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Separation and determination of inorganic germanium and β-carboxyethylgermanium sesquioxide by high-performance ion-exclusion chromatography

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Abstract

A novel method for the separation and determination of inorganic germanium and β-carboxyethylgermanium sesquioxide (Ge-132) by using high-performance ion-exclusion chromatography was proposed. The separation was performed by an isocratic elution and the determination by conductivity detection in combination with visible absorbance detection at 505 nm in series. Several possible detection modes were compared and the conditions of post-column reaction were optimized. Under the specified experimental conditions, the peak area response was proportional to the concentration of inorganic germanium in the range of 0.05–5 μg ml^{-1} and of Ge-132 in the range of 0.1–100 μg ml^{-1}, respectively. The method has been successfully applied to the analysis of tonic oral liquids, and the results obtained were in agreement with those of hydride generation atomic fluorescence spectrometry. Additionally, this method has been used for the preliminary study of Ge-132 transformation in vivo: inorganic germanium was not detected in the urine excreted by rats administered Ge-132.

Keywords: Food analysis; Germanium; Carboxyethylgermanium sesquioxide; Organogermanium compounds; Inorganic cations

1. Introduction

For nearly three decades organogermanium compounds have been studied with increasing interest owing to their extensive physiological and pharmaceutical activities. Among them, β-carboxyethylgermanium sesquioxide (Ge-132) was probably the most important compound having been considered to have an antitumor effect, an inhibitory effect on amyloidosis as well as possessing immunomodulative activity thus allowing its use as an antitumor agent and immune adjuvant for clinical application [1]. Additionally, other organogermanium compounds proved to have similar bioactivity. Therefore, the germanium boom started in Japan in the 1970s [2] and the health foods supplmented with various germanium compounds, including germanium dioxide (GeO_2), were widely used as elixirs firstly in Japan and later in Germany, the USA, the UK and China, among other countries [3]. Unfortunately, since the first case of death caused by intake of a germanium-containing preparation was reported in 1985 [4], at least thirty patients with persistent renal dysfunction following chronic intake of germanium-containing compounds have been found in Japan [5–8] and one in Europe [9]: among them six were fatal. Hence, the germanium-containing preparations and foods were banned in some countries including the UK [2]. In fact, the compound intaken by the majority of the above patients

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was GeO_2 (inorganic germanium), which was confirmed by a variety of analytical methods. Further animal studies [3,8,10] revealed that GeO_2 could cause characteristic nephropathy while Ge-132 did not and Ge-132 could not exacerbate renal dysfunction already present. To date, Ge-132 is considered generally as a safe compound for application and still permitted as an additive in some kinds of tonic oral liquids in Japan and China. Because there is a significant difference in toxicity between inorganic germanium [Ge(IV) form in aqueous solution] and Ge-132 and the ingredient of some health foods containing GeO_2 is labeled illegally as Ge-132 [8] as well as the possibility that Ge-132 converts to inorganic germanium in vivo cannot be absolutely precluded [11], it is vital to establish a method for the determination of Ge(IV) and Ge-132 that can be used for component monitoring of tonic oral liquids and analysis of biological materials including urine.

Although spectrophotometry and electrothermal atomic absorption spectrometry (AAS) were the most commonly used methods for germanium determination their application for real sample analysis was restricted mainly due to their poor speciation analysis capabilities. Two AAS methods were proposed for the determination of Ge(IV) and Ge-132 after specific separation procedures [12,13], and the quantifications were all carried out by AAS after acid digestion. In recent years hydride generation (HG) has proved to be a very useful analytical separation and preconcentration technique for germanium [14], and Ge-132 was found by the present authors not to form volatile germane in acid media upon reduction. On this basis, Ge(IV) was determined directly and total germanium was determined after acid digestion by using HG-AAS [15] or HG-atomic fluorescence spectrometry (AFS) [16]: the difference of the two values was the amount of Ge-132. The latter method has been verified through inter-laboratory collaboration tests and used in the investigation of germanium-containing tonic oral liquids held by The Ministry of Public Health, China [17]. In addition, a differential pulse polarographic method [18] for simultaneous determination of Ge(IV) and Ge-132 was proposed. To the best of our knowledge no ion chromatography for the separation and determination of Ge(IV) and Ge-132 has been reported. In this paper, a novel method for the simultaneous determination of Ge(IV) and Ge-132 by using high-performance ion-exclusion chromatography (HPICE) was proposed and applied to the analysis of tonic oral liquids with satisfactory results. This method was also used successfully for the preliminary study of Ge-132 transformation in vivo and the results indicated its applicability for the further chronic toxicity study of Ge-132.

