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RESEARCH ARTICLES

Determination of total arsenic, soluble arsenic, total mercury and soluble mercury for a Realgar and Cinnabar-containing Traditional Chinese medicine Compound Niuhuang Xiaoyan capsule by Semi-bionic Extraction-ICP-MS

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Abstract: ***Background:*** Compound Niuhuang Xiaoyan capsule (CNC) is a realgar and cinnabar-containing traditional Chinese medicine, the contents of total arsenic and mercury are far away higher than that in the relevant limit standards.

Objective: To study the contents of total arsenic, total mercury, soluble arsenic and soluble mercury in compound Niuhuang Xiaoyan capsules.

Method: Microwave digestion and semi-bionic extraction were used to pretreat the samples of Compound Niuhuang Xiaoyan capsules, and the inductively coupled plasma mass spectrometry method was established to determine the contents of total arsenic, total mercury, soluble arsenic and soluble mercury in Compound Niuhuang Xiaoyan capsules. The accuracy of the established method was further evaluated by using a certified standard reference material prepared from dried citrus leaves (GBW10020(GSB-11)).

Results: The spiked recoveries were within 95-105%. The RSD of repeatability (N=6) and precision (N = 6) were below 5.0%. The correlation coefficients of linear (R) for arsenic and mercury were all above 0.998. The limits of quantification (LOQ) were below 0.1μg/L. The contents of As and Hg were defined in the dried citrus leaves and very near to the standard values provided by the manufacturer. The established methods were applied to the analysis of three batches of compound Niuhuang Xiaoyan capsule produced by three different manufacturers successfully. Insufficient realgar or cinnabar was formulated in some Compound Niuhuang Xiaoyan capsules.

Conclusion: The analysis showed that the contents of soluble arsenic and soluble mercury in artificial gastric juice was significantly less than that of total arsenic and mercury in CNC. The results provided a reference for further study on the toxicology and pharmacokinetics of compound Niuhuang Xiaoyan capsule.

Keywords: Inductively coupled plasma mass spectrometry, Compound Niuhuang Xiaoyan capsule, semi-bionic extraction, total arsenic and mercury, soluble arsenic and mercury

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1. INTRODUCTION

Compound Niuhuang Xiaoyan capsule (CNC) is a traditional Chinese medicine with detoxification, sedative effect, has a significant effect in the treatment of upper respiratory tract infection, pneumonia, tracheitis and other symptoms. Each capsule contains 35.7 mg of Artificial bezoar, 190.6 mg of Scutellaria baicalensis, 62.3 mg of Cape jasmine, 50 mg of Cinnabar, 26.8 mg of Narcre, 66 mg of radix curcumae, 50 mg of realgar, 20 mg of borneolum syntheticum, 71.4 mg of Hydrated gypsum, 95.4 mg of Pulvis cornus bubali concentratus, 4.3 mg of berberine hydrochloride[1]. As Compound Niuhuang Xiaoyan capsule contains mineral components of cinnabar and realgar[2], with HgS and AS_2S_2 as the main constituent, so we find that the contents of total arsenic and mercury are far away higher than that in the relevant limit standards[3-5]. While, the CNC is an oral dose of medicine, only a small amount of

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arsenic in realgar and mercury in cinnabar is dissolved in the gastric juice, most are excreted in the urine and stools [6-10]. Therefore, the determination of soluble arsenic and soluble mercury is more significant than that total arsenic and total mercury in CNC to evaluate the clinical safety.

None has reported the analysis of soluble arsenic, soluble mercury, total arsenic and total mercury in the realgar and cinnabar-containing traditional Chinese medicine. In this paper, we developed a method coupled with ICP-MS to determine the contents of them in CNC to evaluate its clinical safety and provided a reference for further study on the toxicology and pharmacokinetics of CNC.

2. MATERIALS AND METHOD

2.1. Reagents and chemicals

Reference standards solutions for arsenic (As) (1000 mg/L), mercury (Hg)(1000 mg/L) were bought from the National Institute of Metrology of China (Beijing, China). Reference material certified dried citrus leaves (GBW10020(GSB-11)) was purchased from China Geophysical and Geochemical Exploration Institute. Pepsin (pig origin) was purchased from Aladdin Industrial Corporation. Concentrated nitric acid (65%) was purchased from Merck Drugs & Biotechnology Group. Concentrated hydrochloric acid (36%) was purchased from China sinopharm international corporation. Tuning solution (Li, Mg, Y, Ce, Tl, Co, C=1 mg/L, Number: 5185-5959) and internal standards stock solution (^{209}Bi, ^{72}Ge, ^{115}In, ^{6}Li, ^{103}Rh, ^{45}Sc, ^{159}Tb, C=1 mg/L, Number: 5188-6525) were purchased from Agilent Technologies (Palo Alto, USA). Ultarwater was prepared by Millipore Milli-Q system (MA, USA). Other reagents were Guaranteed reagents. The capsules (CNC) were bought from a pharmacy in Zhejiang province, including three manufacturers (codes: A, B and C), which were all the well-known manufacturers in the Chinese market.

