THE RETENTION BEHAVIOR OF ARSENIC COMPOUNDS ON PRP-X100 COLUMN UNDER ALKALINE CONDITION*

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Abstract A systematical investigation of the retention behaviour of arsenic compounds (arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, arsenobetaine etc.) on PRP-X100 column under alkaline condition was carried out. The dependence of the retention times of arsenic compounds on pH with two mobile phases: 20mmol/L ammonium bicarbonate, 2.5mmol/L *p*-hydroxybenzoic acid/1.0mmol/L benzoic acid was studied, and the optimal separations of these five arsenic compounds were achieved.

Key words high performance liquid chromatography, flame atomic absorption spectrophotometer, arsenic compounds speciation

1 INTRODUCTION

Arsenic is ubiquitous in the natural environment. Various arsenic species found in the environment have different toxicological properties. It has been demonstrated that not only the total arsenic concentration, but also the arsenic chemical speciations which becomes more and more important, must be included to assess the toxicity, the environmental impact and the effect of occupational exposure of arsenic. The coupling of high performance liquid chromatography (HPLC) with various arsenic-specific detectors has been proven to be very useful for chemical speciation studies. A large number of HPLC separation methods for the separation of arsenic compounds (anionic compounds)-arsenite, arsenate, methylarsonic acid, dimethylarsinic acid etc. was reported in the past[1~3]. Most of the HPLC separations were carried out under acidic condition or near neutral pH (pH of mobile phase< 7). Although it is known that the pH of the mobile phase is very important for the separation, very little work has been done to systematically study the effect of pH[4]. This is partly because, in most of the studies, silica based anion-exchange columns were used, limiting the mobile phase to a narrow pH range near neutral, where AsB and As(Ⅲ) peaks overlap for most columns. However, with the use of a polymer based column, for instance, Hamilton PRP-X100, mobile phases in the pH range 1-13 can be used without column deterioration. This makes it possible to separate arsenic compounds under alkaline condition.

We here present a systematic study on the retention behaviour of arsenite, arsenate, methylarsonic acid (MA), dimethylarsinic acid(DMA) and arsenobetaine(AB) on a PRP-X100 anion-exchange column under alkaline condition(pH 8-10.8). Flame atomic absorption spectrophotometer(FAAS) was used to detect the arsenic compounds in the column effluent.

2 EXPERIMENTAL

2.1 Reagents

All reagents were of analytical grade or higher purity from Aldrich, Merck or Fluka. NANO pure water was used throughout. Solutions of all arsenic compounds containing 60mg of arsenic per liter were prepared in NANO pure water. All mobile phases were passed through a 0.20μm filter and degassed before use.

2.2 HPLC conditions

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本文收稿日期: 1997-01-09, 修回日期: 1997-04-16

Pump:	Miton Roy CM-4000
Injector:	Rheodyne 6-port injection valve
Loop:	100μL
Column:	Hamilton PRP-X100, 250mm× 4.1mm i.d., 10μm spherical particles of a poly (*N*, *N*, *N*-trimethyl-ammonium methylene) styrene-divinylbenzene
Flow:	1.65mL/min for NH_4HCO_3 mobile phase
	1.5mL/min for *p*-hydroxybenzoic acid-benzoic acid (*p*-HBA-BA) mobile phase
Temperature:	25℃
Connection to FAAS:	stainless steel capillary 0.23mm i.d., 1m long

The column was equilibrated by passing 100mL of each mobile phase through the column before injection of the arsenic species. Each retention time was determined three times(relative deviation< 1%).

2.3 FAAS conditions

The HPLC column exit was connected to the FAAS(Hitachi Z-6100) nebulizer with a stainless steel capillary 1m long, 0.23mm i.d.. The FAAS was operated with an acetylene/air flame at a fuel pressure of 22kPa acetylene and 160 kPa air. The optimal height of the burner head for arsenic monitoring was 10mm. The hollow cathode lamp (S&J Juniper, Essex, UK) was operated at 10mA. Arsenic was measured at 193.7nm. Data were transferred to a personal computer via a Hitachi recorder interface after analog-to-digital conversion.

3 RESULTS AND DISCUSSION

A widely used method for the identification and quantification of arsenic compounds in complex matrices of biological system is liquid chromatography coupling with arsenic specific detectors, such as AAS, AES, ICP-MS. The simplest ion-exchange chromatography of metal species is based on affinity differences of the native analytes on the column. Separation can be conditioned by pH and ionic strength of the eluent which competes with sample species on ion-exchange sites and elutes the sample from the column[5]. A mobile phase ideally should separate arsenic compounds in a reasonable time under isocratic or gradient modes.

In this study, two mobile phases (ammonium bicarbonate, *p*-hydroxybenzoic acidbenzoic acid) were investigated.