2. Experimental

2.1. Apparatus

A Dionex Model DX-500 ion chromatograph (Sunnyvale, CA, USA) equipped with an 110 μl sample loop was employed along with a Dionex PeakNet chromatography workstation for instrument control as well as data acquisition and processing. Separation was performed by a Dionex HPICE-AS1 separation column and the detection by a Dionex ED40 electrochemical detector in conductivity detection mode and a Dionex AD20 absorbance detector placed in series. The detection wavelength was set at 505 nm. Chemical suppression was achieved by a Dionex AMMS-ICE anion micromembrane suppressor and 8.0 mmol l^{-1} potassium hydroxide was used as regenerant. The flow-rates were 0.8 ml min^{-1} and 2.0 ml min^{-1} for eluent and regenerant, respectively. The bead mixing coil for post-column reaction was 130 cm long and the chromogenic reagent was delivered at a flow-rate of 0.4 ml min^{-1}. The system configuration is depicted in Fig. 1.

The preliminary spectrophotometric experiments were carried out by using a Shimadzu UV-120-02 spectrophotometer (Kyoto, Japan) with 1 cm quartz cells at room temperature.

2.2. Reagents

All reagents were prepared from analytical-reagent grade unless specified otherwise. Distilled deionized water was used throughout.

2.2.1. Germanium standard solutions

The inorganic germanium and Ge-132 stock solutions (1 mg ml^{-1}, expressed as germanium) were prepared separately by dissolving appropriate

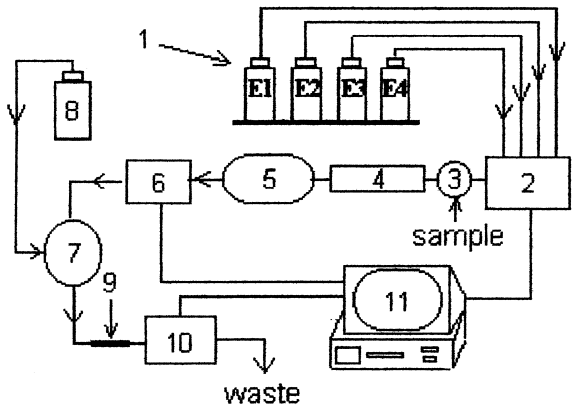

Fig. 1. Schematic of system configuration. 1=Eluents, 2=gradient pump, 3=sample injection valve, 4=column, 5=micromembrane suppressor, 6=conductivity detector, 7=three-way liquid mixing tee, 8=post-column chromogenic reagent, 9=reaction coil, 10=UV–Vis absorbance detector, 11=computer.

amounts of GeO_2 (high purity, Beijing Chemical Factory, China) and Ge-132 (purity 99.4%, obtained from Guangzhou Institute of Military Medicine, China) in hot water. Working solutions were prepared daily by serial dilution of the stock solutions with eluent prior to use.

2.2.2. Phenylfluorone stock solution (2 mmol l^{-1})

0.64 g of phenylfluorone (9-phenyl-2,3,7-trihydroxy-6-fluorone, PF) was dissolved in 500 ml of ethanol, and 50 ml of 6.0 mol l^{-1} sulfuric acid was added previously diluting to 1000 ml with ethanol. This solution was filtered through a 0.45 μm filter prior to use.

2.2.3. Triton X-100 solution (10%, v/v)

50 ml of Triton X-100 was added to 200 ml water and heated in a water bath at 80°C for 10 min, and diluted to 500 ml with water after cooling.

2.2.4. Post-column chromogenic reagent

75 ml of 2 mmol l^{-1} PF was mixed with 30 ml of 10% Triton X-100, and 24 ml of concentrated sulfuric acid was added. Finally the solution was diluted to 500 ml with water.

2.3. Preparation of real samples

The tonic oral liquids were purchased from the local market. The samples were directly diluted with eluent (1:200 for sample 1 and 1:125 for sample 2, respectively), and the diluted solutions were injected after filtration via a 0.2 μm filter.