2.2. Instruments and parameters

Agilent 7700 ICP-MS (Agilent Technologies Co., Ltd, USA); CEM MARS6 (CEM Microwave Technology Co., Ltd, USA); THZ-82 digital display water bath thermostatic oscillator (Changzhou Guohua Electric Co.,Ltd.); Centrifugal (Thermo Fisher Scientific Co., Ltd); Sartorius arium comfort II ultrapure water instrument (Sartorius Scientific Instruments(Beijing)Co., Ltd); Sartorius balance (Sartorius Scientific Instruments (Beijing) Co., Ltd).

The operating conditions of ICP-MS were set as below: RF power: 1500w, sample uptake time: 50s, the coolant gas (Ar) flow rates: 16 L/min, the carrier gas (Ar) flow rate: 1.03 L/min, auxiliary gas (Ar) flow rates: 0.8 L/min, the collision gas (He) flow rate: 5 L/min, atomization chamber temperature: 2℃, double charge ratio: 0.875%, Oxide ratio: 0.525%, He collision mode.

2.3. Solutions preparation

2.3.1. Preparation of artificial gastric juice

The artificial gastric juice was prepared as follows: 16.4 mL diluted hydrochloride acid solution was added into 1000 mL volumetric flask, then 800 mL water and 10 g pepsin were added into this solution and the total volume was adjusted to 1000 mL using water. While, the diluted hydrochloride acid solution was prepared as follows: 234 mL of hydrochloric acid solution was adjusted to 1000 mL with water.

2.3.2. Preparation of digestion solution

In this paper, after considering several factors, we decided that the solutions of the microwave digestion were 4mL HNO3 and 2mL HCL.

2.3.3. Sample preparations by microwave digestion

0.2g of powders in Compound Niuhuang Xiaoyan capsule was weighed accurately in the PTFE digestion tube, digestion solution was poured into the tube. The solution was then mixed, heated 1 hour in the temperature of 90℃, The microwave digestion program was set in the Table.1. Each digested sample was then transferred into a 50 mL volumetric flask and adjusted to 50 mL with ultra-pure water as sample stock solution. The sample stock solution was diluted 2500 folds with 5% (v/v) hydrochloric acid solution to determine the total As and total Hg. The solutions of blank samples and dried citrus leaves were treated with the same method.

Table 1. Microwave digestion program

Stage	Rate/℃/min	Temperature/℃	Hold time/min
1	20	120	5
2	6	150	10
3	6	180	5
4	4	200	15

2.3.4. Sample preparations by Semi bionic extraction in artificial gastric juice

Some Compound Niuhuang Xiaoyan capsules were weighed, 0.2 g of the powders in the capsules were weighed precisely into a 200 mL iodine flask, then 100 mL of artificial gastric juice was added into the flask. The mixed solution was oscillated for 4 h with the temperature of 37℃. Finally, the mixed solution was filtered with 0.45μm micropore membrane. The solutions of blank samples and reference material were treated with the same method.

2.3.5. Preparation of standard solutions and internal standard solutions

Table 2. The recovery for the total As and total Hg analysis.

Element	Original/mg	Added/mg	Found /mg	recovery /%	Average/%	R.S.D/%
Total As	5.5117	4.0000	9.4230	97.78	99.51	2.14
	5.5227	4.0000	9.5920	101.73		
	5.5391	4.0000	9.4230	97.10		
	5.5282	5.0000	10.3400	96.24		
	5.5063	5.0000	10.5060	99.99		
	5.5008	5.0000	10.5060	100.10		
	5.4789	6.0000	11.6460	102.79		
	5.4406	6.0000	11.4470	100.11		
	5.5172	6.0000	11.5010	99.73		
Total Hg	5.3349	4.0000	9.0290	92.35	95.33	4.34
	5.3455	4.0000	9.2280	97.06		
	5.3614	4.0000	9.2940	98.31		
	5.3508	5.0000	9.9870	92.72		
	5.3296	5.0000	9.8990	91.39		
	5.3243	5.0000	9.7660	88.83		
	5.3032	6.0000	11.1200	96.95		
	5.2661	6.0000	11.3300	101.07		
	5.3402	6.0000	11.3000	99.33		

Table 3. The recovery for the soluble As and soluble Hg analysis.

