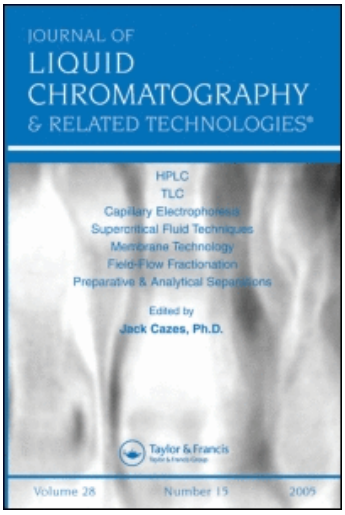

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Separation and Determination of Organogermanium Compounds by Ion Chromatography

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SEPARATION AND DETERMINATION OF ORGANOGERMANIUM COMPOUNDS BY ION CHROMATOGRAPHY

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ABSTRACT

For nearly three decades, organogermanium compounds have become increasingly of interest owing to their extensive physiological and pharmaceutical activity. In this paper, two new high performance ion chromatographic methods for separation and determination of three kinds of organogermanium compounds β-carboxyethylgermanium sesquioxide (I), β-(α-methyl)-carboxyethylgermanium sesquioxide (II) and d-(β-carboxyethyl)germanium hydroxide (III) were proposed. A Dionex DX-300 ion chromatograph equipped with a Dionex PED- II pulsed electrochemical detector (conductivity mode), and a Dionex AI-450 chromatography workstation was employed. The separation was achieved by using ion-exchange or ion-exclusion mechanism.

The detection limits(S/N=3, expressed as germanium) for the three compounds were all below sub- μg/mL level. The methods have been applied to the analysis of tonic oral drinks, and the average recoveries for the three compounds range from 95 - 108%. The results obtained were in agreement with those of hydride generation atomic fluorescence spectrometry (HG-AFS).

INTRODUCTION

Since the late 1960s, the study on organogermanium compounds has been receiving considerable attention. It has been proved that many types of organogermanium compounds possess various kinds of biological activity: analgesic, hypotensive, fungistatic, bactericidal, antiviral, antimalarial, radioprotective, antitumor, interferon-inducing, and immunomodulating.[1] Among them, β-carboxyethylgermanium sesquioxide (Ge-32) was probably the most important compound that has been used as antitumor agent and immune adjuvant for clinical application. It was also approved to add in some kinds of tonic oral drinks in Japan and China due to its very low toxicity. In addition, its analogs such as β-(α-methyl)carboxyethylgermanium sesquioxide have similar bioactivity.[2] Further study revealed that the effects of various organogermanium compounds including β-carboxyethylgermanium sesquioxide and β-(α-methyl)carboxyethylgermanium sesquioxide on some enzymes were somewhat different.[3] In recent years, Chinese scholars have synthesized many kinds of β-carboxyethylgermanium sesquioxide analogs,[4] among which di-(β-carboxyethyl)germanium hydroxide is one of the latest species.[5] It probably has more potential than β-carboxyethylgermanium sesquioxide in clinical application owing to its better solubility in water as well as in organic solvents such as ethanol and acetone.

So far, the majority of the methods for determining organogermanium compounds were in relation to β-carboxyethylgermanium sesquioxide. The indirect method was based on determination of Ge^{4+} after acid digestion.[6] A thin layer chromatographic method coupled with fluorodensitometry detection was proposed for determining the purity of β-carboxyethylgermanium sesquioxide raw material,[7] but it is not suitable for real sample analysis. For the analysis of real drinks samples containing β-carboxyethylgermanium sesquioxide, four methods were reported , one was based on solvent extraction[8] and another on separation by using Dowex 1-X2 resin;[9] the quantifications were all carried out by atomic absorption spectrometry after acid digestion. In another study, we have also found that β-carboxyethylgermanium sesquioxide can not react with tetrahydroborate in acid media. On the basis of this,

