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Determination of 20 trace elements and arsenic species for a realgar-containing traditional Chinese medicine Niuhuang Jiedu tablets by direct inductively coupled plasma–mass spectrometry and high performance liquid chromatography–inductively coupled plasma–mass spectrometry

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ABSTRACT

Niuhuang Jiedu tablet (NHJDT) is a realgar-containing traditional Chinese medicine. A direct inductively coupled plasma-mass spectrometry (ICP-MS) method for the simultaneous determination of 20 trace elements (Mg, K, Ca, Na, Fe, As, Zn, Sr, Ba, Cu, Mn, Ni, Pb, V, Cr, Se, Co, Mo, Cd, Hg) in NHJDT, as well as in water, gastric fluid and intestinal fluid was established. Meanwhile, a high performance liquid chromatography–inductively coupled plasma-mass spectrometry (HPLC–ICP-MS) method was developed for the determination of arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and for the identification of arsenobetaine (AsB) and arsenocholine (AsC) in these extracts. Both methods were fully validated in the respect of linearity, sensitivity, precision, stability and accuracy. The reliability of the ICP-MS method was further evaluated using a certified standard reference material prepared from dried tomato leaves (NIST, SRM 1572a). The analysis showed that some manufacturers formulated lower amount of realgar than required in the Chinese Pharmacopoeia (ChP) in their preparations. In addition, almost same extraction profiles for total As and inorganic As were found in water and in gastrointestinal fluids, while higher extraction rates for other 19 elements were observed in gastrointestinal fluids. Our findings show that the toxicities of Hg, Cu, Cd and Pb in NHJDP are low, while the real As toxicity in NHJDT should be deeply investigated.

1. Introduction

Niuhuang Jiedu tablet (NHJDT) is a patented traditional Chinese medicine used for the treatment of hyperactivity of stagnated fire, aphthae, swelling and gingiva, throat and eyes aching [1]. Each tablet contains 3.33 mg of cultivated calculus bovis, 33.3 mg of realgar, 133 mg of gypsum fibrosum, 133 mg of rheum palamatum, 100 mg of scutellaria baicalensis, 66.7 mg of platycodon grandiflorum, 16.7 mg of borneolum syntheticum and 33.3 mg of gycyrrhiza uralensis. Since the mineral drugs of realgar (with As_2S_2 as the main constituent) and gypsum fibrosum (with $CaSO_4{\cdot}2H_2O$ as the main constituent) were formulated in the pharmaceutical preparation, and increasing cases of arsenic poisoning induced by NHJDT were reported in recent years [2–13], the Sweden's food safety watchdog warned "extremely high" level of arsenic (As) in NHJDT, posing a "very serious health hazard" [14]. Therefore, the determination of As and other mineral elements in NHJDT is critical for clinical safety.

In theory, As_2S_2 and $CaSO_4$ are insoluble in aqueous solutions, whereas only elements dissolved in gastrointestinal tracts could be absorbed by the body. Therefore the determination of these elements in gastrointestinal tracts is more important. Because of the variation of the toxicity levels of different As species, the determination of dissolved elements in gastrointestinal tracts was still not adequate for the evaluation of arsenical toxicity. Inorganic arsenicals, including arsenite (As^{III}) and arsenate (As^{V}), were reported to be very toxic, with As^{III} being even more toxic than As^{V}. Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)

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exhibit only about 1/400 toxicity of inorganic forms, while arsenobetaine (AsB) and arsenocholine (AsC) were almost non-toxic [15]. Therefore, the species analysis for dissolved As was necessary for the toxicity evaluation.

Only few reports have been published about the determination of trace elements in NHJDT. Zheng et al. reported the quantification of 14 elements in NHJDT by inductively coupled plasma atomic-emission spectrometry (ICP-AES) [16], Bai et al. studied the total content of 5 heavy metals (Cu, As, Hg, Cd, Pb) in NHJDT and their extraction profiles in gastrointestinal fluids by inductively coupled plasma-mass spectrometry (ICP-MS) [17]. Although some literatures have reported As speciation analysis in ground water [18], edible oil [19], meat [20,21], marine samples [21–26] and urine [26–28], none has reported the analysis of As species in pharmaceutical formulations. In this paper, we report a method for the determination of 20 elements in NHJDT, its water and gastrointestinal fluids extracts by ICP-MS. We also investigated the different As species in these extracts by high performance liquid chromatography–inductively coupled plasma-mass spectrometry (HPLC–ICP-MS).

