Analytical Bio-Chemistry (ABC) Laboratories (Columbia, NY, USA) was used with the following conditions: column: 340 mm×25 mm I.D. packed with S-X3 Bio-Beads, 200–400 mesh (Bio-Rad, München, Germany); as mobile phase ethyl acetate-cyclohexane (50:50); flow-rate: 5 ml/min; sample input module: up to 23 samples with 24-port valve and 5 ml sample loop; timetable: 24 min dump cycle, 16 min collect cycle, 3.48 min wash cycle.

The parameters for automated solvent evaporation (on-line) were the following: evaporation time, 16 min; evaporation temperature, minimum 50°C, maximum 54.5°C; cool dry time, 10 s; diluent, toluene (mix time 10 s); chamber cleaning, rinse 30 s, 58°C; vacuum setpoint, 26.7 kPa.

For the subsequent clean-up of the GPC fractions the following HPLC system and conditions were used: pump I for separation: Gilson 305 with pump head 10.SC and manometric module 806; pump II for backflushing: Gilson 306 with pump head 10.SC; autosampler: Gilson 231 XL with dilutor 401 C (5 ml syringe) and 2 ml sample loop; fraction collector: Gilson 201 with electric 3-port valve and rack 22; 6-port switching valve: Besta motor valve H. All parts were purchased from Abimed (Langenfeld, Germany). The chromatographic separation was carried out under the conditions, subsequently described: column: Hypersil Si 5 μm, 250×4 mm (Latek, Eppelheim, Germany); mobile phase: 10% toluene and 2% acetone in *n*-hexane (for separation) and 20% acetone in toluene (for backflush). The time program of the used HPLC system is given in Table 1.

2.4. Gas chromatographic determination

For the GC analysis of the HPLC fractions the following instrumentation and conditions were used: Varian 3400 GC with ^{63}Ni ECD system, Varian 8100 autosampler and a septum-equipped programmable injector (SPI); CP-Sil 8 CB and CP-Sil 19 CB capillary columns (Chrompack, Frankfurt, Germany), each measuring 60 m×0.25 mm I.D., 0.25

Table 1
Time program of the HPLC system

Time (min)	Pump I	Pump II	Valve position	Fraction collector	Flow-rate (ml/min)	Fraction volume (ml)
0–3	Active	Stop	Separation	Waste	1	3
3–10	Active	Stop	Separation	Collect	1	7
11–61	Stop	Active	Backflush	Stop	0.5	25
61–91	Active	Stop	Separation	Waste	1	30

μm film thickness, with 5 m retention gap and quartz Y-splitter; carrier gas, hydrogen; make-up gas, nitrogen; oven temperature program, 120°C (2 min), 20 °C/min to 150°C (0 min), 1.7 °C/min to 210°C (0 min), 1.0 °C/min to 230°C (0 min), 5 °C/min to 270°C (20 min); temperature program of the SPI: 100°C (0.2 min), 120 C°/min to 270°C (20 min).

3. Results and discussion

GPC represents a well-suited clean-up technique for fat-containing samples, its separation mechanism is principally based on size exclusion and the chemical reactivity of the stationary phase with the analytes can be practically neglected. Therefore, GPC as a reversible chromatographic technique can be easily automated to run in a continuous and unattended mode. Furthermore, the GPC column mentioned in this study can cope with relatively high quantities of interfering substances (up to 1 g fat per run) with the effect of significant reduction of the determination limits. Due to the relatively large dimensions of the GPC column, the concentration of the GPC fraction is necessary before further clean-up. The careful and controlled evaporation of the collected GPC fraction (with solvent recovery) can be automated by the used GPC combination. In this system the column effluent of the collect cycle is directed to an evaporation module where it is automatically evaporated to dryness under controlled temperature and vacuum, then concentrated in toluene and finally transferred to a sealed vial.