The preliminary study of Ge-132 transformation in vivo was carried out by using three Wistar female rats weighing 180–220 g (obtained from Academy of Military Medical Sciences). The rats were fed a pelleted feed (obtained from Academy of Military Medical Sciences) and allowed to drink water ad lib. After 4 ml of tonic oral liquid (sample 1) were administered by gavage for each rat, the urine excreted was collected separately for three periods: the first was 0–4 h after administration; the second 4–12 h after administration; the third 12–24 h after administration. The urine excreted before administration was also collected as the urine blank sample. All the samples were stored at −22°C until chromatographic analysis. The urine samples were directly diluted with eluent (1:10) immediately after thawing and filtered through a 0.2 μm filter. Aliquots were injected into the HPICE system.

In this study all quantification results were expressed as germanium.

3. Results and discussion

3.1. Selection of separation mode

In aqueous solution Ge-132 existed as trihydroxygermylpropionic acid [$(HO)_3GeCH_2CH_2COOH$] [19,20], whose dissociation constant was reported to be $5.53 \cdot 10^{-5}$ [21], could be determined by high-performance ion-exchange chromatography (HPIC) or HPIEC in combination with conductivity detection, which has been confirmed by our earlier work [22]. For inorganic germanium, although Ge(IV) was the stable oxidation state, the existing forms in aqueous solutions were very complicated [23], at low pH values, it was considered generally to be metagermanic acid (H_2GeO_3) [24] for which pK_{a1} and pK_{a2} values were 8.59 and 12.72 [25], respectively. In principle, being a very weak acid it is not suitable to be determined by conductivity detection either in HPIC or HPICE because of the low conductivity values of the products after suppression or itself, which resulted in very low sensitivity [26].

Two HPIC methods [27,28] coupled with post-column spectrometric detection were proposed for the determination of Ge(IV), while the eluent strengths were too strong to be used to determine monovalent anions such as Ge-132 in basic aqueous solution. In general, HPICE was a relatively attractive alternative for determining the very weak inorganic acids, and it has been applied to the Ge(IV) determination with specific eluents [29]. In this study, HPICE was selected for the simultaneous determination of Ge(IV) and Ge-132.

3.2. Selection of eluent

In HPICE, inorganic acids such as hydrochloric acid and sulfuric acid can be used as eluents. Owing to the presence of sulfuric acid in the post-column chromogenic reagent, sulfuric acid was chosen. We found that the retention time of Ge-132 increased by increasing the concentration of sulfuric acid in the range of 0.05–0.8 mmol l^{-1}, while that of Ge(IV) remained approximately constant. If the concentration chosen is too low the resolution of Ge(IV) and Ge-132 will be inadequate and, if it is too high, resolution will be acceptable but the retention time will be prolonged needlessly. Therefore, the concentration of sulfuric acid selected was 0.2 mmol l^{-1} and the chromatogram obtained is illustrated in Fig. 2a.

3.3. Selection of detection mode

Due to the strong toxicity of Ge(IV), the restriction of its intake is very strict, while the content of Ge-132 in tonic oral liquids is relatively higher because it is added artificially as a synthesized compound. For biological samples their contents are not clear. Hence, our aim is to develop a detection method which can give sensitivities of Ge(IV) and Ge-132 as high as possible.

3.3.1. Conductivity detection

Generally, conductivity detection after chemical suppression has been the first choice of detection modes in HPICE, but the results obtained by this mode are not satisfactory for very weak acids because of the low conductivity of the products after suppression. In this study we have found that Ge(IV) could give a relatively stronger sensitivity by using conductivity detection mode. It was probably because the species in neutral aqueous solution for

