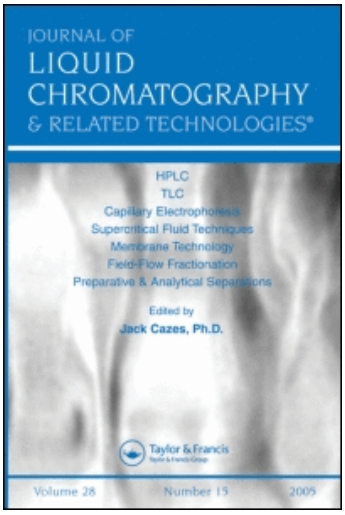

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Separation of Hexabenzyldigermoxane and Hexabenzyldigermanium by HPLC

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Abstract: In this paper optimal conditions of chromatographic determination and separation of hexabenzyldigermoxane and hexabenzyldigermanium by the HPLC method are presented. These compounds cause numerous difficulties in chromatographic processes, because they can be retarded on chromatographic columns and are characterized by long retention times. In our studies, types of stationary phases as well as types of mobile phases and contents were taken into consideration. Obtained results showed unambiguously, that the most selected is the aryl stationary phase RP Si-NAF. It exhibited the highest separation factor (α), i.e., 1.11 for mobile phase acetonitrile/water. However, considered as the standard (reference) phase, RP Si-C_{18} exhibited the maximum separation factor (α), only 1.03 for the solvent system methanol/water. Besides, when the octadecyl column was used, the retention time was three times longer, compared with the use of the analogous aryl column.

Keywords: Stationary phases, Aryl, Octyl, Octadecyl, Benzyl, Germanium derivatives, π-π Interactions, Chromatography

INTRODUCTION

High performance liquid chromatography (HPLC) is still one of the most widespread chromatographic methods. It is applied in different variants: in micro- and macro scale, in aqueous and anhydrous systems, as well as being able to be combined with electromigration techniques. Because a column is the main element of a chromatographic system, the most intensive development of

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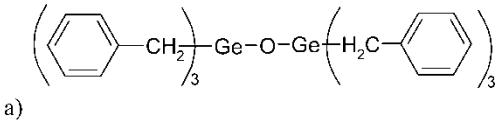
chromatographic methods is connected with the synthesis of new, specific packings.[1–6] Among commonly used packings, materials with chemically bonded phases are the most widespread type of adsorbent. Besides hydrophobic packings with hydrocarbon chains of various length, are also phases with diverse polarity, e.g., with chains ended by different functional groups. However, these packings do not give satisfactory results in every case. During separation of polar compounds, diffusion of bands and tailing of peaks can occur.[4]

Determined compounds, hexabenzyldigermoxane and hexabenzyldigermanium, belong to the category of nonpolar substances (Figure 1).[7] Obtained in above 90% yield, these products have been subjected to spectral analysis: ^{1}H NMR, ^{13}C NMR, IR, MS, and GC/MS. However, in the recorded GC/MS spectrum of hexabenzyldigermanium, neither molecular peaks nor characteristics of these compound peaks were observed. Similar difficulties appeared also in the case of hexabenzyldigermoxane (Figs. 2 and 3). Sporadically, only products of unstable ion disintegration lead to the formation of tribenzylgermanium radical $(PhCH_2)_3Ge^{\cdot}$ and cation $(PhCH_2)_3Ge^{+}$ for the digermanium derivative and $PhCH_2^{\cdot}$ and $(PhCH_2)_5GeO_2^{+}$ for the germoxane derivative. This confirms the conclusion that these compounds easily can be retarded on chromatographic columns, and cause many problems in chromatographic analysis (Fig. 3) and mass spectrometry. Other spectra attested to the presence of the main product. Obtained compounds can be used to simplify transformation into hydro/halogen derivatives and further, as a result of functionalisation, into benzylgermanium compounds with biological activity. Numerous scientific reports confirm biological activity of germanium compounds.[8,9] Analysis showed, that the toxicity of germatranes proved to be low, compared with toxicity of silatranes. Some germanium compounds showed anticancer activity, e.g., Ge-132, invented in Japan.[9]

At present, tumour diseases are among the most frequent reasons of death in humans. Hence, the reason for continuous searching of new anticancer compounds. Difficulties during synthesis, often low yields or quick disintegration, show the necessity of selection of optimal chromatography conditions



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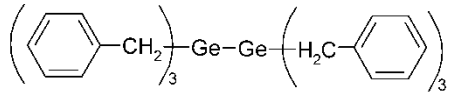

b)

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

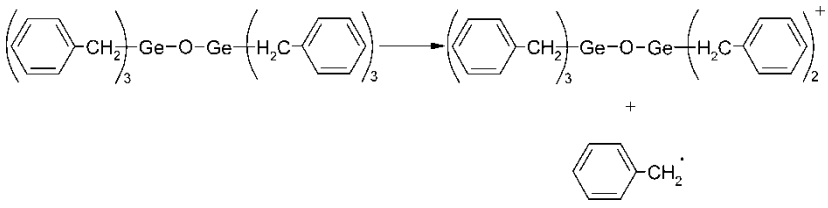

Figure 2. During MS analysis, pattern disintegration of hexabenzyldigermoxane.

for determination and separation of these compounds. This task was the aim of the present work.