The elution profiles of the lipid matrix and some organochlorine compounds (e.g. β-HCH) overlap, thus, for the subsequent GC–ECD and GC–MS analysis, it is essential to purify the GPC fraction in a second step, e.g. by means of adsorption column chromatography. Although various adsorbents have been applied for this step, the GPC/silica-gel column combination has resulted as the best known and almost universally utilizable clean-up technique [1–5]. For the standard GC–ECD residue analysis of halogenated organic compounds in fat-containing matrices this conventional clean-up method requires the elution of the relevant analytes from the silica-gel column by two different solvent systems (a second fraction is necessary for the quantitative recovery of the more polar compounds), which results in two fractions and in two subsequent GC runs. We automated the silica-gel chromatography by an HPLC system with an analytical normal-phase HPLC column (Hypersil Si 5 μm). Several binary solvent

Table 2
Percentage recoveries and R.S.D. values ($n=4$) of 35 compounds from a fortified fat matrix

Compound	Recovery (%)	R.S.D. (%)	Fortification level (μg/kg)
Organochlorine Pesticides			
HCB	77.3	3.3	20.0
α-HCH	80.9	4.3	20.0
β-HCH	82.4	2.9	20.0
γ-HCH	89.1	3.3	20.0
δ-HCH	85.7	3.9	20.0
ε-HCH	83.5	4.9	25.0
o,p'-DDE	84.9	4.6	30.0
p,p'-DDE	87.1	5.6	30.0
p,p'-DDD	86.0	4.2	30.0
o,p'-DDT	87.8	5.5	30.0
p,p'-DDT	89.0	4.6	30.0
Dieldrin	86.1	4.9	30.0
Endrin[a]	89.7	5.4	30.0
cis-Heptachlorepoxide	86.2	4.8	30.0
Oxychlordane	85.6	4.7	30.0
trans-Nonachlor	85.6	5.8	25.1
Polychlorinated Biphenyls (PCB)			
PCB 28	86.2	5.1	30.0
PCB 52	87.5	4.9	30.0
PCB 101	82.7	5.2	30.0
PCB 118	87.8	5.8	30.0
PCB 138	86.9	5.0	30.0
PCB 153	86.9	6.8	30.0
PCB 170[b]	89.3	4.8	20.0
PCB 180	86.7	5.3	30.0
PCB 194	86.2	5.5	30.0
PCB 209	84.8	4.0	30.0
Polychlorinated Terpenes (e.g. Toxaphene)			
Parlar No. 26[a]	82.5	6.8	20.0
Parlar No. 32[a]	85.8	5.3	20.0
Parlar No. 50[a]	84.7	4.4	20.0
Parlar No. 62[a]	85.1	6.7	20.0
Nitro Musks			
Musk Xylene	86.9	5.0	15.2
Musk Ketone	86.2	5.8	15.3
Miscellaneous			
Bromocyclen	80.9	4.6	30.0
Pentachloroanisole	77.7	3.8	30.0
Octachlorostyrene	86.6	5.6	20.0

[a] $n=3$.
[b] $n=2$.

