

学校编码: 10384  
学号: 19120051301831

分类号 \_\_\_\_\_ 密级 \_\_\_\_\_  
UDC \_\_\_\_\_

厦 门 大 学

硕 士 学 位 论 文

几种中成药中砷的含量和形态分析及其体外生物利用度研究

Study on Content and Speciation of Arsenic in Some Chinese Traditional Medicine and in Vitro Bioaccessibility

张建平

指导教师姓名: 王小如 教授

庄峙厦 高工

专业名称: 分析化学

论文提交日期: 2008年5月

论文答辩日期: 2008年6月

学位授予日期:

答辩委员会主席:

评 阅 人:

2008年6月

## 厦门大学学位论文原创性声明

兹提交的学位论文，是本人在导师指导下独立完成的研究成果。本人在论文写作中参考的其他个人或集体的研究成果，均在文中以明确方式标明。本人依法享有和承担由此论文而产生的权利和责任。

声明人（签名）：

年 月 日

# 厦门大学学位论文著作权使用声明

本人完全了解厦门大学有关保留、使用学位论文的规定。厦门大学有权保留并向国家主管部门或其指定机构送交论文的纸质版和电子版，有权将学位论文用于非赢利目的的少量复制并允许论文进入学校图书馆被查阅，有权将学位论文的内容编入有关数据库进行检索，有权将学位论文的标题和摘要汇编出版。保密的学位论文在解密后适用本规定。

本学位论文属于

1、保密（ ），在 年解密后适用本授权书。

2、不保密（ ）

（请在以上相应括号内打“√”）

作者签名： 日期： 年 月 日

导师签名： 日期： 年 月 日

## 摘 要

中药是一个复杂的体系，按来源可分为动物药、植物药、矿物药，按处方可分为单味药、复方药，此外还有原药材、中成药，煎煮、炮制、水飞，片剂、饮液、颗粒、药丸等形式的细分，这成为中药中砷含量和形态分析的瓶颈。通过调研，发现中药中的砷主要有两种来源：一种是在种植、加工、制造过程中被污染而引入可溶性砷盐；另一种以雄黄等矿物类药材及其制剂入药而引入不溶性砷盐。由此，本论文选取六种有代表性的中药：天王补心丹、参茸补肾片、黄芩等三种不含矿物类药材的中药和六神丸、牛黄解毒片、雄黄等三种含矿物类药材的中药来进行相关的探索。

本论文由四个部分组成：1.文献综述；2.中药中砷的含量检测；3.中药中砷的形态分析；4.中药中砷的体外生物有效性研究。

第一章文献综述，主要介绍了砷的性质、运用和毒害，总结了砷含量检测和形态分离的方法，综述了体外生物有效性的研究现状，并在此基础上提出了本论文的研究设想。

第二章主要是对中药中砷盐检测方法进行改进：采用微波消解处理样品，电感耦合等离子质谱法检测中药中的总砷含量，期间对湿法消解、干法灰化、微波消解三种前处理方法进行比较，结果发现：采用微波消解法、以浓硝酸-高氯酸（4:1）为消解液、分两档消解 10 分钟，可以比较准确地反映药丸中砷的水平；建立在线化学蒸气发生，电感耦合等离子体质谱法检测六神丸等含矿物类药材的中药中可溶性砷的含量，该方法较以往的二乙基二硫代氨基甲酸银法简单，方法检出限为 0.36 $\mu\text{g/L}$ 、回收率在 87.4%~97.75%之间、RSD（n=10）在 3%以内。

在第三章中首先建立高效液相色谱-电感耦合等离子体质谱联用装置，对交换柱类型、流动相的组成、洗脱方式等色谱条件和等离子体气流量、干扰扣除等质谱条件进行优化，结果发现：采用 Hamilton PRP X-100（250\*4.1mm，10mm id）阴离子交换柱、以 15mM 磷酸氢二氨(pH=6)为流动相、采用等度洗脱，15min 内六种形态得以分离，线性范围不小于两个数量级，检出限分别为 0.986 $\mu\text{g/L}$ (AsC)、