EXPERIMENTAL

Materials and Methods

Germanium derivatives:[7] hexabenzyldigermoxane (**germ. 1**) (Fig. 1a) and hexabenzyldigermanium (**germ. 2**) (Fig. 1b) were dissolved in dichloromethane (purity for HPLC, Fluka AG, Buchs, Switzerland), obtaining concentration at about 12 $\mu g \cdot mL^{-1}$. The freshly prepared sample was directly subjected to HPLC analysis, at wavelength 242 nm and temperature 20°C. Three stationary phases were studied: octadecyl (S. Witko–J.T. Baker, Łódź, Poland), octyl (S. Witko–J.T. Baker, Łódź, Poland), and aryl (RP Si-NAF, Figs. 4 and 5, Table 1).[10] Dimensions of steel columns were: for RP Si-C_{18}-250 mm × 4.6 mm, for RP Si-C_8 and RP Si-NAF-125 mm × 4.6 mm. Different systems of mobile phase were applied, among others acetonitrile/water (80/20, 75/25) and methanol/water (90/10, 80/20), using flow rates 0.5 and 0.3 $mL \cdot min^{-1}$ (Table 2).

The organogermanium compounds were prepared by the method described in the literature.[7] 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, 129, 138. UV/Vis ($CHCl_3$): λ_{max} ($lg\varepsilon$) = 264 (4.57), 269 (4.55), 292 (4.53). MS (EI), m/z

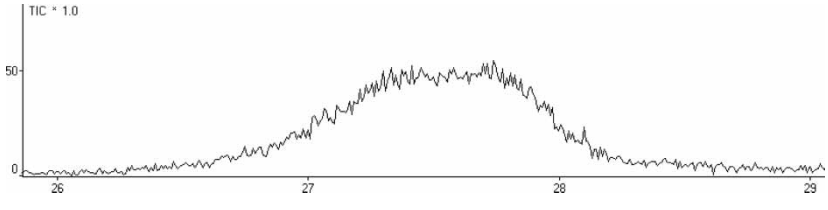

Figure 3. Diagram of GC for hexabenzyldigermoxane.

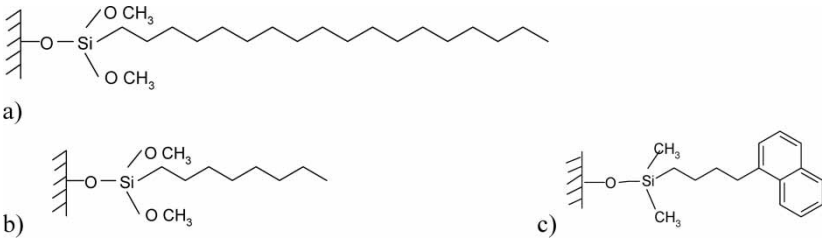

Figure 4. Scheme of chemically bonded stationary phases: a) octadecyl (RP Si-C_{18}), b) octyl (RP Si-C_8), and c) aryl (RP Si-NAF).

(% 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).[7] IR (KBr) 928 cm^{-1} (Ge - O - Ge).[7,11,12]

Hexabenzyldigermanium: ^1H NMR ($CDCl_3$): $\delta = 2.15$ (s, 12H, CH_2Ph), 6.5–6.8 (m, 12H, H-Ph), 7.0–7.3 (m, 18H, H-Ph). ^{13}C NMR ($CDCl_3$): $\delta = 24$ (CH_2Ph), 124, 128.37, 128.42, 140. UV/Vis ($CHCl_3$): λ_{max} ($lg\varepsilon$) = 267 (4.56), 269 (4.55), 277 (4.56), 289 (4.54). MS (EI).[11,12] bp. 182°C (183–184°C).[7,13] 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.