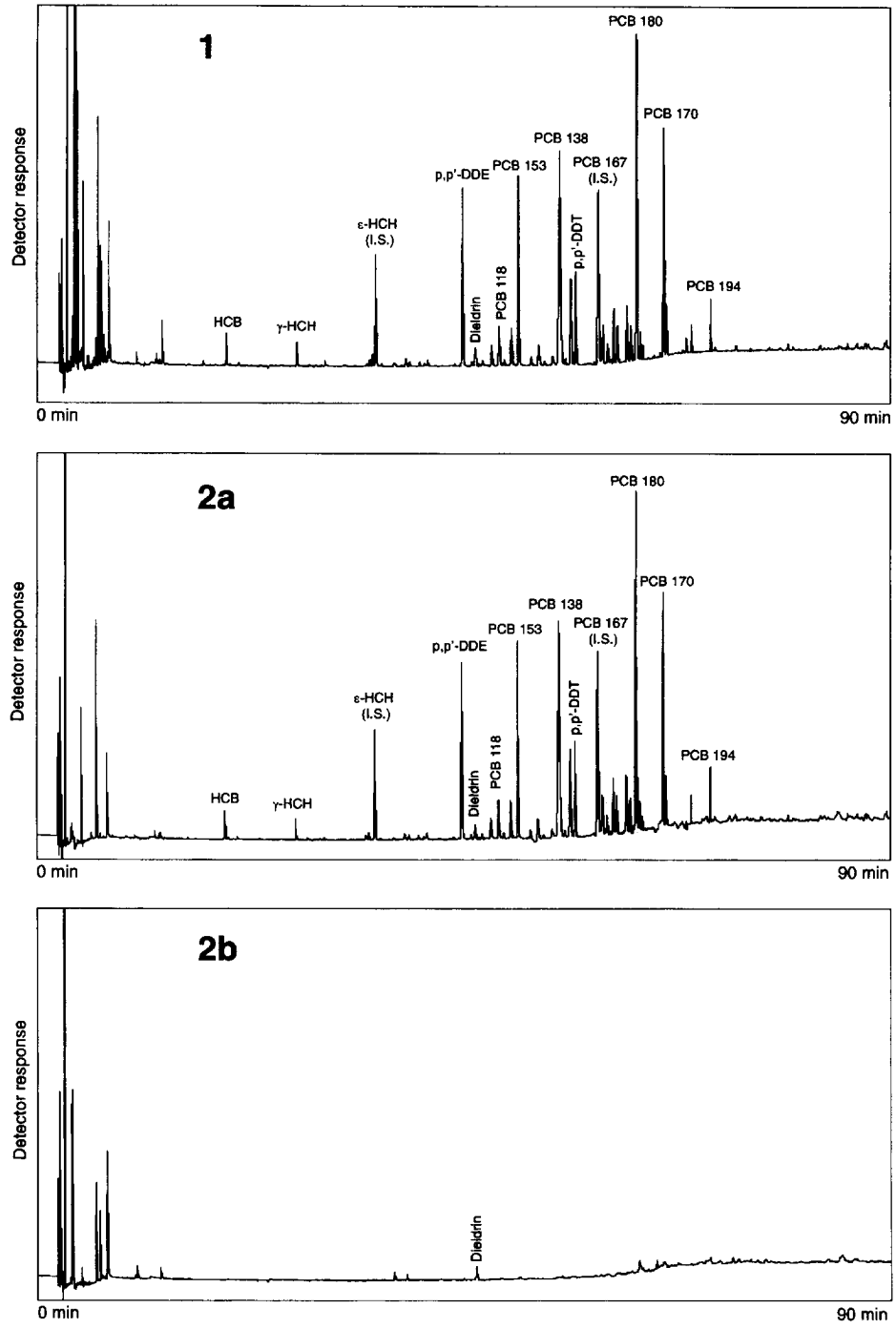

Fig. 1. GC–ECD chromatograms of an egg fat sample, cleaned-up by the automated GPC–HPLC combination (1) and cleaned-up by the conventional GPC/silica-gel column combination (2a, first fraction; 2b, second fraction) (I.S., internal standard; column, CP-Sil 19 CB; for GC conditions, see Experimental).

systems, e.g. *n*-hexane–toluene, *n*-hexane–acetone, were applied with various ratios in the isocratic mode. As matrix absorbed at the column head, the column had to be cleaned after each sample by backflushing with polar solvents and reconditioned before the next injection. As to the selection of the most suitable solvent system for the HPLC clean-up several requirements have to be fulfilled:

1. For the optimum separation of the lipid matrix from the lipophilic analytes the eluotropic strength of the used solvents should not be too strong.
2. The aim of this study was to collect all analytes in one relatively small HPLC fraction (for one subsequent GC run), even though the polarities of the analytes in a multiresidue analysis cover a wide range. In the isocratic mode the eluotropic strength of the HPLC solvents has to be strong enough in order to keep the volume of the collected fraction at a minimum and to achieve high recoveries for all analytes.
3. Low boiling solvents should be used to facilitate the final evaporation step and a high boiling solvent should be used as a keeper.