Element	Original/μg	Added/μg	Found /μg	recovery /%	Average/%	R.S.D/%
soluble As	23.2900	16.0000	38.6746	96.15	97.12	2.73
	23.4529	16.0000	38.5185	94.16		
	23.3831	16.0000	39.0463	97.90		
	23.5925	20.0000	43.1586	97.83		
	23.8717	20.0000	43.9054	100.17		
	23.4063	20.0000	41.9382	92.66		
	23.5692	24.0000	46.7052	96.40		
	23.6623	24.0000	47.1309	97.79		
	23.3365	24.0000	47.5778	101.01		
soluble Hg	0.0838	0.1200	0.1966	94.00	96.18	4.50
	0.0844	0.1200	0.1973	94.09		
	0.0841	0.1200	0.1998	96.39		
	0.0849	0.1000	0.1722	87.31		
	0.0859	0.1000	0.1858	99.91		
	0.0842	0.1000	0.1845	100.28		
	0.0848	0.0800	0.1605	94.62		
	0.0851	0.0800	0.1662	101.33		
	0.0840	0.0800	0.1621	97.67		

1.0 mL of the single element reference standard solution (As was 1000 mg/L, Hg was 1000 mg/L) was diluted to a volume of 100 mL with 5% (v/v) hydrochloric acid solution as the standard stock solution, respectively. The combined standard solutions were prepared by mixing the standard stock solutions and diluting them with a 5% (v/v) hydrochloric acid solution, finally, the mixed standard series solutions were that the As concentrations were 0.00 μg/L, 2.00 μg/L, 4.00 μg/L, 6.00 μg/L, 8.00 μg/L, 10.00 μg/L, respectively, While the Hg concentrations were 0.00 μg/L, 0.50 μg/L, 1.00 μg/L, 2.00 μg/L, 4.00 μg/L, 5.00 μg/L, respectively. Arsenic and mercury were quantified by external calibration. The internal standard solution was prepared by diluting the internal standard stock solution with a 5% (v/v) hydrochloric acid solution to a concentration of 0.1 mg/L of ^{209}Bi, ^{159}Tb, ^{103}Rh, ^{115}In, ^{72}Ge, ^{45}Sc and ^{6}Li.

2.4. Sample Analysis

The standard series solutions, sample solutions prepared by microwave digestion and sample solutions prepared by semi-bionic extraction in artificial gastric juice were introduced into the ICP-MS nebulizer via the sample tube, while the internal standard solution was introduced online via the other tube throughout the analysis.

3. RESULTS

3.1. Linear and correlation coefficient

The standard curve was fitted by the least square method and the calibration curve and linearity parameter for As was *Y=0.0070X, r=0.9997*; while, for Hg was $Y=0.0063X+ 4.455\times10^{-4}$, *r= 0.9986*.

3.2. LOD and LOQ

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated as three and ten times the standard deviation on the measured concentration for eleven replicate blank samples, respectively. The LOD of As was 0.02 μg/L, the LOD of Hg was 0.001 μg/L, the LOQ of AS was 0.05μg/L, the LOQ of Hg was 0.004 μg/L.

3.3. Precision of instrument

The precision of the ICP-MS instrument of As and Hg in CNC was investigated by preparing and analyzing the analytical solutions (As was 2.00μg/L, Hg was 1.00μg/L) six times respectively, the RSD for As was 2.0%, while for Hg was 3.2%, which indicates the precision of instrument was relatively good.

3.4. Repeatability and stability

Six independent solutions (Section 2.3.3. and 2.3.4.) were prepared and analyzed to evaluate the repeatability of the abovementioned method. The results showed the RSD value (n=6) for total As was 0.38%, for total Hg was 2.2%, for soluble As was 1.5%, for soluble Hg was 1.3%. One solution (Section 2.3.3. and 2.3.4.) was prepared and analyzed for 0–2 h, 4 h, 6 h and 8 h, to evaluate the stability of the abovementioned method. The RSD values (n = 6) for total As was 0.64%, for total Hg was 4.0%, for soluble As was 1.7%, for soluble Hg was 1.8%.

3.5. Accuracy

Spiked samples were prepared at 80%, 100%, 120% of the contents for each element by spiking samples with reference standards solutions before dissolution. Then spiked samples were treated by the abovementioned methods (Section 2.3.3. and 2.3.4.). Finally, the solutions of the spiked samples were analyzed as described in Section 2.4. The recovery yields of the total arsenic ranged from 96.24% to 102.79% (average in 99.51%), and from 88.83% to 101.07% for total mercury (average in 95.33%) (Table. 2). The recovery yields of the soluble arsenic ranged from 92.66% to 101.01% (average in 97.12%), and from 87.31% to 101.33% for soluble mercury (average in 96.18%) (Table. 3). The contents of As and Hg were defined in the dried citrus leaves and very near to the standard values provided by the manufacturer. The results showed the established methods were feasible and high in accuracy.

