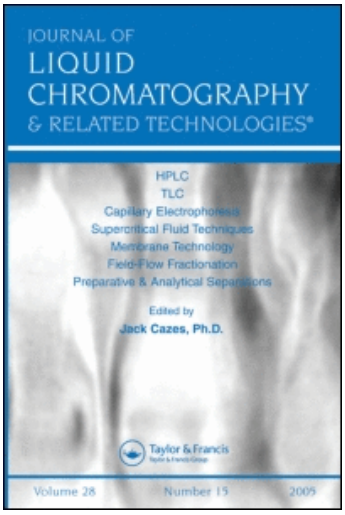

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Separation of Hexabenzylgermanium Derivatives using Aryl Stationary Phases for HPLC

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Abstract: This paper is a continuing investigation concerning optimization of the chromatography of hexabenzylgermanium derivatives. Optimal conditions of chromatographic separation and determination for hexabenzyldigermoxane and hexabenzyldigermanium have been elaborated, utilizing interactions of π-π type between stationary phase and analyzed compound. Because of partial retention of these compounds on a chromatographic column, numerous problems with their determination can appear. During the investigation three aryl stationary phases and various mobile phases were taken into consideration. Obtained results showed, unanimously, that the best selectivity exhibited a column containing porous graphitizing coal. The highest separation factor, 1.73, has been obtained using this column and a mobile phase consisting of aqueous acetonitrile. A slightly lower separation factor has been obtained with the use of aryl stationary phase RP Si–NAF. All obtained results showed a dominating influence of interactions π-π in the investigated chromatography process.

Keywords: Aryl stationary phases, Benzylgermanes, π-π Interactions, HPLC

INTRODUCTION

In anticancer chemotherapy a fundamental part is still played by metalorganic preparations. The most frequently used drugs are based on complexes of platinum and ruthenium. A disadvantage of some anticancer preparations is their significant toxicity, caused by low selectivity.[1] The main aim of contemporary chemotherapy is to find drugs acting more versatile and more

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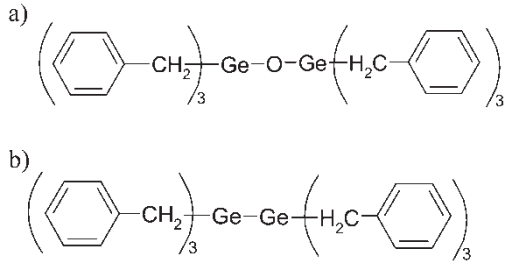

Figure 1. Structures of: a) hexabenzyldigermoxane b) hexabenzyldigermanium.

selective on a degenerated groups of cells. The biological activity of these drugs can be estimated, e.g., by an examination of mechanisms of their interactions with body transport proteins (albumin, transferring). Characterization of these mechanisms is carried out by means of different spectral methods, which must be preceded by preliminary isolation of the tested substance, usually from very complex matrix (Figure 1).

From among all these methods, the most widely used is still high performance liquid chromatography because of the immense development of specific packings.[2–4] Undoubtedly, aryl stationary phases, where interactions of π-π are predominating, belong to this kind of packing. This paper is a continuation of investigations concerning optimization of chromatographic separation and determination of newly obtained hexabenzylgermanium derivatives.[5] Among benzylgermanium derivatives, the highest biological activity shows benzylgermatranes.[6] They exhibit little toxicity, high anesthetic and anticonvulsive activity, and positive influence on memory.[7]

Other germanium compounds, e.g., G-132 can be used in anticancer therapy.[6] Germanium itself is not toxic. Its main application is in electronics, first of all as semiconductor; it is designed for rectifiers, transistors, termistors, and photoelectric cells. Generally, germanium compounds can be considered as a new group of compounds, with not yet explored properties, but which has great expectations of applications.