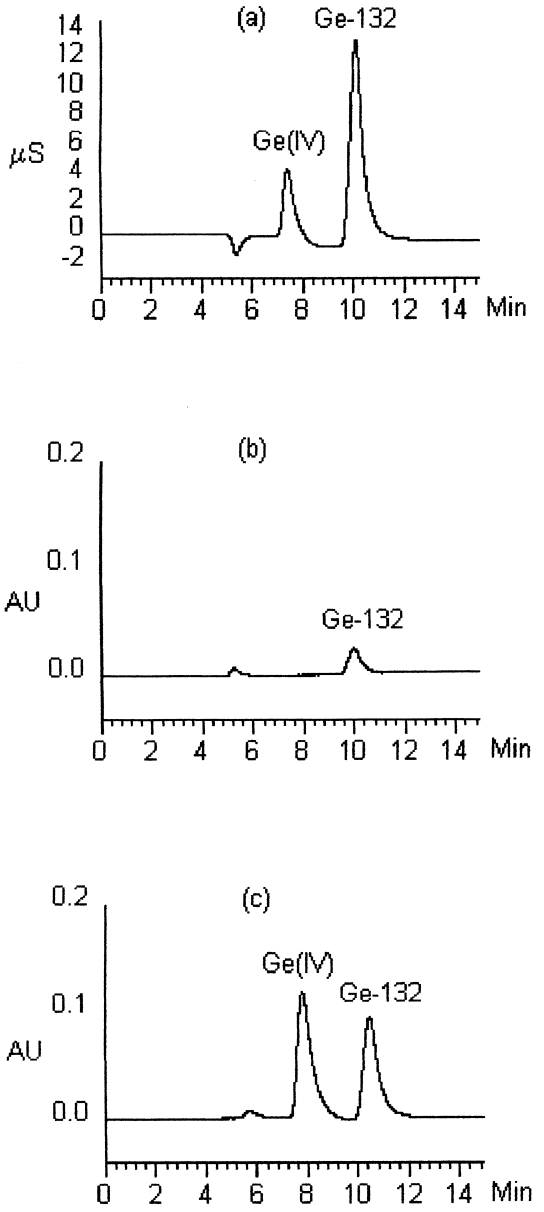

Fig. 2. Chromatograms of 50 μg ml^{-1} Ge(IV) and 50 μg ml^{-1} Ge-132: (a) conductivity detection; (b) direct UV detection at 190 nm; (c) UV detection at 190 nm after suppression. Column: HPICE-AS1; eluent: 0.2 mmol l^{-1} sulfuric acid.

Ge(IV) was the $Ge_5O_{11}^{2-}$ or $Ge_7O_{16}^{4-}$ anion [23,24], which possessed a certain ion mobility resulting in a weak but acceptable conductivity signal (the pH value of the eluent after suppression was changed to 6.60 from 3.24 before suppression). The detection limits, which were defined as the concentrations that gave signals equal to three times the baseline noise, were calculated as 40 ng ml^{-1} and 12 ng ml^{-1} for Ge(IV) and Ge-132, respectively. In addition, because Ge(IV) can form a complex with polyols such as mannitol, which is a reasonably strong acid in aqueous solution [24], it is possible to enhance the detection sensitivity for Ge(IV) by the addition of mannitol in eluent. Results showed that the addition of mannitol did not influence the background conductivity. We also found that for Ge(IV), by increasing the mannitol concentration in the range of 2–50 mmol l^{-1} in 0.2 mmol l^{-1} sulfuric acid eluent, the retention time remained approximately constant while the conductivity response increased, but for Ge-132, by increasing the mannitol concentration in eluent, both the retention time and the conductivity response decreased slightly, which indicated that Ge-132 possibly formed a relatively strong acid complex with mannitol. It has been reported that in aqueous solution Ge-132 can react with fructose [20] which possesses a structure of polyol similar to mannitol. In addition, this assumption can be simply confirmed by a test with a mannitol solution to which has been added just enough sodium hydroxide to give a pink solution with phenolphthalein as indicator. Addition of this solution to a Ge-132 solution treated in the same way results in decolorization of the solution (the reaction may be used for the determination of Ge-132 by titrimetry in the future). Hence, the reason for the decrease of the retention time of Ge-132 by increasing the mannitol concentration can be easily understood, and the slight decrease of conductivity response of Ge-132 can be explained by the relatively larger spatial structure of the formed complex which results in a relatively low ion mobility. On the other hand, the increase of the mannitol concentration also results in the increase of column pressure which causes damage to the separation column [30], but the increase of the conductivity response of Ge(IV) is not significant. Finally, taking all these factors into account, the addition of mannitol in eluent was not employed.

3.3.2. UV detection

Ultraviolet absorbance detection is an alternative to conductivity detection in HPICE and from Fig. 2b we can see that Ge-132 responds weakly by using UV detector while Ge(IV) does not. In another study [31], the present authors have found that the UV responses of some analytes in HPIC could be improved by connecting a suppressor prior to a UV detector as well as the UV background responses could be reduced. In this study, this serial connection mode of conductivity and UV detectors was employed for a trial. The results show that Ge(IV) has ultraviolet characteristic after chemical suppression and the UV response of Ge-132 increases as shown in Fig. 2c. It can be explained by the species transformations of Ge(IV) and Ge-132 after suppression, namely, the metagermanic acid is changed to a $Ge_5O_{11}^{2-}$ or $Ge_7O_{16}^{4-}$ anion and Ge-132 molecule to a monovalent anion. The detailed comparisons of UV responses of 50 μg ml^{-1} Ge(IV) and 50 μg ml^{-1} Ge-132 by using the two UV detection modes at various wavelengths are summarized in Table 1. Besides, it is noteworthy that after suppression the UV background of eluent increased in the range of detection wavelength we studied (190–210 nm), which was different from the result obtained in HPIC [31]. It may be explained by the differences of the eluents and the products after suppression by using the two different separation mechanisms. On the basis of these, it can be concluded that to connect a suppressor prior to a UV detector under some conditions probably will provide a hopeful detection mode which may improve the responses of some