3.6. Samples determination

Based on this validated method, three batches of CNC produced by three different manufacturers (manufacturers' codes: A, Band C) were successfully analyzed. The contents of the total arsenic, total mercury, soluble arsenic and soluble mercury in the sample solutions were listed in Table 4.

Table 4. Results of sample analysis. Mean ± SD (n = 6).

Codes	Total As[a]	Total Hg[a]	Soluble As[b]	Soluble Hg[b]
A	54.73±0.21	232.6±3.5	19.07±0.41	1.036±0.03
B	79.30±0.69	383.9±13.2	106.0±2.37	0.825±0.01
C	82.10±3.03	944.3±29.3	94.94±2.63	2.001±0.06
Dried citrus leaves	1.12±0.015	0.145±0.004	ND[c]	ND[c]

a mg/kg; b μg/kg; c Not detectable.

4. DISCUSSION

4.1. Development of the microwave digestion

Nitric acid is an ideal medium for inductively coupled-plasma mass spectrometry, which can quickly destroy organic substance without causing mass spectrometry interference. Hydrochloric acid is mainly helpful to the digestion of HgS and can improve the solubility and stability of the elements in the sample solutions. In this study, 4ml nitric acid and 2ml hydrochloric acid were used as the digestion system. The digestion solution was clear without wall hanging phenomenon, which indicated the microwave digestion method was satisfied.

4.2. Development of the Semi-bionic extraction

The retention time of food in the stomach is about 4~6 hours. In order to simulate the environment in the stomach, in this study, the time and the water bath temperature of the semi-bionic extraction method were decided to be 4 hours and 37℃.

4.3. Interference and elimination

The interferences of ICP-MS analysis mainly include mass spectral interference and non-interference. In this study, the method to eliminate the interference of mass spectrometry is to tune the instrument to make the mass axis, resolution, sensitivity, oxide and double charge meet the analysis requirements, and helium collision mode is used to analysis the samples. While, the non-mass spectrum interference can be effectively reduced by internal standard solution which was introduced online throughout the analysis by a single tube.

4.4. Elimination of mercury memory effect

Mercury can be changed into elemental mercury in atomic state at room temperature. Elemental mercury will be adsorbed by container and tube wall, so it will have loss and memory effect. In order to ensure the stable existence of Hg in the solution as Hg2+ valence state, in this study, hydrochloric acid was used as a dissolution and dilution solution to reduce the adsorption and memory effects as mercury element can be formed [HgClx]n- patten in the solutions. Meanwhile, blank samples were added between each analysis sample to eliminate mercury memory effect.

4.5. Analysis and discussion of determination result

In the ChP, the CNC weighs 0.4 g per capsule[1], so we can calculate that the concentration of As in CNC should be no less than 31.50mg per capsule, the concentration of Hg in CNC should be no less than 42.24 mg per capsule. The results indicated that the contents of As for A was 21.89mg, for B was 31.72mg, for C was 32.84mg; while the contents of Hg for A was 7.63mg, for B was 42.40mg, for C was 37.98mg. Therefore, some manufacturers were suspected of insufficient feeding. The ratio (soluble As / total As) were 0.25~1.15%, the ratio (soluble Hg / total Hg) were 0.001~0.005%, which was similar to other realgar and cinnabar containing medicines reported in documents [21]. In the table 4., we can find that the contents of soluble As and soluble Hg were much higher than the total content limit which As was 2mg/kg and Hg was 0.2mg/kg [11]. If the soluble As and Hg of Compound Niuhuang Xiaoyan capsules could be absorbed by human body completely, considered the daily oral dose(six capsules per day), we could calculate that the absorptions of As were 492~1044 μg per day, the absorptions of As were 4.8~12 μg per day. Obviously, the contents of As were far beyond the limits of the relevant standards [11-13]. However, all those limits were set for the control of As and Hg pollution in herbal medicine, dietary supplement, food or environment [14-18,21]. As a traditional Chinese medicine, Compound Niuhuang Xiaoyan capsule is not suitable for those standards. In summary, the real As and Hg toxicity of Compound Niuhuang Xiaoyan capsules still need for further study.

CONCLUSION

In this paper, through the optimization of sample pretreatment and tuning of instrument conditions, the determination method of total arsenic, total mercury, soluble arsenic and soluble mercury in compound Niuhuang Xiaoyan capsules was established by ICP-MS. The results of this method are accurate, with high precision, good repeatability and reliable method. The data can provide a reference for further study on the toxicology and pharmacokinetics of Compound Niuhuang Xiaoyan capsule.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base on this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABLITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, financial or otherwise.

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