(1) Ammonium bicarbonate mobile phase(20mmol/L NH_4HCO_3)

The dependence of the retention times of the five arsenic compounds on the pH of the mobile phase(pH 8.3-10.8) was investigated(Fig.1). It was found that AB, arsenite and DMA can be completely separated at pH 8.7, and a good separation (Fig.2) of the five arsenic compounds(arsenite, arsenate, DMA, MA and AB) can be achieved by a gradient elution (pH 8.7 to 9.8) at a flow rate of 1.65mL/min.

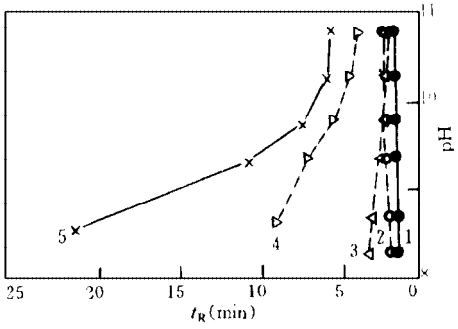

Fig.1 Dependence of the retention times of arsenic compounds on pH with 20mmol/L NH_4HCO_3 buffered aqueous mobile phase, pH adjusted with NH_4OH

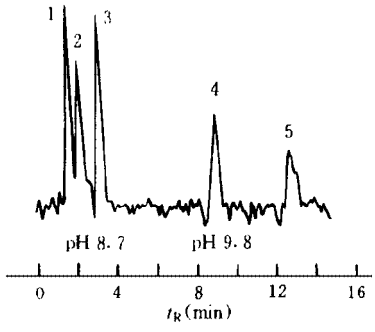

Fig.2 Chromatogram of a mixture(100μL) of arsenic compounds (6μg arsenic each) with 20mmol/L NH_4HCO_3 mobile phase of pH 8.7 to 9.8

1. AB, 2. arsenite, 3. DMA, 4. MA, 5. arsenate.

(2) *p*-hydroxybenzoic acid-benzoic acid(*p*-HBA-BA) mobile phase

The dependence of the retention times of the five arsenic compounds on the pH(8.3-10.3) of the mobile phase (Fig. 3), and on the concentration of *p*-hydroxybenzoic acid and benzoic acid buffers(Fig. 4) were studied, an acceptable separation of AB, arsenite, DMA, MA and arsenate can be achieved with 2.5 mmol/L *p*-hydroxybenzoic acid-1.0 mmol/L benzoic acid (pH 9.0) at a flow rate of 1.5mL/min(Fig. 5). The peaks of arsenic compounds suffer some noises due to the interference on the FAAS detection resulted form the organic matrix of used mobile phase.

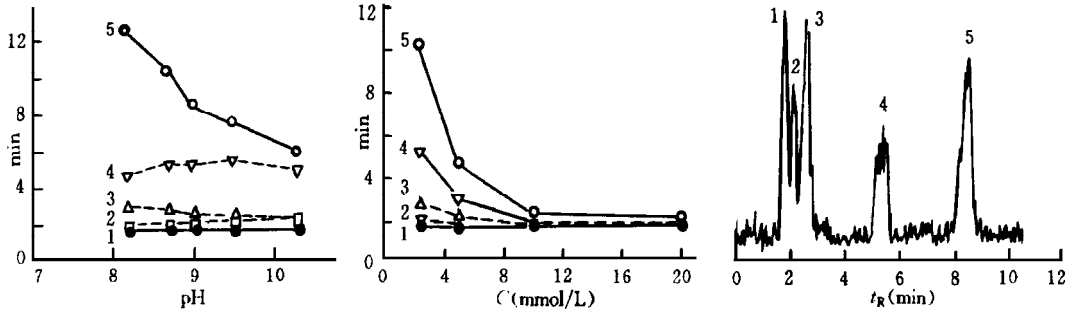

Fig. 3(L)　Dependence of the retention times of arsenic compounds on pH with a mobile phase of 2.5mmol/L *p*-HBA-1.0mmol/L BA

compounds number same as in Fig. 1.

Fig. 4(M)　Dependence of the retention times of arsenic compounds on concentration of *p*-HBA at pH 8.7

compounds number same as in Fig. 1.

Fig. 5(R)　Chromatogram of a mixture (100μL) of arsenic compounds (6μg arsenic each) with a mobile phase of 2.5 mmol/L *p*-HBA-1.0mmol/L BA at pH 9.0

compounds number same as in Fig. 1.

