Separation of Benzyl Derivatives of Germanium for HPLC

Mariusz Kluska[1]*, Krzysztof Pypowski[1], Ireneusz Chrzascik[1], Monika Michel[2], and **Nikolai Erchak[1]**

[1]Institute of Chemistry, University of Podlasie, Siedlce, Poland and [2]Plant Protection Institute, Pesticide Residue Department, Poznan, Poland

Abstract

The paper presents interactions of mono-, di-, tri-, and tetrabenzyl derivatives of germanium with stationary phase surface and results from these processes of determination. As a final result of the work, optimal conditions of chromatographic separation and determination of these compounds by high-performance liquid chromatography (HPLC) method are proposed. During investigations, different types of stationary phase (including octadecyl, octyl, and chemically bonded aryl phases) and mobile phases are considered. As a result of the investigation, the highest selectivity is showed chemically by bonded aryl stationary phase combined with a mobile phase consisting of acetonitrile (capacity factors of di-, tri-, and tetrabenzyl derivatives of germanium $k_1 = 0.86$, $k_2 = 2.53$, $k_3 = 6.45$). However, octadecyl phase, which is considered as reference phase, exhibits the lowest separation factors of determined benzylgermanium compounds ($k_1 = 9.12$, $k_2 = 37.62$, $k_3 = 70.43$).

Introduction

Chromatography is a basic technique for the separation of compounds of various physico-chemical characters. High-performance liquid chromatography (HPLC) is most commonly used from among different modes of chromatography. Common application of this method is a result of preparation of stable and reproducible chemically bonded stationary phases. At a rough estimation, about 90% of all chromatographic analyses are performed by so-called reversed-phase (RP) system (1).

Early papers concerning reversed-phase chromatography were published by Howard and Martin (2), Boldingh (3), and Horvath and Lipsky (4). Crucial achievement in the area of chemically bonded phase preparation has been made by Kirkland and De Stefano (5). These authors have described an adsorbent surface modification by means of poly-*n*-octadecylsilane, obtaining a product called Permaphase ODS. Then, a number of reversed-phase packing has been prepared by Majors (6), using organotrichlorosilanes for this purpose. Recently, progress in preparation of packing materials has been enabled by obtaining the selective adsorbents with properties similar to natural systems.

The new generation of packing material for biochemical separation was proposed by Pidgeon (7). Similar properties possess packing suggested by Buszewski and coworkers (8–10), containing besides silanols and unblocked terminal amino groups, *N*-acylamino groups incorporated in the hydrophobic chain. These phases are successfully used for separation of amines, proteins, and medicines with basic groups (9), as well as polycyclic aromatic hydrocarbons (PAH) with various stereochemistries (10).

Another important group of packing consists of cyclodextrines, according to Armstrong and coworkers (11). It was used to separate chiral compounds or phases with incorporated chiral active center, so-called Pirkle's phases (π-donor, π-acceptor) (12,13). It enables separation and determination of optically active compounds. Then, to separate PAHs, aryl phases (14) and distinctively multidentate phenyl-bonded phases were suggested (15). π -π interactions are dominated, and they are important factors in these phases because of the presence of π electrons. Newly obtained di-, tri-, and tetrabenzylgermanium also belong to π-containing compounds (16).

Benzyl derivatives of germanium are analogs to silicon compounds, which are proved to be practically useful for synthesis of complex organic systems (17). The chemistry of germanium compounds arouses more and more interest. Subtle physicochemical differences between silicon compounds and their germanium analogs cause higher biological activity of the latter (18).

Generally, germanium compounds have relatively low toxicity, have high biological activity, and facilitate healing of wounds. Some of them exhibit anticancer activity, [e.g., spirogermanium, 2-(3-dimethylaminopropyl)-8,8-diethyl-2-aza-8-germanium-

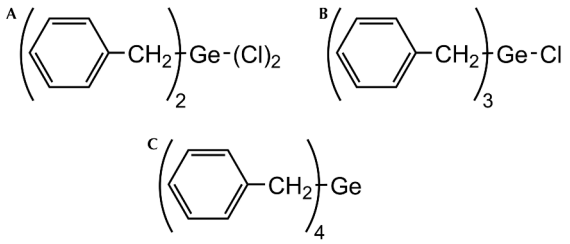

Figure 1. Structures of (A) dibenzyldichlorogermanium, (B) tribenzylchlorogermanium, and (C) tetrabenzylgermanium.