inorganic germanium was determined directly and total germanium was determined after digestion by using hydride generation atomic absorption spectrometry (HG-AAS) or hydride generation atomic fluorescence spectrometry (HG-AFS); the difference of the two values was the amount of β-carboxyethylgermanium sesquioxide.[10,11] The HG-AFS method has been verified through inter-laboratory collaboration tests and applied to the investigation on β-carboxyethylgermanium sesquioxide content in tonic oral drinks held by Ministry of Public Health, P. R. China.[12] Recently, Zhang et al.[13] reported a simple differential pulse polarographic method for determination of β-carboxyethylgermanium sesquioxide based on its forming complex with 3,4-dihydroxy benzaldehyde. Considering the similar syntheses' method, possible transformation in organisms and different toxicity of β-carboxyethylgermanium sesquioxide analogs,[14] it is necessary to establish a method for the separation and determination of β-carboxyethylgermanium sesquioxide analogs. In this paper, two ion chromatographic methods for separation and determination of β-carboxyethylgermanium sesquioxide (I) and its two analogs - β-(α-methyl)carboxyethylgermanium sesquioxide (II) and di-(β-carboxyethyl)germanium hydroxide(III) were proposed, and applied to the analysis of tonic oral drinks. The results obtained were in good agreement with those of HG-AFS.[12] These methods will possibly be used to quality control in manufacture process and study of metabolism in organisms for these three analogs.

MATERIALS AND METHODS

Apparatus

All experiments were performed on a Dionex Model DX-300 ion chromatograph equipped with an 110-μL sample loop and a Dionex Model PED-II pulsed electrochemical detector in the conductivity detection mode. A Dionex AI-450 chromatography workstation was employed for data acquisition, data reduction and control of the ion chromatograph.

The ion-exclusion separation was achieved by using a Dionex IonPac ICE -AS6 column as separation column and 0.075 mmol/L heptafluorobutyric acid as eluent. A Dionex HPICE-AS1 column was used for comparison. The flow rates of eluents were 1.0 mL/min for IonPac ICE-AS6 column and 0.8 mL/min for HPICE-AS1 column, respectively. Chemical suppression was effected by a Dionex AMMS-ICE suppressor with 8.0 mmol/L potassium hydroxide as regenerant flowing at a rate of 1.8 mL/min. For the ion-exchange method, the

gradient program separation was carried out by using a Dionex IonPac AG4A-SC guard column and a Dionex IonPac AS4A-SC separation column. A Dionex AMMS-1 anion micromembrane suppressor was employed, 1.5 mmol/L and 10 mmol/L sodium tetraborate solutions were chosen as eluents, 50 mmol/L sulfuric acid solution as regenerant. The flow rates of eluent and regenerant were set at 1.0 mL/min and 3.0 mL/min, respectively.

Reagents

β-carboxyethylgermanium sesquioxide (purity: 99.4%) was synthesized by Guangzhou Institute of Military Medicine, China. β-(α-methyl) carboxyethylgermanium sesquioxide (purity > 99%) and di-(β-carboxyethyl)germanium hydroxide (purity > 99%) were both synthesized by Changchun Institute of Applied Chemistry of Academia Sinica. The stock solutions (1 mg/mL, expressed as germanium) of organogermanium compounds were separately prepared by dissolving appropriate amounts in hot water. Working solutions were prepared by diluting the stock solutions with water for ion-exchange separation and relevant eluent for ion-exclusion separation. The other reagents were of analytical reagent grade or higher purity except for heptafluorobutyric acid (Pfaltz & Bauer, Inc.) for which purity was 97%. Distilled deionized water was used throughout.

Procedure

A solution containing ^{I}, ^{II}, and ^{III} was injected into the ion chromatograph via a syringe. All calculations were based on peak area measurements. The amount of organogermanium compounds was expressed as germanium.

RESULTS AND DISCUSSION

The molecular structures of the three organogermanium compounds in this study are illustrated in Fig.1a,[5,15] and they can all dissolve in aqueous solution that exist as the form of organic acids[5,16] shown in Fig.1b. The pKa value of ^{I} was reported as 4.26.[17] Though the pKa value of ^{II} was not clear, the existence of α-methyl in ^{II} makes a slight difference of pKa values between ^{I} and ^{II}; to be exact, the acidity of ^{II} is relatively weaker since methyl has repulsive force against electron.