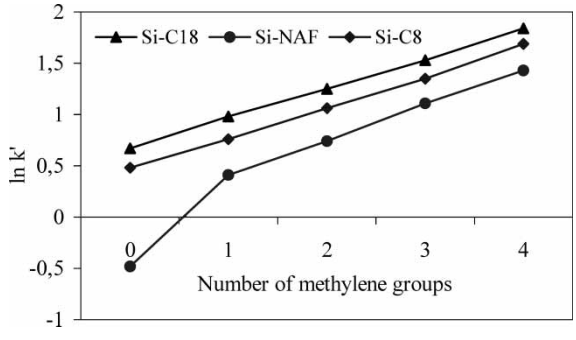

Figure 5. Dependence of ln k′ on the number of carbon atoms in alkyl chain of alkylbenzenes for octyl, octadecyl, and aryl packings. Chromatographic conditions: mobile phase 65/35 vol.% acetonitryle/water, flow rate - 1 mL · min^{-1}, wavelength - 254 nm, temperature - 20°C.

Table 1. Characteristics of bonded phase

Type of packing	Carbon content (vol.%)	Manufacturer of column	Length of column (mm)
RP Si-C_{18}	18.09	S. Witko–J.T. Baker	250 × 4.6
RP Si-C_8	13.49	Home made	125 × 4.6
RP Si-NAF	16.10	Home made	125 × 4.6

Apparatus

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.

The organogermanium compounds were prepared by the method described in the literature;[7] ^{1}H 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, Phenomenex BPX-5 column, 30 m × 0.25 mm I.D. × 0.25 μm FT, total flow rate 52.7 mL · min^{-1}.

IR spectra were recorded on a Nicolet Magna-IR 760 in (KBr) and (polyethylene).

RESULTS AND DISCUSSION

Obtained results are presented in Table 2. Optimal conditions of chromatographic determination and separation of two newly synthesized germanium derivatives have been worked out. Various compositions of mobile phase and three stationary phases: octyl, octadecyl, and aryl, were taken under consideration. The octadecyl stationary phase is considered as a standard phase in determinations of many compounds performed by HPLC. The octyl phase is applied rarely. More and more often, in order to obtain very good separations, so called dedicated stationary phases are used. Such a phase is the aryl chemically bonded stationary phase (RP Si - NAF), destined first of all to determine π electron containing compounds,[10] because in this case, interactions π-π between stationary phase and analyzed compound dominate. It enables separation of numerous mixtures of isomers and shorter retention times. This effect was also exhibited during the separation of hexabenzyldigermoxane and hexabenzyldigermanium (Table 2 and Figs. 6–8). The best results of separation of **germ. 1** and **germ. 2**, when column RP Si-C_{18} was used, could be accomplished with the solvent system methanol/water (90/10) and flow rate 0.3 mL/min. However

Table 2. Chosen dependence ln k′ for **germ. 1** and **germ. 2** from on type of stationary and mobile phase. Chromatographic conditions: −1, wavelength-242 nm, temperature-20°C

Type of stationary phase	Mobile phase, flow [mL min^{-1}]	k_1'	k_2'	$\alpha = k_2'/k_1'$
RP Si-C_{18}	Acetonitryle/water (80/20), 0.5	27.36	27.40	1.00
	Acetonitryle/water (80/20), 0.3	42.75	43.33	1.00
	Methanol/water (90/10), 0.5	42.00	42.8	1.02
	Methanol/water (90/10), 0.3	88.31	90.68	1.03
RP Si-C_8	Acetonitryle/water (80/20), 0.5	27.30	27.84	1.02
	Acetonitryle/water (80/20), 0.3	50.00	52.59	1.05
	Methanol/water (90/10), 0.5	28.33	28.52	1.01
	Methanol/water (90/10), 0.3	55.54	57.31	1.03
RP Si-NAF	Acetonitryle/water (80/20), 0.5	6.23	6.94	1.11
	Acetonitryle/water (80/20), 0.3	15.03	16.58	1.10
	Methanol/water (90/10), 0.5	17.26	17.76	1.03
	Methanol/water (90/10), 0.3	45.69	50.42	1.10

[a]Experiments which did not yield separation of determined compounds or gave retention time longer than 80 min, are omitted in the Table.