* Correspondence to: Dr Mariusz Kluska, Institute of Chemistry, University of Podlasie, 3 Maja 54, 08-110 Siedlce, Poland, e-mail kluskam@ap.siedlce.pl.

spiro[4,5]decan, and Ge-132 (3,3'-(dioxo-3,3-digermoxanediyl) bispropanoic acid)] (19,20). The discovery of a broad spectrum of Ge-132 biological activity stimulated the investigation, particularly in pharmacology for anticancer therapy (21). Ge-132 was known to be a strong analgesic compound that helps the immunological system (22). More studies over this relationship brought the conclusion that Ge-132 derivatives can penetrate the DNA structure. This fact is very essential, taking into consideration the action of anticancer preparations. The results of investigations suggest that Ge-132 is not only active in anticancer treatment but also causes the growth of interferon production, a substance whose inhibit process of protein production in cells as well as in virus organisms. It does show practically no negative influence on cells. These proprieties introduced the idea to include the germane element to pharmaceutical compounds to use its anticancer ability as well as low toxicity. Consequently, it would decrease the harmfulness of the mixtures used in medical treatment.

Ge-132 derivatives show strong activity in treatment with different types of cancer cells (23,24). It is sknown that anticancer mechanism of organogermans is not widely studied. It is supposed that they can be the important element of chemotherapy, one of the most frequently applied methods in cancer treatment in support of surgical action. Therefore, the basic aim of present chemotherapy is development of more extensive and selective working preparations on a degenerate groups of cells. The organometallic preparations still play the fundamental role in anticancer chemotherapy. They usually are the complexes of platinum and ruthenium. However, there is disadvantage concerning high toxicity and result from its low selectivity (25). Therefore, many unfavorable side symptoms are observed during treatment. As cancer diseases are among the most frequent reasons of death, research concerning new remedies and synthesis of compounds most effective against this disease is fully justified. It is connected with a necessity of selection of optimal conditions for chromatographic separation and determination. Therefore, the main aim of this work is to work out optimal conditions for the HPLC separation of di-, tri-, and tetrabenzylgermanium.

Experimental

HPLC analysis of benzyl germanium derivatives consisted of samples of di-, tri-, and tetrabenzylgermanium dissolved in acetonitrile (HPLC purity, Fluka, Buchs, Switzerland) to obtain a concentration of about 20 µg/mL (Figure 1) (16). Analyses were performed at 242 nm and at temperature 21°C. Four RP stationary phases were examined: octadecyl Si-C_{18} (250 × 4.6 mm, S.Witko - J.T. Baker, Lodz, Poland), octyl Si-C_8 (125 × 4.6 mm, home-made packing material, University of Podlasie, Siedlce, Poland), phenylbutyl Si-PB (125 × 4.6 mm, home-made packing material, University of Podlasie, Siedlce, Poland), and naphthylpropyl Si-NAF (125 × 4.6 mm, home-made packing material, University of Podlasie, Siedlce, Poland). Schemes of stationary phases and properties are presented in Figure 2 and Table I, respectively. Two anhydrous systems of mobile phase were applied: acetonitrile and dichloromethane.

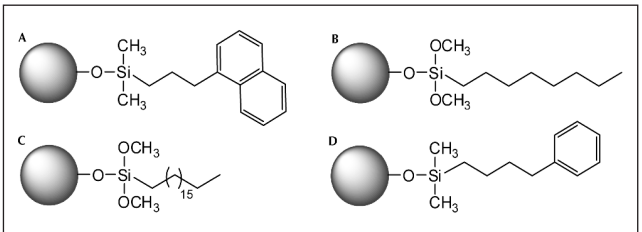

Figure 2. Scheme of chemically bonded stationary phases: (A) naphthylpropyl (Si-NAF), (B) octyl (Si-C_8), (C) octadecyl (Si-C_{18}), and (D) phenylbutyl (Si-PB).

The organogermanium compounds were prepared by the method described in the literature (16).