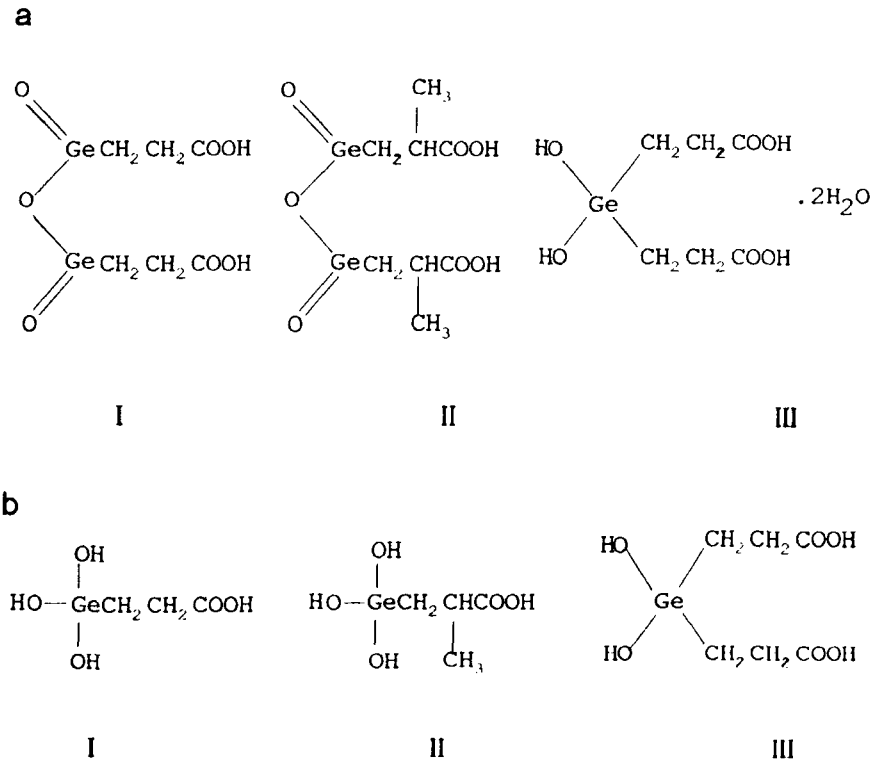

Figure 1. The molecular structures (a) and existing forms in aqueous solution (b) of three organogermanium compounds.

Preliminary study showed that the pKa1value and pKa2 value of [III] are 2.52 and 4.30, respectively.[5] So it was possible to separate one another by either ion-exchange method or ion-exclusion method. The detailed experimental conditions will be discussed separately in the following sections.

Ion-Exclusion Method

In general, ion-exclusion method is commonly used for separation of weakly ionized anions including organic acids and inorganic weak acids. For practical work, it is the most important to choose appropriate separation column and eluent. In this study, two separation columns (HPICE-AS1 and IonPac ICE-AS6) were used for comparison to decide which one can give better performance. The relatively slower flow rate of eluents for HPICE-AS1 is to