Table 1
Effect of UV detection wavelength on UV responses (peak area) of 50 μg ml^{-1} Ge(IV) and 50 μg ml^{-1} Ge-132 by using the two UV detection modes

Wavelength (nm)	Direct UV detection		UV detection after suppression	
	Ge(IV)	Ge-132	Ge(IV)	Ge-132
190	–	904 625	4 905 128	4 541 536
194	–	547 279	4 244 154	2 844 238
198	–	353 335	3 119 126	1 499 212
202	–	258 873	1 991 600	894 171
206	–	206 908	919 068	535 745
210	–	172 421	391 405	348 798

target analytes regardless the UV background response which may be reduced or not. Unfortunately, in this study, the detection limits obtained by this method which detection wavelength was set at 190 nm were found to be 39 ng ml^{-1} for Ge(IV) and 50 ng ml^{-1} for Ge-132, respectively, which were not improved significantly compared with the results obtained from conductivity detection mode. Hence, neither of the two UV detection modes; direct UV detection and UV detection after suppression was studied further.

3.3.3. Post-column spectrometric detection

Spectrophotometry is the conventional method for the determination of Ge(IV) and the most widely used colorimetric method is based on the reaction of PF with Ge(IV) in acid media. Since the late 1960s, many surfactants [32–37] mainly quaternary ammonium salts have been added to the Ge(IV)–PF chromogenic system to increase the detection sensitivity and to accelerate the reaction rate, among which cetyltrimethylammonium bromide (CTMAB) is the most commonly used. The Ge(IV)–PF system with CTMAB as surfactant was firstly applied to the post-column reaction (PCR) detection in HPIC by Sun et al. [28]. However, when this reaction system was used at a temperature below 15°C, precipitation occurred in solution which resulted in practical application difficulties. Additionally, the sensibilization effects of various types of surfactants on the Ge(IV)–PF system were rarely compared in previous studies. Therefore, CTMAB and cetylpyridinium bromide (CPB) as cationic surfactants, sodium laurylsulfonate (SLS) and sodium dodecylsulfate (SDS) as anionic surfactants, Triton X-100 as non-ionic surfactant, along with polyvinyl alcohol (PVA) as special surfactant, were tested in this study. Under the same chromogenic conditions at which the concentrations of all surfactants were 1 g l^{-1} or 0.1% (v/v), we found that the maximum absorption wavelength remained 505 nm in the presence of various surfactants, and the order of sensibilization effects was cationic (CPB and CTMAB), non-ionic (Triton X-100), anionic (SLS and SDS) and special (PVA) surfactants in turn. Although CPB gave the highest sensitivity, it was not suitable for practical application due to its low solubility which resulted in precipitation in solution even above 20°C. The solubility of anionic surfactants was very low, and their sensibilization effects were not satisfactory. By contrast, Triton X-100 gave a relatively higher sensitivity and had a high solubility which could maintain the chromogenic system react normally even at a temperature as low as 10°C. Furthermore, its viscosity was much lower compared with PVA. Finally, Triton X-100 was chosen for the further study and the detection wavelength was set at 505 nm.

3.3.3.1. Acid concentration

The optimal sulfuric acid concentrations for chromogenic reaction were found in the range of 0.1–0.6 mol l^{-1} at which the absorbance was maximum, and the absorbance decreased with further increase in acid concentration. Hence, 0.3 mol l^{-1} sulfuric acid was used for the chromogenic reaction system.

3.3.3.2. PF concentration

PF hardly influenced the absorbance of the Ge(IV)–PF–Triton X-100 system when its concentrations varied from 0.05 to 0.15 mmol l^{-1}, so a 0.10 mmol l^{-1} PF solution was used in all experiments.

3.3.3.3. Triton X-100 concentration

There were no obvious changes in the absorbance when the Triton X-100 concentrations varied from 0.10 to 0.40%. Therefore, the Triton X-100 concentration was set at 0.20%.