Dibenzyldichlorogermanium: ^{1}H NMR, δ (ppm) = 3.02 (s, 4H, CH_2Ge), 7.09 - 7.36 (m, 10H, aromat.). ^{13}C NMR, δ (ppm) = 33.92 (-CH_2-), 126.53, 128.82, 129.07, 133.22 ($C_{aromat.}$). MS (EI), m/z (%) = 328*(3.46), 182 (1.80), 104 (2.15), 91 (100). UV/Vis ($CHCl_3$): λ_{max} (lgε) = 242 (4.62), 267 (4.57), 288 (4.54).

Tribenzylchlorogermanium: ^{1}H NMR, δ (ppm) = 2.61 (s, 6H, CH_2Ge) 6.84 - 7.31 (m, 15H, aromat.). ^{13}C NMR, δ (ppm) = 26.73 (-CH_2-), 125.41, 128.59, 128.62, 136.41 ($C_{aromat.}$). MS (EI), m/z (%) = 382* (2.29), 291 (10.95), 255 (3.55), 165 (3.11), 109 (1.67), 91 (100). UV/Vis ($CHCl_3$): λ_{max} (lg ε) = 242 (4.62), 266 (4.57), 274 (4.56), 290 (4.54).

Tetrabenzylgermanium: ^{1}H NMR, δ (ppm) = 2.19 (s, 8H, CH_2Ge), 6.85 - 7.22 (m, 20H, aromat.). ^{13}C NMR, δ (ppm) = 21 (-CH_2-), 124, 128, 128.3, 140 ($C_{aromat.}$). MS (EI), m/z (%) = 438* (1.41), 347 (87.16), 267 (2.73), 253 (5.43), 165 (79.71), 151 (8.74), 139 (9.37), 91 (100). UV/Vis ($CHCl_3$): λ_{max} (lg ε) = 242 (4.62), 268 (4.55), 290 (4.52).

The infrared spectra of benzylgermanes have been examined in the region of 4000–50 cm^{-1} to assign the characteristic group frequencies in the compounds synthesized. Abbreviations used below are as follows: 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

Chromatographic measurements were performed on a liquid chromatograph SPD-6A (Shimadzu, Kyoto, Japan) equipped with an LC-6A pump, UV detector, a sampling valve Rheodyne model 7125 (Berkeley, CA) with a 20-µL sample loop, and a Shimadzu C-R6 A data recorder. ^{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

Table I. Characteristics of Bonded Phase

Type of packing	Carbon content (vol. %)	Theoretical plate number (NT)	Column dimension (mm)
Si-NAF	16.10	31684 ± 236	125 × 4.6
Si-C_{18}	18.09	15800 ± 200	250 × 4.6
Si-C_8	13.49	44944 ± 392	125 × 4.6
Si-PB	14.94	36187 ± 428	125 × 4.6

GC–MS-QP5050, 70 eV, and column Phenomenex BPX-5 (30 m × 0.25 mm i.d. × 0.25 µm FT, total flow 52.7 mL/min). IR spectra were recorded on a Nicollet Magna-IR spectrophotometer in KBr pellets. Scanning electron microscope (SEM) was used to evaluate images of the stationary phase modified by tribenzylchlorogermanium (LEO 1430VP, Zeiss, Germany). The EDX analysis was done by XFlash 4010 detector (Bruker AXS Microanalysis, Berlin, Germany).

Results and Discussion

Obtained results are presented in Table II. Determined germanium compounds (Figure 1) possess the ability to change a column surface; therefore, there are numerous difficulties in the course of chromatographic analysis (16). Tribenzylchlorogermanium can undergo changes on the surface of a stationary phase because of sterically spacious group $(PhCH_2)_3Ge$- (Figures 3–5). However, germanium compound is easily eluted from columns by a mixture of organic solvent with water in the form of hexabenzyldigermoxane $(PhCH_2)_3Ge$-O-Ge$(PhCH_2)_3$. This conversion ensues with 40% yield (26,27). Applying of anhydrous solvents (e.g., acetonitrile) causes passing of unchangeable tribenzylgermanium through columns. Because of these difficulties, determinations were conducted only with anhydrous solvents (acetonitrile and dichloromethane) with flow rates of 0.3 and 0.1 mL/min. Higher flow caused coelution of all benzyl germanium derivatives.

