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A Study on a Method of Detecting Heavy Metal Content Using Thioglycolic Acid and Quartz Crucible

Guoqiang Fu, Xinyue Qian, Xinyi Liu, Enxu Wang *

Bloomage Biotech (Tianjin) Technology Co., LTD, Tianjin 300000, China
*Corresponding Author: wangenxu@bloomagebiotech.com

ABSTRACT

In the Chinese Pharmacopoeia, the method of detecting heavy metals commonly uses thioglycolic acid. It is often used as a special method for the limit check of heavy metals in raw pharmaceutical or cosmetic raw materials. Traditional use of porcelain crucibles in sample pretreatment may result in false positive reactions. This study used quartz crucibles to replace the traditional porcelain crucibles to study the possibility of contamination. It was found that the false negative reactions caused by crucible material can be eliminated, and the precision of the method was verified by detecting three batches of samples. The method has good repeatability and accuracy. It can replace the use of traditional porcelain crucibles in the method of detecting heavy metals with thioglycolic acid.

KEYWORDS

Heavy metals; Thiocyanate method; Quartz crucible

1. INTRODUCTION

Heavy metals are often encountered in our daily lives and can be found in soil, water, and even in living organisms on the Earth's surface. They accumulate along the food chain [1, 2]. Heavy metals generally refer to metals with a density of 5 or more, although some refer to metals with a density of 4 or more. There are about 45-60 elements, with no strict definition, and some specialized works may define the scope of heavy metals very broadly. For example, arsenic is a metalloid, but because of its toxicity and certain properties similar to heavy metals, it is often included in discussions of heavy metals. In terms of environmental pollution, the heavy metals referred to are primarily mercury, cadmium, lead, chromium, and the metalloid arsenic, all of which have significant biological toxicity. They also refer to certain other heavy metals with moderate toxicity, such as zinc, copper, tin, nickel, and vanadium [3]. Heavy metals enter the body through diet, breathing, and the skin. They can directly bind to proteins and enzymes that contain hydroxyl and amino groups, thereby affecting their normal physiological and biochemical functions and even rendering them inactive. This can lead to symptoms such as disorders in protein and carbohydrate metabolism. Other important life substances such as nucleic acids, hormones, and neurotransmitters can also bind to or be affected by heavy metals, causing human illnesses [4, 5]. The Minamata disease, which once caused widespread global panic, was an environmental pollution disease caused by heavy metal mercury and cadmium in human water sources. In recent years, it has been found that certain heavy metals have carcinogenic effects, which has aroused people's concern about the toxicity of heavy metals.

In the production process of raw materials or cosmetic raw materials, there may be lead, zinc, cadmium, copper and other heavy metal impurities in the raw materials, auxiliary materials, packaging materials, and metal equipment, pipelines, and other metal tools used in the production

process. If there is a risk of heavy metal pollution in the raw materials, auxiliary materials, and packaging materials, and the production system, the products produced will inevitably have heavy metal residues, which will cause harm to the human body. If we simply want to remove these pollutants, it will inevitably cause difficulties in production operations. However, within the safe residue limit of heavy metals, which adheres to the principle of not causing adverse effects, it is allowed to have certain limits of heavy metal residues, so it is necessary to conduct tests on the limits of the products [6], and it will also be one of the key points of daily product quality risk control.

Currently, the most commonly used method for detecting heavy metals in raw materials and cosmetic raw materials is the Heavy Metal Test in the Chinese Pharmacopoeia. Among them, Method 0821 specifies three detection methods, with the second method being the most commonly used. The main principle is to use heavy metals, which refer to metallic impurities that can react with thioacetamide or sodium sulfide under specified experimental conditions to produce a colored reaction. Most methods use the sample after the ash residue has been determined to detect heavy metals or directly use the method. Most laboratories use porcelain crucibles for detection, and acid is often used in the process. However, the porcelain crucible used in production is generally coated with a glaze on the surface. Currently, laboratory crucibles are used repeatedly, but the detection of heavy metals or ash residue in the Chinese Pharmacopoeia will use acid, which will cause the glaze on the surface of the porcelain crucible to corrode under high-temperature incineration conditions, revealing the inside material, which is usually a fired refractory kaolinite-type bone ash raw material. This material will make the reaction solution cloudy and cause false positive reactions, leading to deviations in the test results. After comparing with the laboratory, we used quartz crucibles to replace the porcelain crucibles, and the blank test no longer showed positive reactions. The reaction solution was clear and did not interfere with the test results. After using quartz crucibles in the laboratory, we verified the accuracy and precision, both of which met the requirements.

2. EXPERIMENT

2.1. Experimental Principle

The heavy metals referred to in this method are those that can react with thioglycolic acid or sodium thiosulfate to produce a colored complex under specified experimental conditions.