EXPERIMENTAL

Materials and Methods

As reported in the literature,[5] germanium derivatives were subjected to chromatographic separation using aryl stationary phases. For this purpose hexabenzyldigermoxane (germ. 1) and hexabenzyldigermanium (germ. 2) were dissolved in dichloromethane (HPLC purity, Fluka AG, Bucks, Switzerland), obtaining concentration at about 15 μg/mL. Freshly prepared samples were

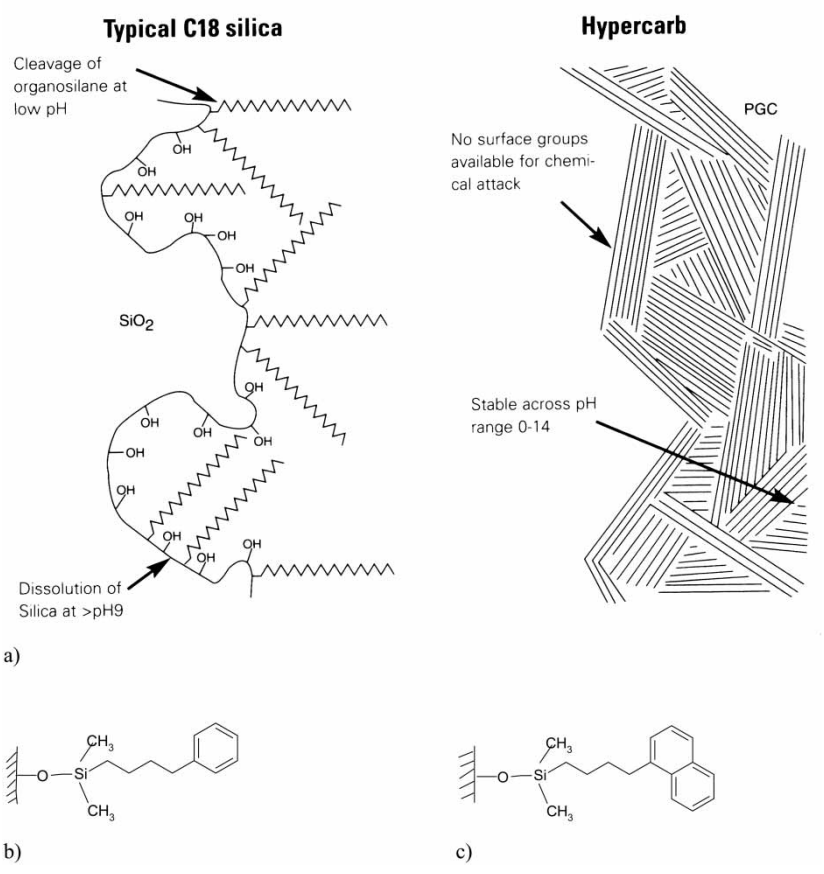

Figure 2. Scheme of chemically bonded stationary phases: a) typical C_{18} silica and hypercarb (RP Si–PGC) b) phenylbutyl (RP Si–PB) c) naphthylpropyl (RP Si–NAF).

directly subjected to HPLC analysis, at wavelength 242 nm and temperature 20°C.

Three aryl stationary phases were studied: commercial column Hypercarb, packed with porous graphitized coal (RP Si–PGC, Thermo Electron Corporation UK–Figure 2a), phenylbutylic (RP Si–PB, Figure 2b[8]), and naphthylpropylic (RP Si NAF, Figure 2c).[8] Dimensions of steel columns were: for RP Si–PGC–100 × 4.6 mm, for RP Si–PB and RP Si–NAF - 125 × 4.6 mm (Table 1). Different systems of mobile phases were applied: aqueous acetonitrile (100/0, 90/10, 80/20, 75/25) and aqueous methanol (100/0, 90/10, 80/20), using flow 0.5 and 0.3 mL/min (Table 2).

The organogermanium compounds were prepared by the method described in the literature[5] and their references.

Table 1. Characteristics of aryl bonded phase

Phase code	Carbon (%)	Manufacturer of column	Column dimensions (mm)
RP Si–PB	14.9	home made	125 × 4.6
RP Si–NAF	16.1	home made	125 × 4.6
RP Si–PGC	100	Thermo Electron Corporation	100 × 4.6

Hexabenzyldigermoxane

^{1}H NMR ($CDCl_3$): $\delta. = 2.07$ (s, 12H, CH_2Ph), 6.8–6.9 (m, 12H, H-Ph), 7.0–7.3 (m, 18H, H-Ph). ^{13}C NMR ($CDCl_3$): $\delta = .25$ (CH_2Ph), 124, 128,