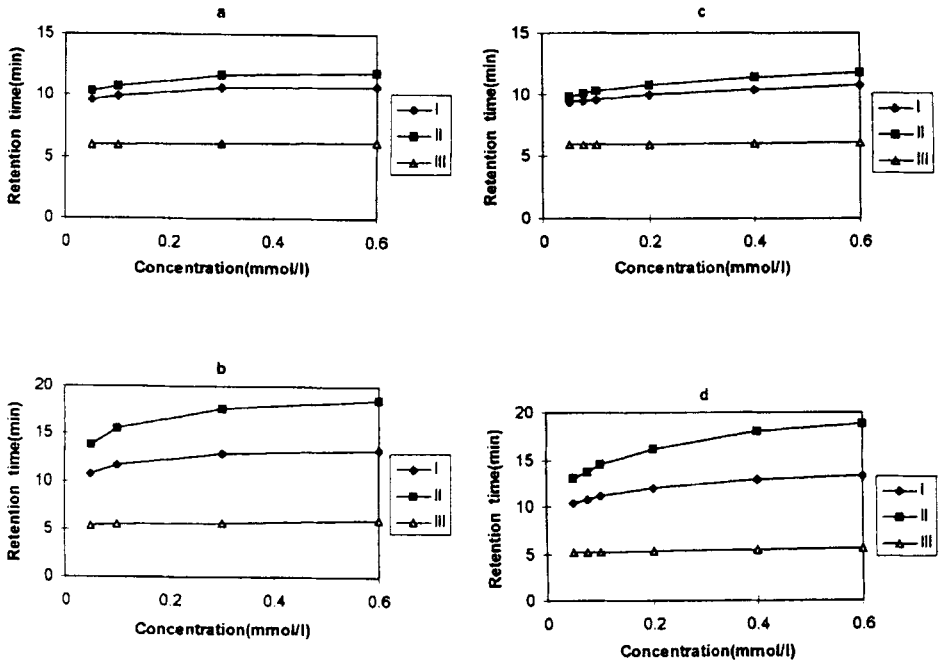

Figure 2. Effect of eluent concentration on retention time. (a) HPICE-AS1 column, sulfuric acid eluent; (b) IonPac ICE-AS6 column, sulfuric acid eluent; (c) HPICE-AS1 column, heptafluorobutyric acid eluent; (d) IonPac ICE-AS6 column, heptafluorobutyric acid eluent.

maintain not-too-high column pressure. In addition, different concentrations of sulfuric acid and heptafluorobutyric acid were employed for eluent selection. The relationships between retention time and eluent concentration by using various columns are shown in Fig.2.

It is well known that in ion-exclusion chromatography, the higher the pKa value of analyte the greater the retention. As expected, the three compounds were eluted out in sequence of ^{III}, ^{I}, ^{II}, and the retention time of all compounds increased by increasing the eluent concentration (Fig.2). It can be also observed from Fig. 3 that the baseline separation of ^{I} and ^{II} was not achieved on HPICE-AS1 column by using different concentrations of eluents although the relatively optimum eluent condition was found to be 0.1 mmol/L sulfuric acid or 0.2 mmol/L heptafluorobutyric acid. The cases of poor resolution for ^{I} and ^{II} may be the slight difference of acidity and poor efficiency of ion-exclusion stationary phase containing only sulfonic acid groups.

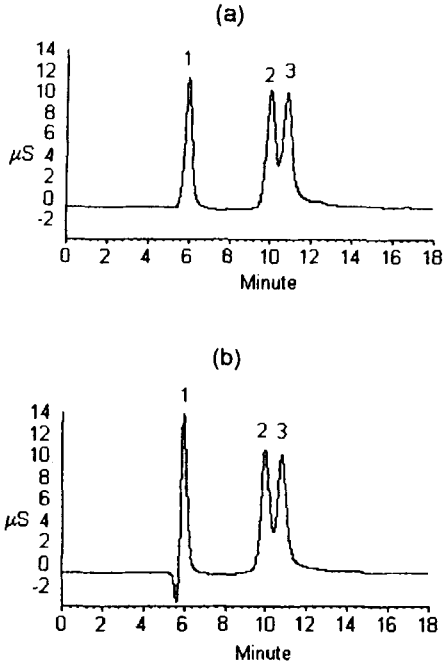

Figure 3. Separation of organogermanium compounds on HPICE-AS1 column. Peaks: 1 = ^{III} (20 μg/mL); 2= ^{I} (50 μg/mL); 3= ^{II} (50 μg/mL). Eluents: (a) 0.2 mmol/L heptafluorobutyric acid; (b) 0.1 mmol/L sulfuric acid.

Compared with HPICE-AS1 column, the IonPac ICE-AS6 column has hydrophobic functional groups within the resin structure which promote adsorption and hydrogen bonding besides sulfonic acid groups. These two additional retention mechanisms allow resolution of organic acids which are poorly resolved by ion-exclusion alone. In fact, as shown in Fig. 4, it was utilization of this separation method that reach the baseline resolution of the three compounds. Hence, the further study was carried out on the IonPac ICE-AS6 column.

In view of eluent, heptafluorobutyric acid is the typical choice in ion-exclusion chromatography due to the low conductivity of the product after chemical suppression which resulted in low detection limit, but the higher price restricts its intensive application. The cheaper sulfuric acid was used for comparison with heptafluorobutyric acid in this study, and the baseline separation of three compounds can be achieved with both of the acids as shown in Fig. 4.

