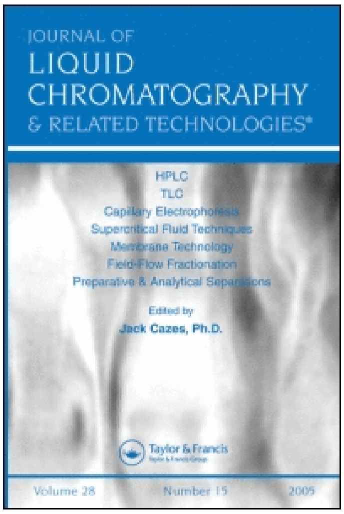

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Chromatographic Separation of Isomers of Tribenzylgermanium Nitrile Derivatives using Chemically Bonded Aryl Stationary Phases

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Abstract: The present work concerns the development and optimisation of an HPLC method with UV detection in order to separate new organogermanium compounds (3-tribenzylgermanyl-butyronitrile and 4-tribenzylgermanyl-butyronitrile). Organic germanium compounds have found application as pharmaceutical drugs, as biostimulators in agriculture, and in the electronic industry. Three types of stationary phase: octyl, octadecyl, and aryl chemically bonded phases have been investigated. The octadecyl phase is the standard phase in most HPLC methods. Also, detailed analysis of the influence of the mobile phase has been carried out. Preliminary experiments have shown that regardless of the mobile phase composition, octyl and octadecyl stationary phases are not suitable for efficient separation. The use of an aryl chemically bonded stationary phase provided the best resolution of isomers. The greatest separation ratio (1.64) has been achieved for acetonitrile/water mixture (70/30) as the mobile phase.

Keywords: Organogermanium compounds, Stationary phases: octyl, octadecyl, and aryl, Chromatography, $\pi-\pi$ Interactions

INTRODUCTION

Recently, an investigation of organogermanium compounds became a great challenge for the organic chemists. This field is very attractive to researchers because of the possibility of using germanium compounds for model studies concerning properties of the elements of the 14th group (carbon, silicon,

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germanium, lead, and tin).[1] Organogermanium compounds are a novel class of organic compounds, possessing various industrial applications. A number of germanium compounds are used in the electronic, semiconductor industry.[2] Germanium compounds have low toxicity; they are known for their biologically activity of germanium and for having a positive influence on living organisms.[3,4] Germanium compounds are less toxic than silicon compounds. Germanium occurs as the trace element in almost all living organisms.[5–8] It is well absorbed by bacteria, fungi, and seaweed. The most number of germanium compounds occurs in therapeutic plants such as ginseng, aloe, and garlic.[9] Also tomato juice, broad beams soya, and fish were proven to contain germanium compounds. Germanium compounds have been found to have a positive influence on thrombocyte activity; they can stimulate healing of injuries. They were reported to have antibacterial properties. Some of the germanium organic compounds were proven to exhibit antitumour activity, for instance 2-aza-8-germanspiro[4,5]decane-2-propamine-8,8-diethyl-N,N-dimethyl dichloride (Spirogermanium) or 3,3′–(1,3-dioxo-3,3-digermoxanediyl)bispropanoic acid (Ge-132).[4] Nowadays, when a number of cancer cases increases, the research on the antitumour medicines is of great importance. There is the need for the synthesis of more and more complicated structures, especially analogues of natural products and pharmacologically active[10,11] compounds that require the development of new methods of synthesis and analysis of organogermanium compounds. The aim of the present work was the chromatographic separation of two isomers of organogermanium compounds: 3-tribenzylgermanyl-butyronitrile and 4-tribenzylgermanyl-butyronitrile (Figure 1).

EXPERIMENTAL

Newly synthesised organogermanium compounds (Figure 1a and 1b) were dissolved in methylene chloride (HPLC-grade, Fluka AG, Switzerland). The sample of a concentration of 10 μg/mL was prepared and analyzed by high performance liquid chromatography at the following conditions: wavelength 268 nm, column oven temperature 20°C. The three types of stationary phases octadecyl (S. Witko – J.T. Baker, Łódź, Poland), octyl (S. Witko – J.T. Baker, Łódź, Poland), and aryl (RP SG–NAF, Figure 2, prepared in the

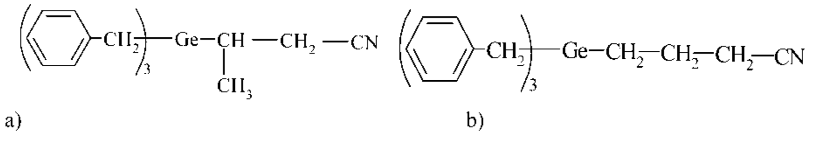

Figure 1. Structures of: a) 3-tribenzylgermanyl-butyronitrile b) 4-tribenzylgermanyl-butyronitrile.