The retention times of arsenate decrease with increasing pH for both of the mobile phases. On the basis of the pK values for arsenic acid (2.2, 6.9, 11.5), arsenic acid deprotonated and mainly existed as $HAsO_4^{2-}$ ion in the pH range of 8.0-10.8 (Fig. 6), thus, with the pH increase, the doubly charged *p*-HBA ion(*p*-HBA, pK 4.48, 9.32) and CO_3^{2-} (H_2CO_3, pK 6.37, 10.25) will more effectively compete with the analyte anions on the positively charged groups at the surface of the stationary phase. The result of this competition is a decrease of retention times for arsenate with increasing pH. The observed decrease of retention times of arsenate with increasing of *p*-HBA concentration (Fig. 4) also supports this explanation.

The retention times of methylarsonic acid show different pH dependence. With ammonium bicarbonate (Fig. 1) mobile phase, the retention time decreases monotonical from pH 8.7 to 10.8. This also can be attributed to the competition between analyte anions and CO_3^{2-} ion for the positively charged groups on the surface of the stationary phase. With *p*-HBA-BA mobile phase, almost no appreciable change of retention times was observed. This is probably because of the similar pK values of methylarsonic acid (pK 4.1, 9.1) and *p*-HBA (pK 4.48, 9.32). In the pH range being studied, they deprotonate almost at same pH value, the ratio of $HA^-_{MA}/HA^-_{p\text{-HBA}}$ and the ratio of $A^{2-}_{MA}/A^{2-}_{p\text{-HBA}}$ keep in a relative stable level, therefore, the competition between analyte and mobile phase ions for the positively charged ion-exchange sites on the surface of the stationary phase stands in a state of relative equilibrium, and it results in the stable retention times of methylarsonic acid with increasing pH.

With a pK of 6.3, dimethylarsinic acid is present as $(CH_3)_2AsO_2^-$ ion under alkaline condition, there-

fore, electrostatic interactions between $(CH_3)_2AsO_2^-$ and the cationic sites (ammonium groups) on the stationary phase should influence the retention times. With increasing pH, the absolute amount of CO_3^{2-} and $A_{p\text{-HBA}}^{2-}$ gradually increase in the mobile phases, the doubly charged CO_3^{2-}, $A_{p\text{-HBA}}^{2-}$ have a higher affinity for the positively charged groups on the surface of the stationary phase than the singly charged $(CH_3)_2AsO_2^-$ ion, thus a gradual decrease of retention times of dimethylarsinic acid for both mobile phases is observed(Fig. 3).

Under acidic conditions, arsenite migrates with the solvent front, because arsenite is present as neutral H_3AsO_3(pK 9.2). However, with the increasing of pH value to alkaline conditions(pH 8.0-10.8), arsenite does not remain undissociated, it was shown that certain extent of interaction between arsenite and the stationary phase took place (see Fig. 1 and 3), arsenite doesn't co-elute with AB(pK 2.18[6], AB is probably present as a neutral zwitterion in the pH range 8-10.8) in the void time(volume), therefore, arsenite and AB can be separated.

4 CONCLUSION

Investigation of the retention behavior of arsenobetaine, arsenite, methylarsonic acid, dimethylarsenicacid and arsenate on the PRP-X100 anion-exchange column, influenced by the pH, the concentration, and the nature of buffer solutions, show that arsenic compounds have different retention behavior under alkaline condition from that under acidic condition, and optimal separation of the five arsenic compounds(FAAS detection) is possible with 20mmol/L ammonium bicarbonate buffer at pH 8.7 and 9.8 at a flow rate of 1.65mL/min, with 2.5mmol/L *p*-HBA-1.0mmol/L BA mobile phase at pH 9.0 at a flow rate of 1.5mL/min.