Based on the above discussions, the optimum compositions of the post-column chromogenic reagent for Ge(IV) determination were 0.10 mmol l^{-1} PF, 0.30 mol l^{-1} sulfuric acid and 0.2% Triton X-100.

3.3.4. Spectrometric detection of Ge-132

In this study we found that Ge-132 solution could instantly give an orange color after the addition of PF and Triton X-100 solutions similar to Ge(IV) solution. Considering that the restriction for Ge(IV) is more strict thus the post-column reaction should give a sensitivity of Ge(IV) as high as possible: the optimal experimental conditions for Ge-132 determination such as the varieties of surfactant together with the concentrations of acid, PF and surfactant

were not studied further. Under the optimal conditions for Ge(IV) determination, the maximum absorption wavelength for the Ge-132–PF–Triton X-100 system was 500 nm, which was slightly different from that of the Ge(IV)–PF–Triton X-100 system.

3.3.5. Establishment of the detection method

Since the PF–Triton X-100 system can be used for the post-column spectrometric detection of both Ge(IV) and Ge-132, the detection limits obtained by this mode were compared with those obtained by conductivity detection mode for evaluation. By using PCR mode for which detection wavelength was set at 505 nm considering the optimum sensitivity of Ge(IV), the detection limits for Ge(IV) and Ge-132 were 0.72 ng ml^{-1} and 72 ng ml^{-1}, respectively. That is to say, for Ge(IV) determination the post-column spectrometric detection was more sensitive than the conductivity detection but vice versa for Ge-132 determination. Taking all these factors into account, a compromise was made that a conductivity detector and an absorbance detector were connected serially, i.e., the former for detection of Ge-132 and the latter for trace Ge(IV) as well as Ge-132. Fig. 3 shows the chromatograms obtained under these conditions. It can be found that the peaks of Ge(IV) and Ge-132 in Fig. 3b are slight tailing peaks which may be caused by the following reasons: (1) the increase of void volume due to the addition of a post-column reaction coil may cause serious peak diffusion and (2) the use of surfactant produces the adsorption on the wall of the post-column reaction coil. Nevertheless, baseline separation is achieved.

3.4. Repeatability and linearity

A standard solution containing 0.5 μg ml^{-1} Ge(IV) and 10 μg ml^{-1} Ge-132 was analyzed seven times successively under the same conditions and the results obtained are summarized in Table 2. We can find that the repeatability of the peak area responses is better than that of peak height responses for both analytes by using either detection method. As a result, the peak area measurements for all calculations in this study were chosen. Good linearities between the concentrations of Ge(IV) as well as of

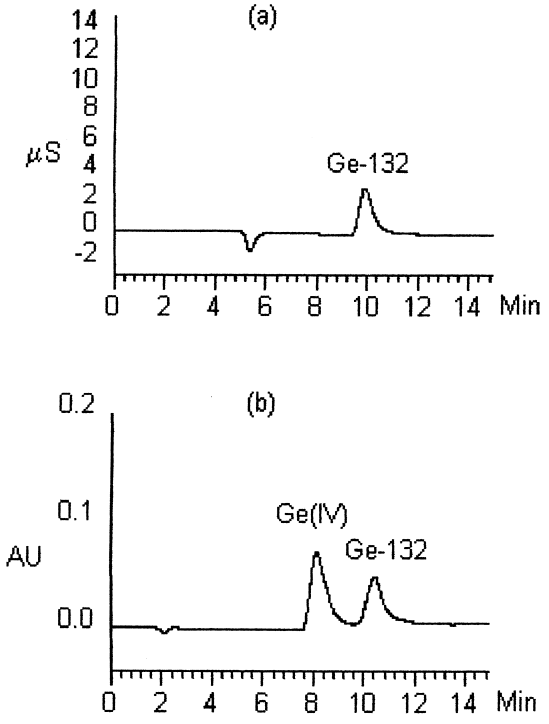

Fig. 3. Chromatograms of 0.50 μg ml^{-1} Ge(IV) and 10 μg ml^{-1} Ge-132: (a) conductivity detection; (b) post-column spectrometric detection at 505 nm. Column: HPICE-AS1; eluent: 0.2 mmol l^{-1} sulfuric acid.

Ge-132 and the peak area responses are achieved as shown in Table 3.