0.756 $\mu\text{g/L}$ (AsB)、0.568 $\mu\text{g/L}$ (As III)、0.893 $\mu\text{g/L}$ (DMA)、0.941 $\mu\text{g/L}$ (MMA)、0.742 $\mu\text{g/L}$ (As V), RSD (n=10) 均小于 10%。然后将所建立的联用装置运用于海产品、生物样品、化妆品等实际样品, 与同类文献具有可比性, 所建立的装置符合研制方法的预期目标, 同时还发现: 海产品总砷含量较高, 但超过 99% 的砷是有机砷; 生物样品由污染源而定; 化妆品中主要是无机砷。最后将所建立的联用装置运用于中药实际样品, 期间对石油醚快速溶剂萃取、甲醇/水 (1:1) 超声溶剂提取、盐酸回流三种不同的前处理方法进行比较, 结果发现: 在天王补心丹等不含矿物类药材的中药中, 除含 As (III)、As (V) 外还存在低浓度的 DMA 和微量的 AsC, 采用甲醇/水 (1:1) 超声提取较合理; 而在六神丸等含矿物类药材的中药中, 主要是无机砷, DMA、AsC 等基本可以忽略, 采用盐酸回流较合理。

第四章建立中药体外生物有效性的研究方法, 采用电感耦合等离子体质谱对中药在胃肠道溶解部分进行了总砷测定, 研究中药的生物利用度; 采用高效液相色谱-电感耦合等离子体质谱对中药在胃肠道溶解部分进行砷的形态分析, 观察消化前后各形态的变化情况, 结果发现: 中药中的砷在人体胃肠道环境下不能完全溶解, 其总砷的生物利用度在 80% 以下; 主要在胃过程中溶出; 在天王补心丹等不含矿物类药材的中药中部分有机砷可能转化成无机砷; 在六神丸等含矿物类药材的中药中部分不溶态的二硫化二砷可能被溶解。

本文的创新点在于:

- (1) 对二乙基二硫代氨基甲酸银法进行改进, 将在线化学蒸气发生-电感耦合等离子体质谱法运用于中药中可溶性砷的检测, 操作简单, 灵敏度高;
- (2) 对几种中药的体外生物有效性进行探索, 进一步加强各种形态在人体内代谢的研究, 为砷的安全性评价提供一定的参考价值。

**关键词:** 中药; 砷; 电感耦合等离子体质谱; 高效液相色谱;

厦门大学博硕士学位论文摘要库

---

## Abstract

In the field of arsenic speciation analysis, experts and scholars from home and abroad have done much research, however, few of which covered Chinese traditional medicine.

Subject paper focuses on analysis of arsenic in some Chinese traditional medicine. Total four chapters: 1. Literature review; 2. Determination of arsenic in Chinese traditional medicine; 3. Analysis of arsenic speciation in Chinese traditional medicine; 4. Study on in vitro bioavailability of arsenic in Chinese traditional medicine.

Chapter I was an overview of the research background, the property, application and toxicity of arsenic, the content and speciation detection methods, and the study of in vitro bioavailability were introduced in this chapter.

Chapter II comprises of two parts: 1). Total arsenic of Chinese traditional medicine were treated with microwaving digestion method and determined by inductively coupled plasma mass spectrometry. Comparison of these treatment methods were conducted to find the best one. The results showed microwaving digestion is more convenient, quick and finally selected in this study as the treatment method. 2). Soluble arsenic of Chinese traditional medicine was treated with hydrochloric acid and determined by online chemical vapor generation - inductively coupled plasma mass spectrometry. Compared to Ag-DDC, this method is more simple, the detection limit reaches  $0.36\mu\text{g/L}$ , the recovery obtains 87.4%, and RSD ( $n=10$ ) is within 3%.