Table 2. Chosen dependence ln k for germ. 1 and germ. 2 from one type of stationary and mobile phase. Chromatographic conditions: – flow – 0.5 or 0.3 mL · min^{-1}, wavelength – 242 nm, temperature – 20°C

Type of stationary phase	Mobile phase	k_1	k_2	$\alpha = k_2/k_1$
RP Si–NAF	acetonitryle/water (80/20), 0.5 mL · min^{-1}	6.23	6.94	1.11
	acetonitryle/water (80/20), 0.3 mL · min^{-1}	15.03	16.58	1.10
	methanol/water (90/10), 0.5 mL · min^{-1}	17.26	17.76	1.03
	methanol/water (90/10), 0.3 mL · min^{-1}	45.69	50.42	1.10
RP Si–PB	acetonitryle/water (80/20), 0.5 mL · min^{-1}	14.25	14.72	1.03
	acetonitryle/water (80/20), 0.3 mL · min^{-1}	23.67	25.33	1.07
	methanol/water (90/10), 0.5 mL · min^{-1}	21.55	23.23	1.08
	methanol/water (90/10), 0.3 mL · min^{-1}	54.96	60.05	1.09
RP Si–PGC	acetonitryle/water (80/20), 0.5 mL · min^{-1}	2.12	3.66	1.73
	acetonitryle/water (80/20), 0.3 mL · min^{-1}	3.22	4.27	1.33
	methanol/water (90/10), 0.5 mL · min^{-1}	5.86	7.04	1.20
	methanol/water (90/10), 0.3 mL · min^{-1}	15.13	16.92	1.12

[a]In the Table only optimal data of chromatographic separation and determination of hexabenzylgermanium derivatives were placed.

129, 138. UV/Vis ($CHCl_3$): λ_{max} (lgε) = 242 (4.62), 264 (4.57), 269 (4.55), 292 (4.53).

MS (EI), m/z (% rel. int.), for $C_{42}H_{42}^{74}Ge_2O$: $[M\text{-}CH_2Ph]^+$ 619 (17), 528 (1), 437 (3), 347 (14), 256 (1), 165 (39), 91 (100). bp. 134°C (134–135°C).[5] IR (KBr) 928 cm^{-1} (Ge-O-Ge).[5,9,10]

Hexabenzyldigermanium

^1H NMR ($CDCl_3$): δ = .2.15 (s, 12H, CH_2Ph), 6.5–6.8 (m, 12H, H-Ph), 7.0–7.3 (m, 18H, H-Ph). ^{13}C NMR ($CDCl_3$): δ = .24 (CH_2Ph), 124, 128.37, 128.42, 140. UV/Vis ($CHCl_3$): λ_{max} (lgε) = 242 (4.62), 267 (4.56), 269 (4.55), 277 (4.56), 289 (4.54).

MS (EI).[9,10] bp. 182°C (183–184°C).[5,11] IR (polyethylene) 238 cm^{-1} (Ge-Ge).

The infrared spectra of benzylgermanes have been examined in the region 4000–50 cm^{-1} to assign the characteristic group frequencies in the compounds synthesized. Benzyl Derivatives: Abbreviations: w–weak; m–medium; s–strong; b–broad: 3099 w, 3082 w, 3066 m, 3050 s, 3018 s, 2936 m, 2899 m, 2293 w, 1948 w, 1874 w, 1816 w, 1754 w, 1595 s, 1578 s, 1491 s, 1450 s, 1414 m, 1334 m, 1317 m, 1210 s, 1181 s, 1146 s, 1056 s, 1030 m, 999 w, 908 m, 805 s, 761 s, 698 s, 559 m, 542 m, 460 bs, 444 bs, 342 w, 207 w, 204 w, 150 m, 144 m.

Apparatus

The organogermanium compounds were prepared by the method described in the literature;[7] ^1H NMR spectra were recorded on a Bruker-200 in $CDCl_3$, with HMDS as internal standard. MS spectra were performed with a Shimadzu Mass-Spectrometer GC/MS-QP5050, column Phenomenex BPX-5 30 m × 0.25 mm I.D. × 0.25 μm FT, total flow 52.7 mL · min^{-1}.

The infrared (IR) spectra were recorded on a Nicollet Magna-IR 760 in (bromide of potassium) and (polyethylene).