3.5. Analysis of real samples

3.5.1. Tonic oral liquids

Two kinds of tonic oral liquids which contained Ge-132 (declared on labels by manufacturers) were analyzed under the specified experimental conditions and the chromatograms of 1:200 diluted solution of sample 1 are shown in Fig. 4. No inorganic germanium was found and the accuracy of the proposed

Table 2
Relative standard deviations (%) of Ge(IV) and Ge-132 ($n=7$)

Analyte	Conductivity detection		PCR	
	Peak height	Peak area	Peak height	Peak area
Ge(IV)	–	–	2.40	1.26
Ge-132	2.38	1.18	2.55	2.28

Table 3
Linearity of Ge(IV) and Ge-132

Analyte	Concentration level ($\mu g\ ml^{-1}$)	Correlation coefficient	
		Conductivity detection	PCR
Ge(IV)	0.05; 0.1; 0.3; 0.5; 2; 5	–	0.9999
Ge-132	0.1; 1; 5; 10; 50; 100	0.9997	0.9969

method was evaluated by comparing the results with those obtained by using HG-AFS [17]. The results as shown in Table 4 reveal an excellent agreement between the two methods. Moreover, the values of Ge-132 content for sample 1 compare well with the value of 2.140 mg ml^{-1} labeled by the manufacturer.

The study of spiked recovery was also used to evaluate the proposed method and sample 1 diluted solution (1:200) was chosen. The results also shown in Table 4 are satisfactory.

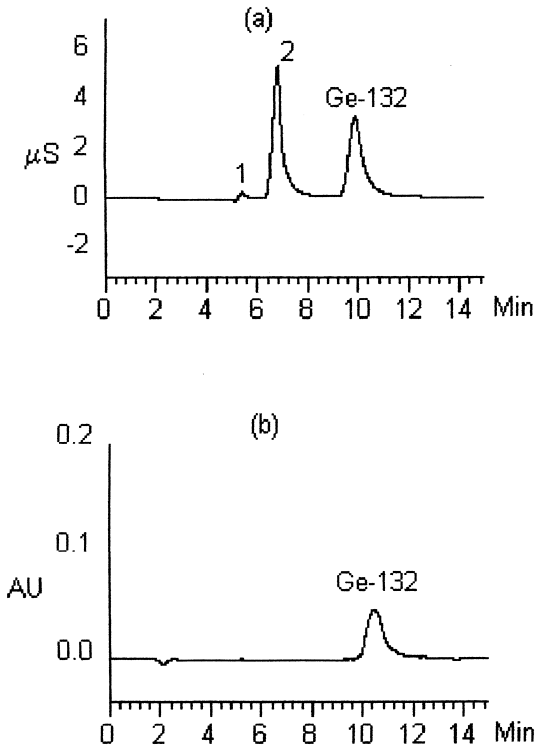

Fig. 4. Chromatograms of sample 1 diluted solution (1:200): (a) conductivity detection; (b) post-column spectrometric detection at 505 nm. Peaks: 1, 2=unknown peaks. Column: HPICE-AS1; eluent: 0.2 mmol l^{-1} sulfuric acid.

3.5.2. Urine

The clinical studies [4–9] revealed that the persistent renal dysfunction could be found among all the cases after chronic intake of GeO_2, and the most important excretion path either for GeO_2 or Ge-132 was through the kidney [8,38,39]. In addition, Zhang [11] warned of the potential risk of Ge(IV) transformed from Ge-132 in vivo. Therefore, by monitoring the Ge(IV) content in urine, it is possible to aid diagnosis of some specific renal diseases and to study the possible transformation of Ge-132 in vivo.

The urine excreted by the three rats administered with tonic oral liquid (sample 1) were collected and analyzed. The chromatograms obtained are shown in Fig. 5. No pretreatment procedure was needed. Neither Ge(IV) nor Ge-132 was found in urine blank samples and no Ge(IV) was detected in the all urine samples excreted by the rats administered with Ge-132 during the test period. The results of the diluted solutions (1:10) are listed in Table 5. From Fig. 5a, we can find that the unknown peaks (mainly peak 1) interfere with the detection of Ge(IV) and Ge-132 even their concentrations above the detection limits by using a conductivity detector.

The spiked recovery study was carried out by using a group of urine blank sample diluted solutions (1:10) added with 0.5 $\mu g\ ml^{-1}$ Ge(IV) and 5 $\mu g\ ml^{-1}$ Ge-132. The recoveries (mean±standard deviation, $n=5$) were found to be 92.20±5.20% for Ge(IV) as well as 88.30±1.91% for Ge-132 by using PCR detection, and 94.65±5.82% for Ge-132 by using conductivity detection, respectively.