1. Experimental

2.1. Reagents and chemicals

Reference standard solutions for arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), arsenocholine (AsC), sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), barium (Ba), mercury (Hg) and lead (Pb) were purchased from the National Institute of Metrology of China (Beijing, China). Certified tomato leaves reference material (SRM 1572a) was purchased from the National Institute of Standards and Technology (Gaithersburg, USA). Suprapure concentrated nitric acid (65%) and hydrofluoric acid (40%), optima-grade hydrochloric acid, potassium dihydrogen phosphate and sodium hydroxide were obtained from the Beijing Reagent Company (Beijing, China). Agilent tuning solution (10 $\mu g\,L^{-1}$ of Li, Y, Ce, Tl, Co) and internal standards stock solution (10 $mg\,L^{-1}$ of ^{6}Li, ^{45}Sc, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb, ^{209}Bi) were purchased from Agilent Technologies (Palo Alto, USA). Water was purified with a Millipore Milli-Q system (18 MΩ, Bedford, USA). Pepsin was purchased from Sigma (St. Louis, USA) and pancreatin was bought from Merck (Darmstadt, Germany).

The tablets (NHJDT) were purchased from a pharmacy in Beijing, including two kinds of film coated tablets (manufacturers' codes: TRT and LD) and three kinds of sugar coated tablets (manufacturers' codes: ZT, HT and YB), which were all the most popular brands in the Chinese market.

2.2. Sample preparation

2.2.1. Preparation of artificial gastric and intestinal fluids

Artificial gastric and intestinal fluids were prepared as described in ChP [29]. For the artificial gastric fluid preparation, 16.4 mL of diluted hydrochloride acid solution (234 mL of hydrochloric acid solution adjusted to 1000 mL with water) was mixed with 800 mL of water. 10 g of pepsin was dissolved in this solution and the total volume was adjusted to 1000 mL using water. The artificial intestinal fluid was obtained by dissolving 6.8 g of potassium dihydrogen phosphate in 500 mL of water and the pH was adjusted to 6.8 with 0.1 M of sodium hydroxide solution (*Solution A*). 10 g of pancreatin was then dissolved in an appropriate amount of water (*Solution B*) and *solution A* and *solution B* were mixed and the volume was adjusted to 1000 mL with water.

2.2.2. Preparation of analytical solutions of NHJDT and NIST reference materials

0.1 g of the above mentioned tablets powder or NIST tomato leaves reference material (SRM 1572a) was accurately weighed in a microwave digestion tube, into which 6 mL of suprapure concentrated nitric acid (65%) solution and 0.2 mL of hydrofluoric acid (40%) were added. The solution was then mixed, heated at 95 °C for 1 h, and cooled to room temperature. The solution was subjected to a 4 stage microwave digestion program (power 1200 W) using a CEM MARS x-press microwave digester (CEM, Matthews, USA), as follows: (1) the temperature was raised from room temperature to 120 °C in 5 min and maintained at this temperature for 5 min; (2) the temperature was then increased to 150 °C in 5 min at which it was held for 10 min; (3) the temperature was raised to 180 °C in 5 min, followed by a plateau at this temperature for 15 min; (4) finally, the temperature was brought to 200 °C in 5 min and held for 5 min. The digested sample was then transferred into a 50 mL volumetric flask and the volume was adjusted to 50 mL with water. The determination of total As was carried out after the 500 folds dilution of the prepared solutions using a 10% (v/v) nitric acid solution, while the other 19 elements were determined by directly analyzing the prepared solutions. Blank solutions containing no NHJDT and reference material were prepared and analyzed within the same batch of the samples.

2.2.3. Samples extraction in artificial gastric and intestinal fluids and in water

The sample extraction in artificial gastric fluid was carried out as follows. First, 20 tablets (sugar coating removed) were weighed, finely grinded, and about 0.8 g of the powder (equivalent to a single dose) was transferred into a 200 mL conical flask containing 100 mL of artificial gastric fluid. The solution was vortexed at 37 °C for 1 h and filtered. The residue was then rinsed 3 times with the artificial gastric fluid solution and the filtrate was further diluted to 200 mL with water.

For the sample extraction in the artificial intestinal fluid, the filtered residue was transferred into a 200 mL conical flask containing 100 mL of artificial intestinal fluid. The solution was then vortexed at 37 °C for 4 h, filtered and the residue rinsed 3 times with the artificial intestinal fluid. The filtrate was then diluted to 200 mL with water.

The sample extraction in water was carried out by transferring 0.8 g of the above mentioned tablets powder in a 200 mL conical flask containing 100 mL of water, and vortexed at 37 °C for 5 h. The solution was then filtered and the residue rinsed 3 times with water. The filtrate was then diluted to 200 mL using water. These extracts were diluted 10 folds with a 10% (v/v) solution of nitric acid for the determination of total As, while the other 19 elements and arsenic species were determined by the direct analysis of the extracts after filtration through a 0.22 μm PTFE membrane filter (Anachem, Cheshire, UK). Blank extracts containing no NHJDT were prepared and analyzed within the same batch of the samples.

2.3. Equipment and operating conditions of HPLC and ICP-MS

The total content of each element was directly determined using an Agilent 7500a ICP-MS (Agilent Technologies, Palo Alto, USA), while the As species were separated on an Agilent 1100 unary pump HPLC system (Agilent Technologies, Palo Alto, USA) and an Agilent G3154-65001 anion exchange resin column (150 mm × 4.6 mm i.d., 5 μm, Agilent Technologies, Palo Alto, USA) prior to ICP-MS analy-