In the third chapter, the association device of high performance liquid chromatography-inductively coupled plasma mass spectrometry were set up for the analysis of arsenic speciation, conditions were optimized, and the result showed: using Hamilton PRP X-100 ( $250\times 4.1\text{mm}$ , 10mm id) anion exchange column, with 15mM  $(\text{NH}_4)_2\text{HPO}_4$  (PH=6) as the mobile phase, can analyse six arsenic speciation within 15 min, and the linear range is not less than two orders of magnitude, the detection limit is  $0.986\mu\text{g/L}$  (AsC),  $0.756\mu\text{g/L}$  (AsB),  $0.568\mu\text{g/L}$  (AsIII),  $0.893\mu\text{g/L}$  (DMA),  $0.941\mu\text{g/L}$  (MMA),  $0.742\mu\text{g/L}$  (AsV), RSD ( $n=10$ ) is within 10%. Then the association device of high performance liquid chromatography -

inductively coupled plasma mass spectrometry was applied to analyse arsenic speciation in aquatic products, biological samples and cosmetics. The results showed that: more than 99% of the arsenic in seafood is organic, such as AsB and AsC, minimal toxicity; biological samples decided by sources; cosmetics only contain inorganic arsenic. Finally the association device was applied to Chinese traditional medicine. Compare of the three treatment methods, ASE, SON, HCL, were conducted to find the best one. The results showed that the main forms of arsenic in HuangQin are As (III) and As (V), as well as low concentrations of AsC and DMA, can use SON; but in XiongHong they are As (III) and As (V), DMA and AsC can be ignored, so using HCL is more appropriate.

In Chapter IV, the method of in vitro bioaccessibility was established to determine the capability of solution of Chinese traditional medicine in gastrointestinal track. After Chinese traditional medicine were digested in gastrointestinal track, the content and speciation of arsenic in the digestion fluid were analyzed. Arsenic in gastrointestinal fluid is not completely dissolvable. Bioaccessibility of total arsenic was below 80%. Inorganic arsenic increased after gastrointestinal digestion. It is possible that some organic arsenic transferred into inorganic arsenic.

The main innovation of this thesis is: (1) Developed online chemical vapor generation - inductively coupled plasma mass spectrometry technique to determine soluble arsenic in Chinese traditional medicine. Compared to Ag-DDC, this method is more simple, convenient and quick. (2) The solution and transfer of arsenic in Chinese traditional medicine by method of in vitro bioaccessibility have a great significance to further study how arsenic in high arsenic food can change and release from matrix and whether it will transfer from one speciation to another in human gastrointestinal track.

**Keyword:** Chinese traditional medicine, Arsenic, Inductively coupled plasma mass spectrometry, High performance liquid chromatography.



# 目 录

<b>第一章 文献综述 .....</b>	<b>1</b>
1 引言 .....	1
2 砷 .....	2
2.1 砷的性质 .....	2
2.2 砷的使用 .....	2
2.3 砷的毒性 .....	3
3 中药中砷含量的测定 .....	4
3.1 有机破坏 .....	5
3.2 检测方法 .....	6
4 中药中砷形态的分析 .....	8
4.1 砷的形态 .....	8
4.2 分离方法 .....	9
4.3 检测方法 .....	10
4.4 联用技术 .....	12
5 体外生物有效性研究 .....	13
5.1 生物有效性的定义 .....	13
5.2 生物有效性的测定方法 .....	14
5.3 生物有效性的体外模型 .....	15
6 实验规划 .....	18
参考文献 .....	18
<b>第二章 中药中砷含量的测定 .....</b>	<b>23</b>
<b>第一节 中药中总砷含量的测定 .....</b>	<b>23</b>
1 引言 .....	23
2 实验部分 .....	24
2.1 主要试剂 .....	24
2.2 主要仪器 .....	25