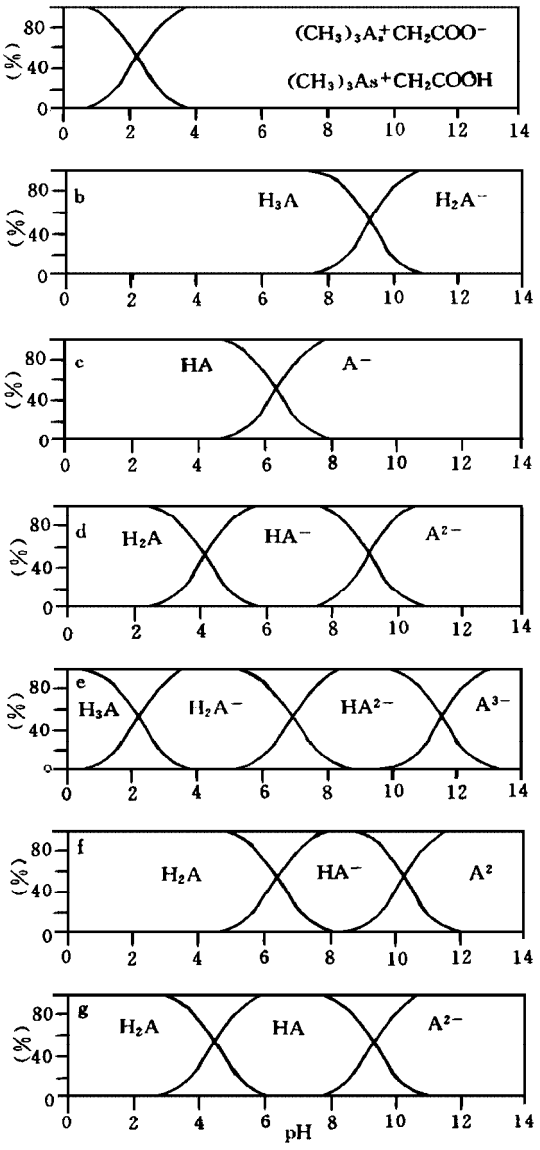

Fig. 6 Species distribution diagram for (a) arsenobetaine (pK 2.18), (b) arsenous acid(pK 9.2), (c) methylarsonic acid (pK 6.3), (d) dimethylarsinic acid(pK 4.1, 9.1), (e) arsenic acid(pK 2.2, 6.9, 11.5), (f) carbonic acid(pK 6.37, 10.25), and (g) *p*-hydroxybenzoic acid(pK 4.48, 9.32) in the pH range 0-14

The coupling of the above-mentioned two HPLC systems with ICP-MS, in which the sub-ng level of As compounds can be monitored, and the applications of these HPLC-ICP-MS systems to the arsenic speciation of biological and environmental samples are in progress.

Acknowledgement The authors thank OAD for awarding a scholarship to J. Zheng.

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碱性条件下砷化合物在 PRP-X100 柱上的保留行为

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提　要　系统地研究了碱性条件下(pH 8~ 10.8) As^{3+}, As^{5+}, MA, DMA 和 AB 等砷化合物在 PRP-X100 阴离子交换柱上的保留行为。用火焰原子吸收光谱(FAAS)测定从 HPLC 分离的砷化合物, 即通过一根 1m × 0.23mm i.d. 不锈钢毛细管, 将 HPLC 柱出口与 FAAS 的雾化器连接起来, 采用乙炔/空气火焰, 在 193.7nm 处测定。具体研究了两个流动相(20mmol/L NH_4HCO_3 和 2.5mmol/L 对-羟基苯甲酸-1.0mmol/L 苯甲酸水溶液), 并系统研究了 pH 值和缓冲液浓度对上述 5 个砷化合物的保留时间的影响, 发现对于 20mmol/L NH_4HCO_3 体系, 在 pH 8.7 时, AB, As^{3+}, DMA 可以得到完全分离, 通过梯度洗提(pH 8.7 到 9.8, 流速 1.65mL/min), 在 15min 内上述 5 个砷化合物可以得到良好分离; 对于 2.5mmol/L 对-羟基苯甲酸-1.0mmol/L 苯甲酸体系, 在 pH 9.0 及流速 1.5mL/min 时, 可以在 12min 内将上述 5 个砷化合物分离。

关键词　高效液相色谱法, 火焰原子吸收光谱, 砷化合物形态

分类号　O658/O62

会　议　通　知

受中国化学会委托, 中国化学会毛细管电泳专业组(筹)决定于 1998 年 10 月 6~ 7 日及 8~ 11 日相继召开第三届全国毛细管电泳及相关微分离分析技术学术报告会(CCE'98)和第二届亚太毛细管电泳及相关微分离分析技术学术报告会(APCE'98)。其中 10 月 8 日将举办内容丰富的各种短训班。具有相当规模的分析仪器和相关技术展览会将贯穿两个会议的始终。这儿所指的相关技术包括毛细管电色谱、微板毛细管电泳和用于微分离分析的各种色谱技术(HPLC 等)。一批相关领域国际顶尖科学家和海外华裔学者、学生将应邀参加。

两会自即日起开始征稿, 凡在这一领域技术、应用、仪器和附件的研制及基础理论等方面尚未公开发表的论文均可应征, 应征稿件摘要按已发的第一轮通知所示规格编写并于 1998 年 4 月 1 日前挂号寄出, 提交大会学术委员会审定。被接受的稿件将编入相应的报告会文集。

会议将热忱欢迎在学博士、硕士研究生参加并将根据会议财力在费用上给予尽可能多的方便。会议将热忱欢迎无论文者参加。有意参加者请与大会秘书组联系。秘书组负责人: 薛俊女士和许旭博士, 通讯地址: 辽宁省大连市中山路 457 号中国科学院大连化学物理研究所(邮编 116023), Tel. 0411-4671991 转 861, Fax. 0411-3622302, Email g602@rose.dicp.ac.cn。

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1997 年 11 月