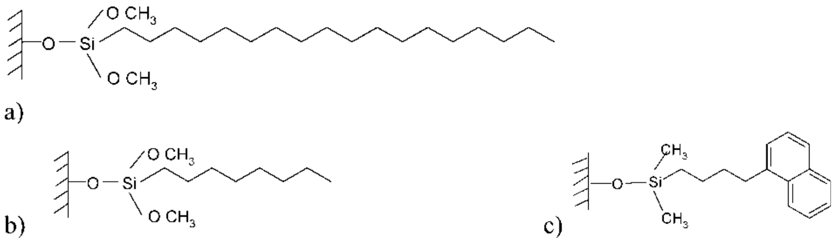

Figure 2. Scheme of chemically bonded stationary phases: a) octadecyl (RP SG-C_{18}), b) octyl (RP SG-C_8), and c) aryl (RP SG-NAF).

Department of Environmental Chemistry and Ecoanalitics, Faculty of Chemistry, Nicolaus Copernicus University,[12,13] Table 1) chemically bonded phases were tested. The sizes of steel columns were RP SG-C_{18}–250 × 4.6 mm, RP SG-C_8–125 × 4.6 mm, and RP SG-NAF–125 × 4.6 mm (Table 1). The mobile phase optimisation was carried out using acetonitrile/water (55/45, 65/35, 70/30, 100/0) and methanol/water (85/15, 100/0) mixtures at different flow rates. The organogermanium compounds were prepared by the method described in the literature.[14]

3-Tribenzylgermanyl-butyronitrile: ^{1}H NMR ($CDCl_3$): $\delta.$ = 1.14 (d, 3H, CH_3), 1.27–1.42 (m, 1H, Ge-CH), 2.10 (t, 2H, CH_2-CN), 2.33 (s, 6H, CH_2Ph), 6.78–7.28 (m, 15H, H-Ph.). UV ($CHCl_3$): λ_{max} = 254, 261, 268, 276 nm.

MS (EI): m/z (% rel. int.): [M^+] 415 (3), 373 (3), 347 (8), 324 (25), 280 (3), 257 (7), 179 (3), 165 (83), 139 (8), 91 (100), 65 (20).

4-Tribenzylgermanyl-butyronitrile: ^{1}H NMR ($CDCl_3$): δ= 0.69–0.78 (m, 2H, Ge-CH_2-C), 1.27–1.42 (m, 2H, C-CH_2-C), 2.10 (t, 2H, CH_2-CN), 2.27 (s, 6H, CH_2Ph), 6.78–7.28 (m, 15H, H-Ph.). UV ($CHCl_3$): λ_{max} = 254, 261, 268, 276 nm.

MS (EI): m/z (% rel. int.): [M^+] 415 (1), 324 (44), 296 (9), 255 (11), 165 (32), 139 (4), 115 (6), 91 (100), 65 (16).

Table 1. Characteristics of bonded phase

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

^{13}C NMR ($CDCl_3$): 12.2, 15.7 (CH_3), 17.5 (CH), 20.5, 20.7, 21.7, 119.3 (CN), 124.4, 124.7, 128.0, 128.1, 128.3, 128.5, 128.7, 139.2, 139.6.

Equipment

NMR spectra were recorded on a Bruker-200 in $CDCl_3$, with HMDS as the internal standard. MS-spectra were performed with a Shimadzu Mass-Spectrometer GC/MS-QP5050, column: Phenomenex BPX-5 30 m × 0.25 mm ID × 0.25 μm FT, total flow 52.7 mL/min.