2.3 实验方法 .....	26
<b>3 结果与讨论 .....</b>	<b>26</b>
3.1 仪器条件 .....	26
3.2 样品前处理 .....	26
3.3 方法测试 .....	28
3.4 实际测量 .....	29
4 小结 .....	30
<b>第二节 中药中可溶性砷的测定 .....</b>	<b>30</b>
1 引言 .....	30
2 实验部分 .....	31
2.1 主要试剂 .....	31
2.2 主要仪器 .....	31
2.3 实验方法 .....	32
3 结果与讨论 .....	32
3.1 样品前处理 .....	32
3.2 氢化物发生条件的选择 .....	32
3.3 仪器条件 .....	33
3.4 方法测试 .....	34
3.5 实际测量 .....	35
4 小结 .....	35
参考文献 .....	36
<b>第三章 中药中砷形态的分析 .....</b>	<b>37</b>
<b>第一节 高效液相色谱-电感耦合等离子体质谱联用装置的建立 ....</b>	<b>38</b>
1 引言 .....	38
2 实验部分 .....	39
2.1 主要试剂 .....	39
2.2 主要仪器 .....	39
2.3 实验步骤 .....	39
3 结果与讨论 .....	40

---

3.1 分离结果 .....	40
3.2 色谱优化 .....	41
3.3 ICP 优化 .....	44
<b>4 结论 .....</b>	<b>45</b>
<b>第二节 高效液相色谱-电感耦合等离子体质谱联用装置的运用 ....</b>	<b>46</b>
1 引言 .....	46
<b>2 海产品 .....</b>	<b>46</b>
2.1 主要仪器 .....	46
2.2 主要试剂 .....	46
2.3 结果 .....	46
2.4 小结 .....	47
<b>3 生物样品 .....</b>	<b>48</b>
3.1 主要仪器 .....	48
3.2 主要试剂 .....	48
3.3 结果 .....	48
3.4 小结 .....	50
<b>4 化妆品 .....</b>	<b>50</b>
4.1 主要仪器 .....	50
4.2 主要试剂 .....	50
4.3 实验结果 .....	50
4.4 小结 .....	51
<b>第三节 中药中砷的形态分析 .....</b>	<b>52</b>
1 引言 .....	52
<b>2 实验部分 .....</b>	<b>52</b>
2.1 主要仪器 .....	52
2.2 主要试剂 .....	52
2.3 实验方法 .....	53
<b>3 结果与讨论 .....</b>	<b>54</b>
3.1 样品前处理 .....	54

3.2 不含矿物类药材的中药的提取情况 .....	54
3.3 含矿物类药材的中药的提取情况 .....	57
4 小结 .....	60
参考文献 .....	60
<b>第四章 中药中砷的体外模拟生物有效性研究 .....</b>	<b>64</b>
1 引言 .....	64
2 实验过程 .....	65
2.1 主要试剂 .....	65
2.2 主要仪器 .....	65
2.3 试验步骤 .....	66
3 结果与讨论 .....	67
3.1 方法测试 .....	67
3.2 消化的情况 .....	68
4 小结 .....	73
参考文献 .....	74
小 结 .....	75
展 望 .....	76
在学期间发表的论文 .....	77
致 谢 .....	78

---

# CONTENTS

<b>Chapter I Literature review.....</b>	<b>1</b>
<b>1 Introduction.....</b>	<b>1</b>
<b>2 Arsenic .....</b>	<b>2</b>
2.1 Property .....	2
2.2 Application .....	2
2.3 Toxicity.....	3
<b>3 Determination of arsenic in Chinese traditional medicine.....</b>	<b>4</b>
3.1 Pretreatment of sample .....	5
3.2 Detection methods .....	6
<b>4 Analysis of arsenic speciation in Chinese traditional medicine .....</b>	<b>8</b>
4.1 Introduction of arsenic speciation .....	8
4.2 Separation technique of arsenic speciation .....	9
4.3 Detection methods of arsenic speciation .....	10
4.4 Association technology.....	12
<b>5 Research on in vitro bioavailability of arsenic.....</b>	<b>13</b>
5.1 Definition .....	13
5.2 Detection methods .....	14
5.3 Simulative experiment.....	15
<b>6 Objective of this research.....</b>	<b>18</b>
<b>References .....</b>	<b>18</b>
<b>Chapter II Determination of arsenic in Chinese traditional medicine .....</b>	<b>23</b>
<b>Section A Determination of total arsenic.....</b>	<b>23</b>
<b>1 Introduction.....</b>	<b>23</b>
<b>2 Experiment .....</b>	<b>24</b>
2.1 Equipment .....	24
2.2 Reagent and sample.....	25
2.3 Experiment step.....	26
<b>3 Results and Discussion.....</b>	<b>26</b>
3.1 Condition of equipments .....	26