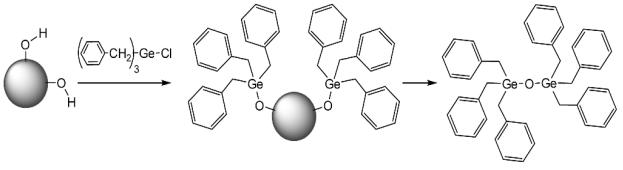

Figure 3. Scheme of normal stationary phase surface modification by tribenzylchlorogermanium.

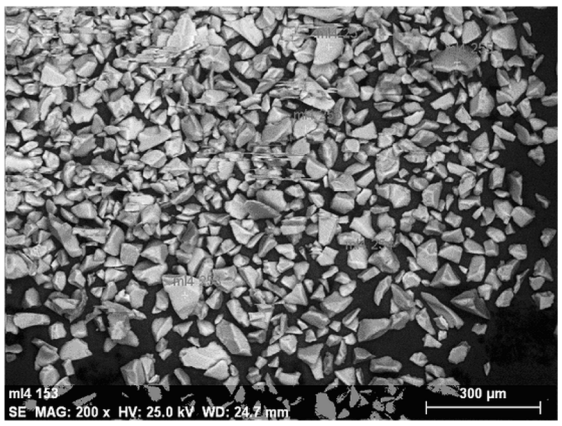

Figure 4. Image of the stationary phase modified by tribenzylchlorogermanium obtained by SEM microscope.

Dibenzyl germanium derivatives showed characteristic behavior. Dibenzylgermanyl substitute is so strongly bonded to stationary phase surface, breaking of this bond and elution of dibenzylgermoxanes (A), (B), (C) can be done only by using polar solvents (28). Their structures undergo transformations, one

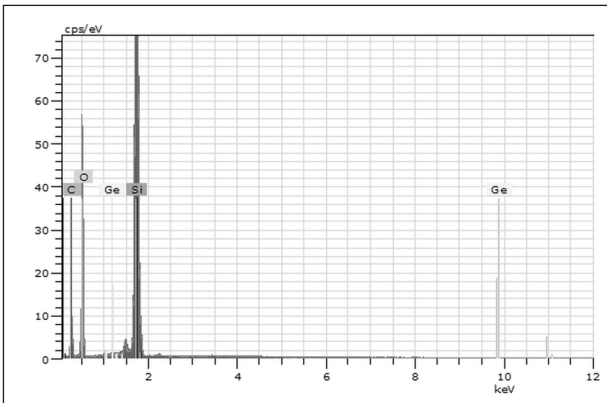

Figure 5. Diagram of the stationary phase confirmed modification by tribenzylchlorogermanium.

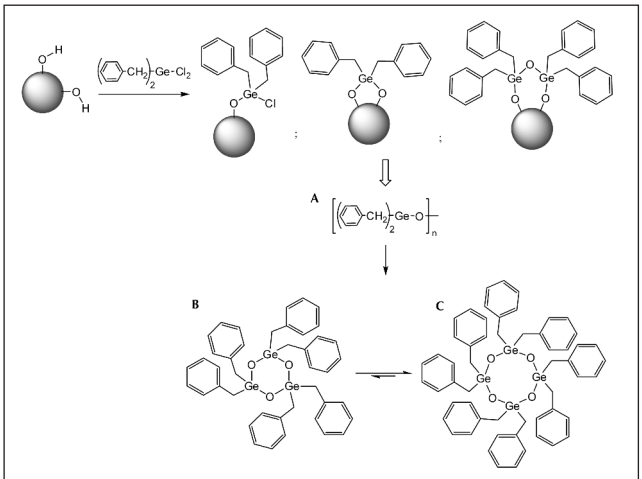

Figure 6. Scheme of normal stationary phase surface modification by dibenzyldichlorogermanium.

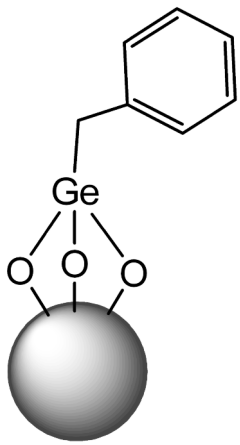

Figure 7. Scheme of normal stationary phase surface modification by benzyltrichlorogermanium.

into another (Figure 6). Because of low stability, their identification was difficult. In most cases, the structure C was obtained. The structures of analytes after elution were examined again. They answered the structures of analyzed benzyl derivatives of germanium.