In this study, although no Ge(IV) was detected in urine, it was not appropriate to make any conclusions about the transformation possibility of Ge-132 in

Table 4
Tonic oral liquids analysis

Sample	Analyte	Content (mg ml^{-1})[a]		Add (μg ml^{-1})	Recovery (%)[b]		Result of HG-AFS (mg ml^{-1})
		Conductivity detection	PCR		Conductivity detection	PCR	
1	Ge(IV)	–	ND[c]	0.5	–	102.41±2.40	ND
	Ge-132	2.046±0.050	2.160±0.059	10	101.42±2.11	96.15±2.94	2.038
2	Ge(IV)	–	ND				ND
	Ge-132	1.273±0.016	1.269±0.054				1.243

[a] Average of six determinations±standard deviation.
[b] Average of five determinations±standard deviation.
[c] ND=not detected.

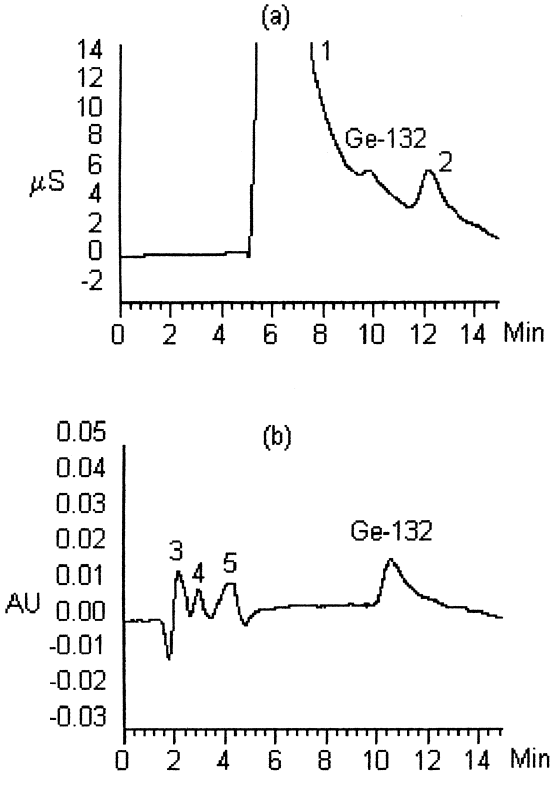

Fig. 5. Chromatograms of rat urine (rat 2, 1st collection) diluted solution (1:10): (a) conductivity detection; (b) post-column spectrometric detection at 505 nm. Peaks: 1–5=unknown peaks. Column: HPIEC-AS1; eluent: 0.2 mmol l^{-1} sulfuric acid.

Table 5
Rat urine diluted solution (1:10) analysis

Rat	Collection time	Content of Ge-132 (μg ml^{-1})[a]	
		Conductivity detection	PCR
1	1	4.40±0.24	4.47±0.29
	2	ND[b]	0.60±0.030
	3	ND	ND
2	1	8.30±0.27	8.11±0.19
	2	ND	1.97±0.15
	3	ND	0.11±0.0063
3	1	4.04±0.45	4.40±0.11
	2	ND	0.56±0.030
	3	ND	ND

[a] Average of three determinations±standard deviation.
[b] ND=not detected.

vivo at present because of the insufficient animal number of subjects as well as the short time for test. The results obtained in this section only indicates the potential of practical application in the future study on pharmacokinetic and chronic toxicity of Ge(IV) and Ge-132.

4. Conclusions

A HPICE method for the separation and determination of Ge(IV) and Ge-132 has been developed. The procedure employs 0.2 mmol l^{-1} sulfuric acid as eluent and conductivity detection for Ge-132 coupled

with post-column spectrometric detection of trace Ge(IV) and Ge-132. The chromogenic system in the presence of Triton X-100 avoids the precipitation occurring in the presence of the conventional cationic surfactants. A new chromogenic reaction between Ge-132 and phenylfluorone with Triton X-100 is reported for the first time. The method proposed has the practical advantage of a board applicability which is useful in both tonic oral liquid analysis and the preliminary metabolism study of Ge-132 in vivo. It can probably be used for the study of pharmacokinetic and chronic toxicity of Ge(IV) and Ge-132.

Acknowledgements

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