Chromatographic measurements were performed on a liquid chromatograph SPD-6A (Shimadzu, Kyoto, Japan) equipped with a gradient pump LC-6A, UV detector, a sampling valve Rheodyne (Berkeley, CA, USA), model 7125, with a 20 μL sample loop, and a Shimadzu C-R6A data recorder.

RESULTS AND DISCUSSION

In this paper we have reported optimal data of chromatographic separation and determination of two synthesized compounds: hexabenzyldigermoxane and hexabenzyldigermanium. Obtained results are compared in Table 2. During selection of optimal conditions of the chromatographic process, various

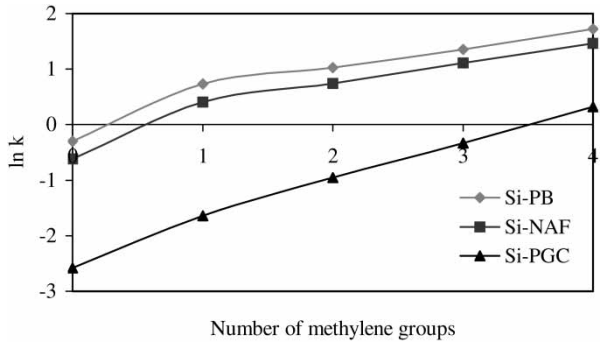

Figure 3. Dependence ln k on the number of carbon atoms in alkyl chain of alkylbenzenes for phenylbutyl, naphthylpropyl and hypercarb packings. Chromatographic conditions: mobile phase 75/25 vol.% acetonitryle/water, flow − 0.5 mL × min^{-1}, wavelength −254 nm, temperature − 20°C.

compositions of mobile phase were tested: aqueous acetonitrile (100/0, 90/10, 80/20, 75/25) and aqueous methanol (100/0, 90/10, 80/20), using flow 0.5 and 0.3 mL/min and three aryl stationary phases: phenylbutylic, naphthylpropylic, and porous graphitized coal (Hypercarb column).

Application of columns commonly considered as standard with octadecyl or octyl packing, did not yield satisfactory effects concerning the separation and determination of the above mentioned compounds,[5] because of long retention time and unsatisfactory resolution of analyzed samples. Therefore, attempts were taken to use aryl packing with various contents of π electrons. According to the subject literature, aryl stationary phases are dedicated to determination of compounds containing aromatic ring.[8] Predominating interactions in the chromatographic process become interactions of π-π types (Figure 3); this enables the separation of isomers, as well as significantly shorten the time of analysis of determined compounds. This observation was confirmed (Table 2 and Figures 4–6) in the case presented here.

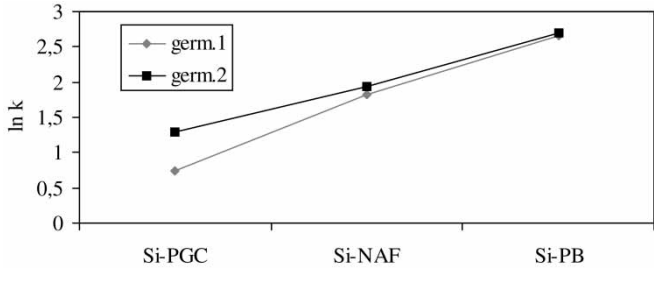

Figure 4. Effect of the separation of (germ. 1) and (germ. 2) with the use of aryl stationary phases RP Si–PB, RP Si–NAF and RP Si–PGC. Mobile phase: acetonitrile/water (80/20), flow rate: 0.5 mL · min^{-1}, detection −242 nm (see Table 2).

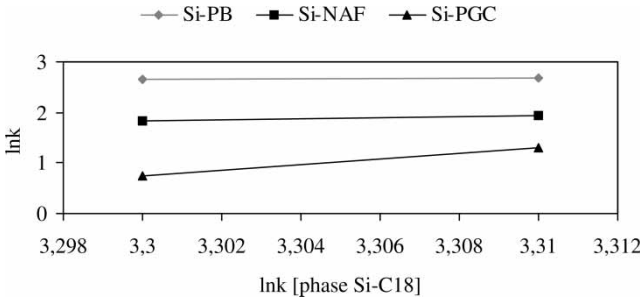

Figure 5. Dependence of ln k of the RP Si–PB and RP Si–NAF and RP Si–PGC phases on ln k obtained for the octadecyl phase for germ. 1 and germ. 2.