However, monobenzyl derivatives of germanium form the strongest bond with the stationary phase surface. These bonds were resistant to all systems (Figure 7). Therefore, separation of monobenzyl germanium derivative was not considered in presented results (28). Application of concentrated hydrochloric or sulfuric acid led to inorganic germoxanes.

The main aim of this paper was to optimize conditions in order to separate and determinate three newly synthesized benzyl germanium derivatives (di-, tri-, and tetrabenzylgermanium). Searching for optimal process conditions, two mobile phases with various flow and four stationary phases: octyl, octadecyl, phenylbutyl, and naphthylpropyl, were considered. Acetonitrile as a mobile phase gave the best separation and shapes of determined peaks.

Currently, octadecyl stationary phase is recognized as a standard in numerous HPLC determinations. Slightly rarer octyl phase is used. In the present work, aryl chemically bonded stationary phases (Si-NAF and Si-PB) were also considered. These phases are assigned first of all to determinate π electron containing compounds (Figure 8–9) (14). Dominating effects in such chromatographic process with π-π interactions between the previously mentioned stationary phases and analyzed compounds are shown in Table II, Figures 9–10.

When three benzyl germanium derivatives were determined together with the use of acetonitrile as the mobile phase (flow 0.3 mL/min) and Si-C_{18} column, retention time of tetrabenzylgermanium was 48.582 min. For the same solvent system and Si-NAF, retention time of this compound was 7.863 min (Table II).

Table II. Chosen Dependence of k' for di-(k_1'), tri-(k_2'), and tetra-(k_3') benzyl Derivatives of Germanium from One Type of Stationary and Mobile Phase*

Mobile phase/flow rate (mL/min)	k_1'	k_2'	k_3'	$\alpha_1 = k_2'/k_1'$	$\alpha_2 = k_3'/k_2'$
Stationary phase: Si-C_{18}					
Acetonitrile/0.3	9.12	37.62	70.43	4.13	1.87
Acetonitrile/0.1	27.13	116.69	215.34	4.30	1.85
Dichloromethane/0.3	9.18	38.12	71.14	4.15	1.87
Dichloromethane/0.1	28.93	119.34	220.08	4.13	1.84
Stationary phase: Si-C_8					
Acetonitrile/0.3	1.49	5.30	10.26	3.56	1.94
Acetonitrile/0.1	3.31	10.24	24.17	3.09	2.36
Dichloromethane/0.3	1.64	5.38	11.08	3.28	2.06
Dichloromethane/0.1	3.39	10.52	25.11	3.10	2.39
Stationary phase: Si-NAF					
Acetonitrile/0.3	0.86	2.53	6.45	2.94	2.55
Acetonitrile/0.1	5.51	10.03	17.92	1.82	1.78
Dichloromethane/0.3	0.93	2.67	6.62	2.877	2.48
Dichloromethane/0.1	5.76	10.41	19.69	1.81	1.89
Stationary phase: Si-PB					
Acetonitrile/0.3	1.78	4.51	9.35	2.53	2.07
Acetonitrile/0.1	8.23	16.96	23.15	2.06	1.37
Dichloromethane/0.3	1.98	4.86	9.91	2.46	2.04
Dichloromethane/0.1	8.58	17.70	26.38	2.07	1.50

* Chromatographic conditions: flow rate, 0.3 or 1.0 mL/min; wavelength, 242 nm; temperature, –21°C.

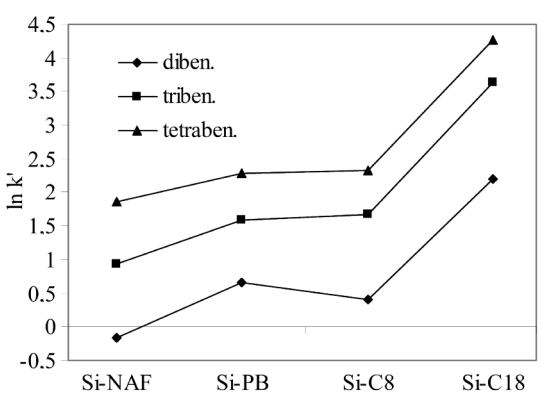

Figure 8. Effect of the separation of di-, tri-, and tetrabenzylgermanium with the use of stationary phases Si-NAF, Si-PB, Si-C_8 and Si-C_{18}. Mobile phase: acetonitrile (100 vol. %); flow rate 0.3 mL/min, wavelength 242 nm, temperature 21°C.