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

RESULTS AND DISCUSSION

Table 2 shows the optimal conditions for the chromatographic separation of the compounds. The optimisation of HPLC conditions included use of

Table 2. Chosen dependence k′ for 3-tribenzylgermanyl-butyronitrile (k'_1) and 4-tribenzylgermanyl-butyronitrile (k'_2) from on type of stationary and mobile phase. Chromatographic conditions: wavelength −268 nm, flow rate: 0.5 ml × min^{-1}, temperature −20°C

Type of packings	[a]Mobile phase/	k_1'	k_2'	$\alpha = k_2'/k_1'$
RP SG-C_{18}	Methanol/water (75/25)	35.72	36.45	1.02
	Methanol/water (70/30)	36.25	37.87	1.04
	Acetonitryle/water (70/30)	53.15	54.88	1.03
	Acetonitryle/water (60/40)	55.20	57.97	1.05
RP SG-C_8	Methanol/water (75/25)	29.22	30.37	1.04
	Methanol/water (70/30)	41.44	43.14	1.04
	Acetonitryle/water (70/30)	27.46	28.32	1.03
	Acetonitryle/water (60/40)	37.66	39.33	1.04
RP SG-NAF	Methanol/water (75/25)	13.34	15.24	1.14
	Methanol/water (70/30)	21.03	23.82	1.13
	Acetonitryle/water (70/30)	9.94	16.30	1.64
	Acetonitryle/water (60/40)	17.64	23.28	1.32

[a]Experiments which did not yield separation of determined compounds or gave retention time longer than 50 min, are omitted in the Table.

different compositions of mobile phases and three types of stationary phases: octadecyl, octyl, and aryl chemically bonded phases. Octadecyl stationary phase is the standard phase for most of HPLC analysis. At different flow rates, and with different mobile phase's the octadecyl stationary phase did not provide efficient separation. Using pure methanol or a methanol/water mixture resulted with tailing and diffusion of the peaks, independently on the flow rate. Similar observations were made for pure acetonitrile or acetonitrile/water mixture. The stationary phase was the principal factor that affects the resolution, so the use of octyl stationary phase has been studied, but the separation was not improved. Nowadays, the dedicated phases (used for certain groups of compounds) are produced and aryl chemically bonded stationary phase (RP SG–NAF) is an example of such a phase. It is designated especially for π – electrons containing compounds (Figure 3).[13] Π–Π interactions are the dominating interactions between stationary phase and the analysed compounds. These interactions improve the resolution and shorten retention time. Use of an aryl stationary phase and methanol or methanol/water mobile phase resulted with tailing and diffusion of peaks. High resolution was obtained when the mobile phase is changed for a more polar phase: acetonitrile/water (70/30). The effect of the mobile phase was shown in Figures 4 and 5. Optimisation of the HPLC conditions leads to the conclusion that the aryl chemically bonded stationary phase gives the best resolution (1.64) of the organogermanium compounds (Table 2). The most efficient separation has been observed for the acetonitrile/water mixture (70/30) as the mobile phase. The separation on RP SG-C_{18} and RP SG-C_8

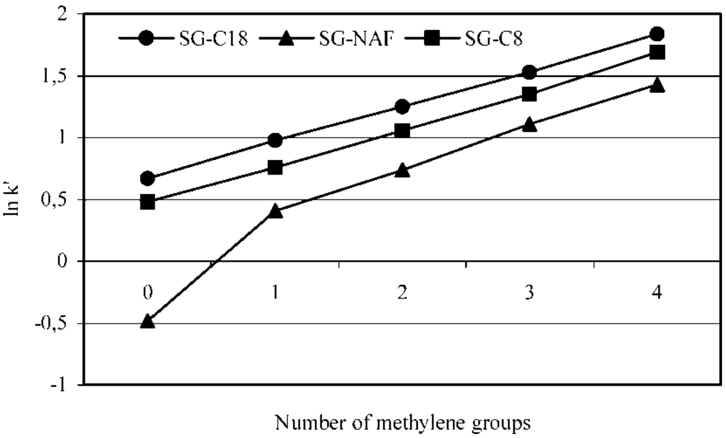

Figure 3. Dependence of ln k′ on the number of carbon atoms in the alkyl chain of alkyl-benzenes for octyl, octadecyl, and aryl packings. Chromatographic conditions: mobile phase 65/35 vol.% acetonitryle/water, flow rate:1 mL × $\min^{-1}$, wavelength −254 nm, temperature −20°C.