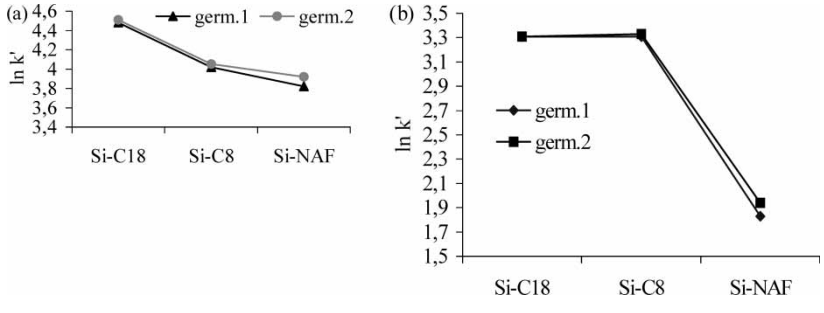

Figure 6. Effect of the separation of **(germ. 1)** and **(germ. 2)** with the use of stationary phases RP Si-C_{18}, RP Si-C_8, and RP Si-NAF. Mobile phase: (a) methanol/water (90/10, flow rate: 0.5 mL·min^{-1}, (b) acetonitrile/water (80/20), flow rate: 0.3 mL · min^{-1}, detection −242 nm (see Table 2).

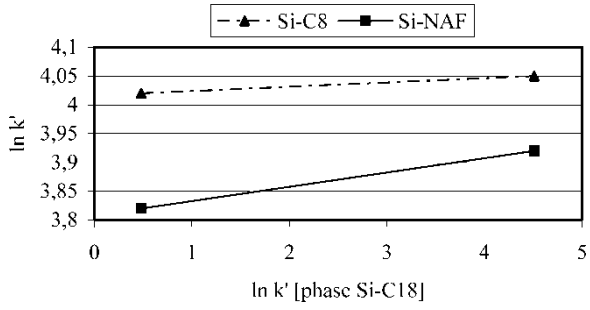

Figure 7. Dependence of ln k′ of the RP Si-C_8 and RP Si-NAF phases on ln k′ obtained with the octadecyl phase for hexabenzyldigermoxane and hexabenzyldigermanium.

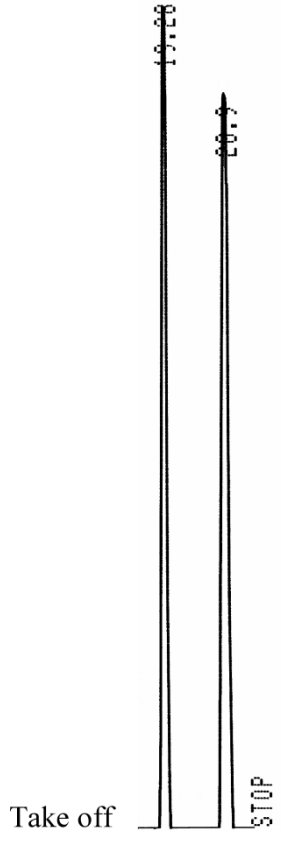

Figure 8. A chromatogram of separation of the hexabenzyldigermoxane (19.280 min) and hexabenzyldigermanium (20.900 min) on the stationary RP Si-NAF phase. Mobile phase: acetonitrile/water (80/20 vol.%); flow rate - 0.3 mL · min^{-1}, wavelength - 242 nm, temperature-20°C.

retention time for **germ. 1** was 60.728 and 62.343 for **germ. 2**. The use of the RP Si-C_8 column only slightly shortened the retention times of the analyzed compounds, and the best results were obtained for solvent system acetonitrile/water, flow rate 0.3 mL·min^{-1} (Fig. 7). Optimal separation and the shortest retention times were obtained by the use of the RP Si-NAF column, regardless of mobile phase composition or its flow rate. Optimal separation on the above mentioned column (separation factor $\alpha = 1.11$) was achieved for the mobile phase consisting of aqueous acetonitrile and flow rate 0.5 mL · min^{-1} (Fig. 7). Retention times of the analyzed compounds were 8.670 and 9.528. Similar results were obtained for other compositions of mobile phases. Data for solvent systems methanol/water 80:20 and acetonitrile 70:30 were omitted, because of the long, over 80 minutes, time of retention. Other data concerning separation of above mentioned compounds are presented in Table 2.

Optimalisation of separation conditions of hexabenzyldigermoxane and hexabenzyldigermanium, according to data (capacity factors) presented in Table 2, led to the conclusion, that aryl chemically bonded phase RP Si-NAF enables the highest selectivity, both for solvent system methanol/water (90/10, $\alpha = 1.10$) as well for solvent system acetonitrile/water (80/20, $\alpha = 1.11$). However, the phase RP Si-C_{18}, considered as standard phase, is characterized by a very long time of retention. Slightly shorter times of analysis, than for the phase RP Si-C_{18}, were obtained for the phase RP Si-C_8.

CONCLUSIONS

In order to perform chromatographic separation of hexabenzyldigermoxane and hexabenzyldigermanium in a relatively short time, the use of aryl chemically bonded phase is recommended. The use of this phase could shorten the time of analysis almost threefold, compared with the octadecyl phase, considered by many analysts as the standard phase.

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