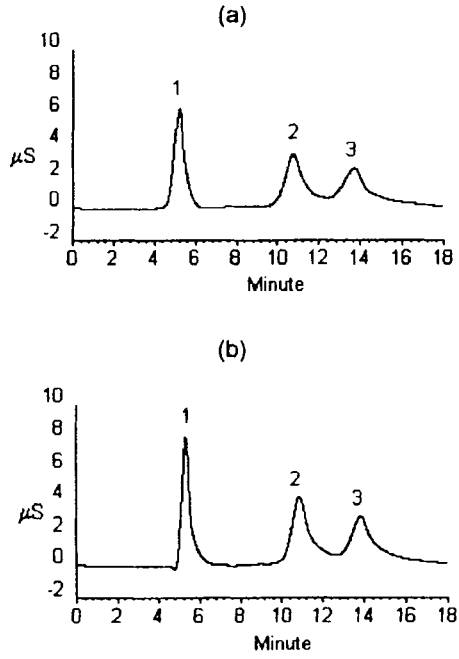

Figure 4. Separation of organogermanium compounds on IonPac ICE-AS6 column. Peaks as in Fig. 3. Eluents: (a) 0.075 mmol/L heptafluorobutyric acid; (b) 0.05 mmol/L sulfuric acid.

The further study on eluents concentration indicated that ^{I} and ^{II} would be partially overlapping (Rs<1.5) if the acid concentrations were too low, that is to say, the concentrations of heptafluorobutyric acid and sulfuric acid were below 0.07 mmol/L and 0.04 mmol/L, respectively. When the acid concentrations were too large, the peaks of ^{I} and ^{II} would become tailed peaks. Though the addition of organic modifier could improve the peak shapes of ^{I} and ^{II}, the resolution of ^{I} and ^{II} became poor because the decrease of retention time of ^{II} was greater than that of ^{I}, which can be explained by the stronger hydrophobicity of ^{II}. The optimum experiment conditions should meet two aspects of demands, baseline separation (Rs>1.5) and run time as short as possible. So, the eluent concentrations adopted were 0.075 mmol/L for heptafluorobutyric acid and 0.05 mmol/L for sulfuric acid, respectively, and heptafluorobutyric acid was preferred for real sample analysis because an apparent negative water peak would appear very near to the peak of ^{III} resulting in an inaccurate result by using sulfuric acid.

Ion-Exchange Method

It is also possible to analyze many organic acids by using anion-exchange method. Under the typical basic anion-exchange eluent conditions, the three organogermanium compounds existed as monovalent anions for ^{I}, ^{II}, divalent anion for ^{III}, respectively. It was proved by experiments that the commonly used eluents such as sodium carbonate, sodium carbonate/sodium bicarbonate and sodium bicarbonate could not give good separation for ^{I} and ^{II} which could be explained by weak acidity, identical charge and structure similarity of ^{I} and ^{II}, as well as high ionic strength of these eluents. The separation of ^{I} and ^{II} was improved by using sodium tetraborate eluent, and their baseline resolution was achieved when the concentration of eluent was as low as 1.5 mmol/L. Contrary to ion-exclusion method, the elution order was ^{II}, ^{I} and ^{III}. Since ^{III} exists as divalent anion in basic solution, the retention would be greater on stationary phase. Therefore, though three organogermanium compounds were separated by 1.5 mmol/L sodium tetraborate, the whole run time would last 70 min and the peak of ^{III} would be too flat to be identified and quantitatively measured. A better solution to this problem was utilization of a gradient program which consisted of two eluents: 1.5 mmol/L sodium tetraborate (E1) and 10 mmol/L sodium tetraborate (E2). The separation was completed within 40 min.

Because the maximum flow rate of regenerant used for AMMS-1 suppressor must not be higher than 3.0 mL/min, the suppression ability was limited. To avoid severe change of baseline, 10 mmol/L sodium tetraborate was adopted as the higher ionic strength eluent. The detailed gradient program was listed in Table 1, and the chromatogram was shown in Fig. 5. It needed about 10 min for equilibration prior to another injection.

Linearity, Precision, and Detection Limits

Under the optimum experimental conditions of both methods, the three compounds all showed good linearity. The detection limits, which are defined as the concentrations that give the peak intensity three-fold the baseline noise, were also calculated. The results are summarized in Table 2. It can be found that the detection limits obtained by using ion-exchange method are lower than those by ion-exclusion method though its run time is much longer.