These conditions were widely fulfilled by the ternary solvent system *n*-hexane–toluene–acetone (88:10:2, v/v/v). 7 ml of the HPLC effluent were collected for the recovery of the polar compounds, e.g., dieldrin, endrin and musk ketone [6,7]. The mean recoveries of 35 compounds, such as organochlorine pesticides, polychlorinated biphenyls (PCBs), polychlorinated terpenes, e.g., toxaphene, nitro musks [6,7], and bromocyclen [8] from a fortified fat sample (Table 2) using the above-described automated clean-up system ranged from 77 to 90% with R.S.D. values of 2.9 to 6.8%. The recovery rates may be improved by further optimization of both the evaporation procedures as critical steps of the clean-up, especially for the more volatile analytes. The collection of all analytes of interest in one fraction results in a considerable saving of analysis time (only one GC run). Most samples showing relatively simple contamination patterns require no fractionating separation of PCBs from organochlorine pesticides [9] provided that the analysis is carried out by a high-resolution capillary column GC system. This automated clean-up procedure is used in the daily routine for the multiresidue analysis of fat-containing matrices (e.g., fat from fish, milk, butter, cheese, egg, meat, liver, including human fat and milk).

The high efficiency of the applied automated technique and some of the shortages observed in the use of the conventional clean-up procedure are demonstrated by three GC–ECD chromatograms of an egg fat sample with an unusual PCB pattern (Fig. 1). The conventional clean-up technique (GPC and silica-gel column chromatography) requires for the elution of all analytes from the silica-gel column two different solvent systems resulting in two fractions and in two subsequent GC runs. These chromatograms often show negative and matrix peaks and a disturbed base line. The implementation of an automated solvent evaporation of the GPC fraction and, in particular, of the HPLC separation into the conventional clean-up technique of the GPC/silica-gel column combination does not only produce an automation and time-saving effect but also improves the purity of the extracts and consequently the quality of the GC analysis.

4. Conclusions

The conventional clean-up technique in multiresidue analysis consisting of GPC and subsequent silica-gel column chromatography can be significantly improved by using an off-line GPC–HPLC combination including the automated solvent evaporation of the GPC fraction. It produces very clean extracts and delivers high recovery rates for many organochlorine pesticides and environmental pollutants. The described method can, therefore, be successfully applied for the routine residue analysis of various fat-containing biological matrices.

References

[1] W. Specht and M. Tillkes, Fresenius J. Anal. Chem., 301 (1980) 300.
[2] W. Specht and M. Tillkes, Fresenius J. Anal. Chem., 322 (1985) 443.
[3] B. Jansson, R. Andersson et al., Fresenius J. Anal. Chem., 340 (1991) 439.
[4] D.M. Holstege, D.L. Scharberg, E.R. Richardson and G. Möller, J. Ass. Off., Anal. Chem., 74 (1991) 394.

[5] T.A. Ternes, J. Hany, W. Baumann and R. Nagel, Fresenius J. Anal. Chem., 351 (1995) 790.
[6] G. Rimkus, B. Rimkus and M. Wolf, Chemosphere 28, (1994) 421.
[7] G.G. Rimkus and M. Wolf, Chemosphere, 30 (1995) 641.
[8] B. Pfaffenberger, H. Hühnerfuss, B. Gehrcke, I. Hardt, W.A. König and G. Rimkus, Chemosphere, 29 (1994) 1385.
[9] E.A. Hogendoorn, G.R. van der Hoff and P. van Zoonen, J. High Resolut. Chromatogr., 12 (1989) 784.