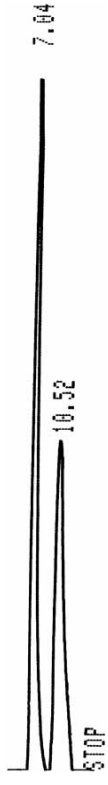

Figure 6. A chromatogram of separation of the hexabenzyldigermoxane (7.04 min) and hexabenzyldigermanium (10.52 min) on the stationary RP Si–PGC phase. Mobile phase: acetonitrile/water (80/20 vol.%); flow $-0.3\ \text{mL} \cdot \text{min}^{-1}$, wavelength -242 nm, temperature -20°C.

Application of aryl stationary phases enabled accomplishment of separation and determination of germanium derivatives in a very short time (at about 10 min). From among three tested stationary phases, the highest selectivity in determination of hexabenzylgermanium compounds exhibited the phase containing porous graphitized coal (RP Si–PGC). When it was used with aqueous acetonitrile (80/20) as a solvent system with flow 0.5 mL/min, the separation factor 1.73 has been achieved. A lower separation factor (1.11) gave naphthylpropyl stationary phase (RP Si–NAF). Satisfactory separation also yielded phenylbutyl phase. Obtained results show the predominant influence of interactions of π-π types, the separation factor of analyzed hexabenzylgermanium derivatives increases and the retention time decreases with increased number of π electrons contained in the stationary phase. Application of RP Si–PGC column (Hypercarb) almost twofold shortened retention time of analyzed compounds, compared with results obtained with RP Si–PB phase (phenylbutyl). However, retention times obtained on naphthylpropyl and hypercarb columns were similar, under 10 min, whereas better separation of germanium derivatives could be observed on Hypercarb.

To recapitulate, application of the octadecyl stationary phase to the analysis of above mentioned compounds[5] did not allow for elaborating of optimal conditions of their chromatographic separation and determination. During the analysis diffusion and tailing of peaks appeared independently of flow and stationary phase composition. Only application of aryl stationary phases dedicated to separation of π electrons containing compounds enabled elaboration of optimal conditions of chromatography of analyzed hexabenzyl germanium compounds. This effect is shown on graphs in Figures 4–6.

CONCLUSIONS

Hexabenzyl derivatives of germanium: hexabenzyldigermoxane and hexabenzyldigermanium can be conveniently separated and determined by high performance liquid chromatography using aryl stationary phases. All tested phases gave satisfactory results. However, the highest selectivity and the best separation could be achieved with the phase containing porous graphitized coal (Hypercarb).

REFERENCES

1. Hay, M.P.; Gamage, S.A. J. Med. Chem. **2003**, *46*, 169.
2. Buszewski, B.; Gilpin, R.K.; Jaroniec, M. Chem. Anal. **1994**, *39*, 653.
3. Armstrong, D.W.; Bertrand, G.L.; Ward, K.D.; Ward, T.J. Anal. Chem. **1995**, *67*, 45.
4. Pirkle, W.H.; Welch, Ch. J. Chromatogr. **1992**, *589*, 45.
5. Kluska, M.; Pypowski, K.; Erchak, N. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30*, 1777.

6. Karlow, S.S.; Zajcewa, G.S. Khim. Geterotsikl. Soedin. **2001**, *11*, 1451 (Russia).
7. Lukvevics, E.; Jgnatovich, V.; Shul'ga, T.; Mitchenko, O. J. Organomet. Chem. **2002**, *659*, 165.
8. Gadzała-Kopciuch, R.M.; Kluska, M.; Wełniak, M.; Buszewski, B. Elsevier–Mat. Chem. & Phys. **2005**, *89*, 228.
9. Bruschkies, B. Chem. Ber. **1934**, *67*, 1041.
10. Cross, R.J.; Glockling, F. J. Organomet. Chem. **1965**, *3*, 146.
11. Ferguson, D.; Glidewell, Ch. Acta Cryst. C52, **1996**, 1889.

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