For ion-exclusion method, the relative standard deviations for seven replicated analyses of a mixed standard solution containing 50 μg/mL ^{I}, 50 μg/mL ^{II} and 10 μg/mL ^{III} were 1.74 % for ^{I}, 1.97% for ^{II} and 2.45 % for ^{III}, respectively.

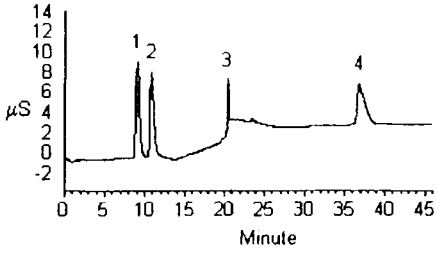

Figure 5. Separation of organogermanium compounds on IonPac AG4A -SC and IonPac AS4A-SC columns. Peaks: 1= ^{II} (20 μg/mL); 2= ^{I} (20 μg/mL); 3= system peak; 4= ^{III} (5 μg/mL).

Table 1

Gradient Program for Ion-Exchange Method

Time (Min)	E1 (%)	E2 (%)
0	100	0
11.0	100	0
20.5	0	100
45.0	0	100
45.1	100	0

For ion-exchange method, the relative standard deviations for seven replicated analyses of another mixed standard solution which contained 20 μg/mL ^{I}, 20 μg/mL ^{II} and 10 μg/mL ^{III} were 1.27 % for ^{I}, 2.87% for ^{II} and 1.22% for ^{III}, respectively.

Sample Analysis

Two kinds of tonic oral drinks which contained ^{I} (declared on labels by manufacturers) were centrifuged at 4000 rpm for 10 min to remove the suspension; 0.25 mL supernatant of sample 1 or 0.50 mL supernatant of sample 2 was transferred accurately into a 25 mL volumetric flask, and diluted to volume with 0.075 mmol/L heptafluorobutyric acid for ion-exclusion or water for ion-exchange separation. The diluted solution was injected after filtration through a 0.2 μm Gelman filter.

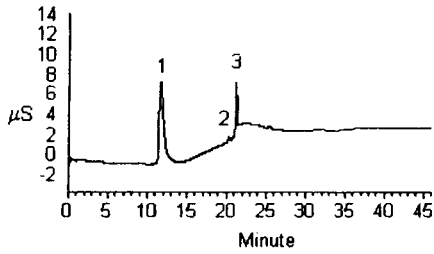

Figure 6. Chromatogram of sample 1 diluted solution on IonPac AG4A -SC and IonPac AS4A-SC columns. Peaks: 1= [I]; 2= chloride; 3= system peak.

Table 2

Linearity and Detection Limits for Organogermanium Compounds

Method	Analyte	Concentration Range (μg/mL)	Correlation Coefficient	Detection Limit (μg/mL)
Ion-exclusion	I	4 - 100	0.9994	0.14
	II	6 -100	0.9984	0.24
	III	1- 20	0.9999	0.053
Ion-exchange	I	2-50	0.9992	0.038
	II	2-50	0.9979	0.035
	III	1-20	0.9997	0.025

Since the most important synthesis path of [I] and its analogs was hydrolysis of relevant chlorides,[14,16] the real samples may contain chloride ion. The peak of chloride appeared in a chromatogram of real samples when using ion-exchange method (shown in Fig.6). When using ion-exclusion method, the chloride eluted out very near void volume, which interfered with the determination of [III].

So in this method, the real samples should be pretreated by using Dionex OnGuard-Ag Cartridge to remove chloride prior to dilution. As shown in Fig.7, the unknown peak which appeared in the chromatogram did not interfere with the determination of [III] in spiked sample.

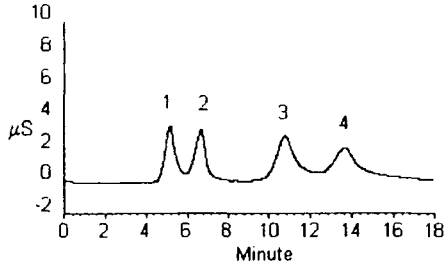

Figure 7. Chromatogram of spiked sample 1 diluted solution on IonPac ICE-AS6 column. Peaks: 1= [III], 2= unknown; 3= [I], 4= [II]. The spiked concentrations: [I] 20 μg/mL; [II]40 μg/mL; [III]10μg/mL.