3.2 Pretreatment of sample .....	26
3.3 Linearity range and detection limit .....	28
3.4 Result.....	29
<b>4 Conclusion .....</b>	<b>30</b>
<b>Section B Determination of soluble arsenic.....</b>	<b>30</b>
<b>1 Introduction.....</b>	<b>30</b>
<b>2 Experiment .....</b>	<b>31</b>
2.1 Equipment .....	31
2.2 Reagent and sample.....	31
2.3 Experiment step.....	32
<b>3 Results and Discussion.....</b>	<b>32</b>
3.1 Pretreatment of sample .....	32
3.2 Conditions of HG .....	32
3.3 Condition of equipments .....	33
3.4 Linearity range and detection limit .....	34
3.5 Result.....	35
<b>4 Conclusion .....</b>	<b>35</b>
<b>References.....</b>	<b>36</b>
<b>Chapter III Analysis of arsenic speciation in Chinese traditional medicine .....</b>	<b>37</b>
<b>Section A Establishment of the association device .....</b>	<b>38</b>
<b>1 Introduction.....</b>	<b>38</b>
<b>2 Experiment .....</b>	<b>39</b>
2.1 Equipment .....	39
2.2 Reagent and sample.....	39
2.3 Experiment step.....	39
<b>3 Results and Discussion.....</b>	<b>40</b>
3.1 Experimental result.....	40
3.2 Chromatographic optimization.....	41
3.3 ICP optimization.....	44
<b>4 Conclusion .....</b>	<b>45</b>
<b>Section B Application of the association device .....</b>	<b>52</b>

---

<b>1 Introduction</b> .....	<b>46</b>
<b>2 Seafood</b> .....	<b>46</b>
2.1 Equipment .....	46
2.2 Reagent and sample.....	46
2.3 Experimental result.....	46
2.4 Conclusion.....	47
<b>3 Biomaterial</b> .....	<b>48</b>
3.1 Equipment .....	48
3.2 Reagent and sample.....	48
3.3 Experimental result.....	48
3.4 Conclusion.....	50
<b>4 Cosmetic</b> .....	<b>50</b>
4.1 Equipment .....	50
4.2 Reagent and sample.....	50
4.3 Experimental result.....	50
4.4 Conclusion.....	51
<b>Section C Application in Chinese traditional medicine</b> .....	<b>52</b>
<b>1 Introduction</b> .....	<b>52</b>
<b>2 Experiment</b> .....	<b>52</b>
2.1 Equipment .....	52
2.2 Reagent and sample.....	52
2.3 Experiment step.....	53
<b>3 Results and Discussion</b> .....	<b>54</b>
3.1 Pretreatment of sample .....	54
3.2 Extraction result.....	54
3.3 Comparision of speciation.....	57
<b>4 Conclusion</b> .....	<b>60</b>
<b>References</b> .....	<b>60</b>
<b>Chapter IV Study of arsenic in vitro bioavailability in Chinese traditional medicine</b> .....	<b>64</b>
<b>1 Introduction</b> .....	<b>64</b>
<b>2 Experiment</b> .....	<b>65</b>

2.1 Reagent and sample.....	65
2.2 Equipment .....	65
2.3 Experiment step.....	66
<b>3 Results and Discussion.....</b>	<b>67</b>
3.1 Linearity range and detection limit .....	67
3.2 Result of assimilation .....	68
<b>4 Conclusion .....</b>	<b>73</b>
<b>References.....</b>	<b>74</b>
<b>Conclusion .....</b>	<b>75</b>
<b>Prospection .....</b>	<b>76</b>
<b>Publications .....</b>	<b>77</b>
<b>Acknowledgements .....</b>	<b>78</b>

厦门大学博士论文摘要



Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to [etd@xmu.edu.cn](mailto:etd@xmu.edu.cn) for delivery details.

厦门大学博硕士论文摘要库