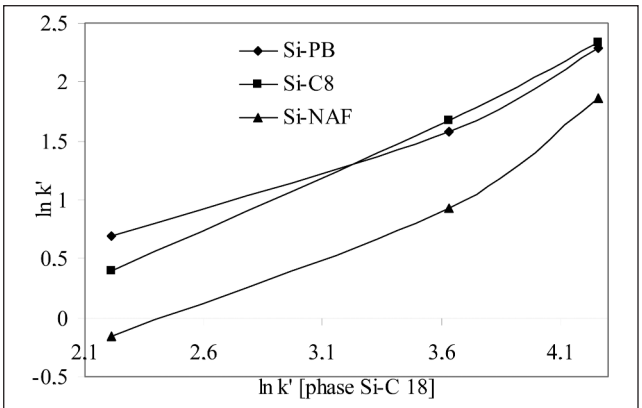

Figure 9. Dependence of ln k' of the Si-$C1_8$ and Si-NAF phases on ln k' obtained for the octadecyl phase for di-, tri-, and tetrabenzyl derivatives of germanium. Mobile phase: acetonitrile (100 vol. %); flow rate 0.3 mL/min, wavelength 242 nm, temperature 21°C.

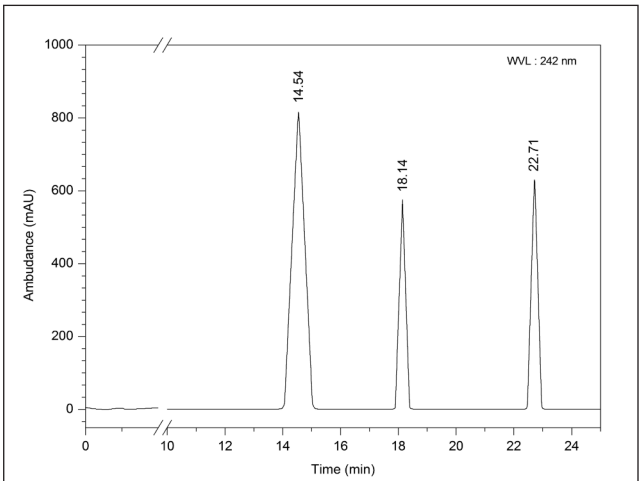

Figure 10. Chromatogram of separation of the di-, tri, and tetrabenzyl derivatives of germanium on the Si-NAF stationary phase. Mobile phase: acetonitrile (100 vol. %); flow rate 0.1 mL/min, wavelength 242 nm, temp. 21°C.

Retention times of di- and tribenzylgermanium derivatives were shorter. Decreasing of flow intensity to 0.1 mL/min caused extension of retention times. But in the case of tetrabenzylgermanium, flow was 147.131 min on the Si-C_{18} column (Table II) and 22.713 min on the Si-NAF column (Table II, Figure 10).

Shorter retention times, compared to the octadecyl column, were achieved by the use of octyl and phenylbutyl columns. Anhydrous dichloromethane was also used as a solvent and resulted in similar separations. Higher flow (above 0.3 mL/min) allowed to obtain shorter retention times, but separation was not satisfied.

Optimization of separation conditions of three benzyl germanium derivatives, according to the data presented in Table II, showed that independently a type of mobile phase, the highest selectivity can be accomplish using aryl stationary phase. Besides the best selectivity, shorter retention times are another advantage of this phase. Slightly less separation factor (α) and longer retention times yielded the phenylbutyl and octyl phases. However, octadecyl phase was recognized as a standard and showed the lowest selectivity and the longest retention times.

Conclusions

Investigated benzyl germanium derivatives exhibited an ability to change the stationary phase surface. This ability decreases with the increase of a number of benzyl groups (tetrabenzylgermanium is the least active). Optimization of HPLC separation of these compounds showed, that they can be determined first of all on columns with aryl phase and alternatively with octyl phase. Application of chemically bonded aryl phase instead of octadecyl phase enabled to one-sixth of analysis time. Therefore, recommendation of aryl (naphthylpropyl) chemically bonded phase to the determination of these compounds seems to be justified.

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