Table 3

Analysis of Organogermanium Compounds in Tonic Oral Drinks

Sample	Analyte	Concentration (μg/mL)[a]		Added (μg/mL)	Recovery (%)[b]		Result of HG-AFS (μg/mL)[c]	Label Claim (μg/mL)
		Exclusion	Exchange		Exclusion	Exchange		
1#	I	21.59±0.19	21.29±0.44	20	107.8±3.69	95.35±1.20	20.38±0.39	21.40±1.07
	II	ND	ND	40	97.92±2.31	101.0±1.54	----	----
	III	ND	ND	10	98.36±1.08	96.79±0.61	----	----
2#	I	24.42±0.48	25.74±0.14				24.86±0.46	----
	II	ND	ND				----	----
	III	ND	ND				----	----

[a] Average of five determinations ± standard deviation.
[b] Average of four determinations ± standard deviation.
[c] Average of three determinations ± standard deviation.

The recoveries of the methods were determined in quadruplicate by spiking standard solutions in 1:100 diluted sample 1 solutions using both methods.

The results obtained are shown in Table 3; they were consistent with those of HG-AFS[12] and the manufacturer's label claim.

CONCLUSIONS

Two procedures have been developed for the separation and determination of three kinds of organogermanium compounds with ion chromatography. Either method is suitable for the analysis of tonic oral drinks and will probably be applied for metabolism study in future.

ACKNOWLEDGMENT

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REFERENCES

1. M. P. Egorov, P. P. Gaspar, "Germanium: Organometallic Chemistry" in **Encyclopedia of Inorganic Chemistry**, Vol. 3, R. B. King, eds., John Wiley & Sons, Inc., Chichester, 1994, pp.1317.

2. H. Orimo, K. Miyao, Ger. Pat., DE 3 720 151 (1987).

3. T. Komuro, N. Kakimoto, T. Katayama, T. Hazato, Biotechnol. Appl. Biochem., **8**, 379-386 (1986).

4. S. G. Zhang, C. X. Lu, J. W. Cai, J. Z. Ni, Y. X. Nie, Y. L. Chen, Yingyong Huaxue, **10,** 83-85 (1993).

5. S. G. Zhang, Chin. Pat. Appl. No. 95100844.7 (1995).

6. Q. Sun, H. T. Wang, S. F. Mou, J. Chromatogr. A, **708**, 99-104 (1995).

7. P. S. Xie, Y. Z. Yan, C. M. Yu, M. Q. Shen, J. Planar Chromatogr. - Mod. TLC, **3**, 141-143 (1990).

8. H. Oshima, I. Saito, N. Kawamura, M. Yamada, Eisei Kagaku, **36**, 219-225 (1990).

9. K. Itano, K. Imura, K. Sasaki, Shokuhin Eiseigaku Zasshi, **33**, 231 -236 (1992).

10. D. Q. Zhang, Z. M. Ni, Anal. Chim. Acta, **330**, 53-58 (1996).

11. Q. C. Chen, H. F. Yang, Guangpuxue Yu Guangpu Fenxi, in press.

12. Q. C. Chen, H. F. Yang, Zhongguo Gonggong Weisheng, **11**, 543 (1995).

13. L. Zhang, H. Q. Fang, H. Y. Chen, S. T. Zhu, Fenxi Huaxue, **23**, 369-373 (1995).

14. S. G. Zhang, Huaxue Tongbao, **9**, 11-21 (1993).

15. F. Glockling, **Gmelin Handbook of Inorganic and Organometallic Chemistry, Ge Organogermanium Compounds, Part 5**, Springer-Verlag, Berlin and Heidelberg, 1993, pp. 299-300.

16. S. G. Zhang, G. Q. Shangguan, J. Z. Ni, Huaxue Xuebao, **48**, 879-883 (1990).

17. N. Wang, G. Q. Shangguan, Y. J. Chen, Y. M. Chen, Jining Yixueyuan Xuebao, **15**, 30-32 (1992).

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