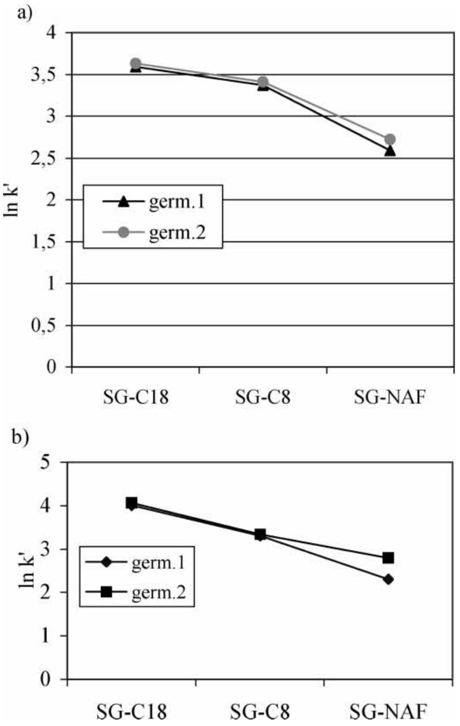

Figure 4. Effect of the separation of 3-tribenzylgermanyl-butyronitrile (germ. 1) and 4-tribenzylgermanyl-butyronitrile (germ. 2) with the use of stationary phases RP SG-C_{18}, RP SG-C_8, and RP SG-NAF. Mobile phase: (a) methanol/water (75/25), (b) acetonitrile/water (70/30), flow rate: 0.5 mL × min^{-1}; detection −268 nm (see Table 2).

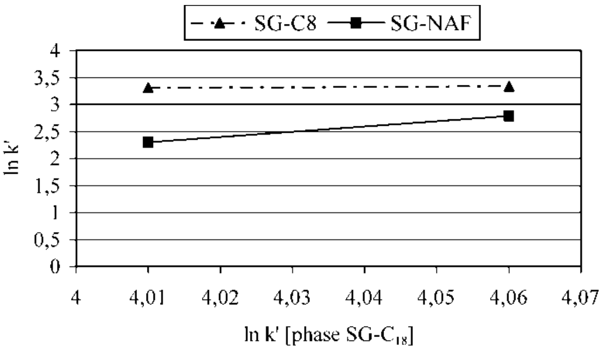

Figure 5. Dependence of ln k′ of the RP SG-C_8 and RP SG-NAF phases on ln k′ obtained for the octadecyl phase for 3-tribenzylgermanyl-butyronitrile and 4-tribenzylgermanyl-butyronitrile.

columns was incomplete, even if chromatogram showed the presence of two compounds. The properties of organogermanium compounds are not well known, and this is the reason why they cause many problems during chromatographic separation (Figure 6).

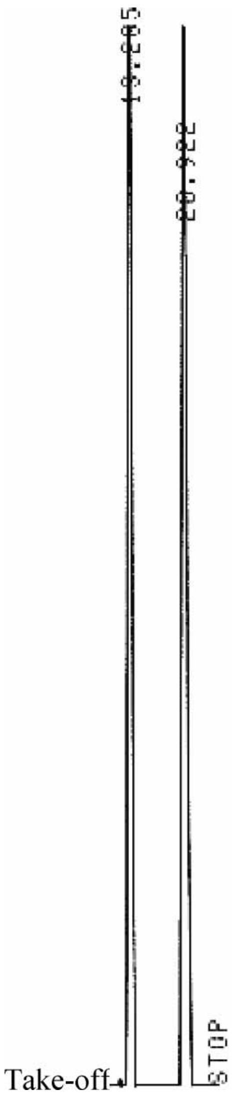

Figure 6. A chromatogram of separation of the 3-tribenzylgermanyl-butyronitrile (13.205 min.) and 4-tribenzylgermanyl-butyronitrile (20.922 min.) on the stationary RP SG-NAF phase. Mobile phase: acetonitrile/water (70/30 vol.%); flow rate:1 mL × min^{-1}, wavelength–268 nm, temperature–20°C.

CONCLUSION

The aryl chemically bonded stationary phase gives the best resolution for the separation of the isomers 3-tribenzylgermanyl-butyronitrile and 4-tribenzylgermanyl-butyronitrile. Octadecyl and octyl stationary phase do not provide the desirable results.

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