Thioglycolic acid hydrolyzes in weak acidic conditions (pH 3.5 acetic acid buffer solution) to produce hydrogen sulfide, which reacts with trace amounts of heavy metal ions to form a yellow to brown-black suspension of sulfide. The color produced by the same amount of standard lead solution treated in the same manner is compared [7, 8].

$$CH_3CSNH_2 + H_2O \xrightarrow{pH\ 3.5} CH_3CONH_2 + H_2S\uparrow$$

$$Pb_2 + H_2S \xrightarrow{pH\ 3.5} PbS\ \text{Yellow-brownish}$$

2.2. Experimental Equipment and Reagents

Electronic scale, electric furnace, muffle furnace, ceramic crucible (for the first use), ceramic crucible (used 3 times), ceramic crucible (used 10 times), quartz crucible, graduated cylinders (25 mL), pipettes, lead nitrate, thioacetamide, sodium hydroxide, glycerin, concentrated sulfuric acid, concentrated nitric acid, ammonia water.

2.3. Preparation of Experimental Reagents

Standard stock solution of lead nitrate: Precision weigh 0.1599 g of lead nitrate and place it in a 1,000 mL volumetric flask. Add 5 mL of nitric acid and 50 mL of water, dissolve it, and then dilute it with water to the mark. Shake well and use it as the stock solution.

Standard working solution of lead nitrate (10 μg/mL): Precision measure 10 mL of the stock solution and place it in a 100 mL volumetric flask. Add water to dilute to the mark. Shake well and use it on the same day.

Acetate buffer solution (pH 3.5): Take 25g ammonium acetate, dissolve it in 25 mL of water, then add 38 mL of 7 mol/L hydrochloric acid solution. Adjust the pH value to 3.5 with 2 mol/L hydrochloric acid solution or 5 mol/L ammonia solution, dilute it with water to 100 mL, and mix well for use.

Thiocyanamide reagent: Take 4 g of thiocyanamide and dissolve it in water to make 100 mL. Store it in the refrigerator. Before use, take 5.0 mL of the mixture (composed of 15 mL of 1 mol/L sodium hydroxide solution, 5.0 mL of water, and 20 mL of glycerol) and add 1.0 mL of the thiocyanamide solution. Heat it on a water bath for 20 seconds, cool it, and use it immediately.

Ammonia solution: Take 400 mL of concentrated ammonia solution, add water to make it 1,000 mL, and you have it.

Phenolphthalein indicator: Take 1 g of phenolphthalein, dissolve it in 100 mL of ethanol, and you have the indicator.

2.4. Experimental Procedures

(1) Sample Preparation: Precision weigh 1 g of the sample and place it in a crucible on a stove. Slowly roast it until it is completely carbonized. Allow it to cool, then add 0.5 mL-1.0 mL of sulfuric acid to moisten it. Heat it over a low flame until the sulfuric acid is completely removed. Then add 0.5 mL of nitric acid and heat it over a low flame until the nitric oxide gas is completely removed. Allow it to cool, and then roast it at 500°C-600°C until it is completely ash.

(2) Experimental Tube: After the ash is completely cooled, add 2.0 mL of hydrochloric acid to the crucible and heat it over a water bath until it is evaporated. Then add 15 mL of water, drop by drop of ammonia solution until a faint pink color appears, then add 2.0 mL of acetic acid buffer solution (pH 3.5) and heat it slightly to dissolve it. Transfer the entire contents to a Test tube and add water to dilute it to 25 mL, which serves as the experimental tube.

(3) Positive Control: In the crucible, add 0.5 mL-1.0 mL of sulfuric acid and 0.5 mL of nitric acid, heat it over a low flame until it is evaporated. Allow it to cool, then add 2.0 mL of hydrochloric acid to the crucible and heat it over a water bath until it is evaporated. Then add 15 mL of water, drop by drop of ammonia solution until a faint pink color appears, then add 2.0 mL of acetic acid buffer solution (pH 3.5) and heat it slightly to dissolve it. Add a certain amount of lead standard solution and dilute it with water to 25 mL, which serves as the positive control.

(4) Negative Control: All steps except adding a certain amount of lead standard solution are performed in the same way as the positive control. Add 2.0 mL of thioacetamide reagent to the test tube, positive control, and negative control tube, mix well, and leave for 2 minutes. Place them all on a white paper and observe the color reaction from above downwards.

(5) Criteria for Determination: The negative control tube should not have any color, and the color of the sample tube should not be deeper than that of the positive control tube.

2.5. Validation of Accuracy and Precision of Quartz Crucible Replacement for Porcelain Crucible Method

2.5.1. Validation of Method Accuracy

Three batches of samples were tested, and the recovery rate of three batches of samples was determined at different concentrations. The recovery rate was used to evaluate the quartz crucible replacement method for porcelain crucible detection, with a requirement of 80%-110% recovery rate.

2.5.2. Validation of Method Precision

For three batches of samples, six replicate tests were conducted, and the precision was evaluated using the relative standard deviation. The relative standard deviation should be less than 20%.

3. EXPERIMENTAL RESULTS

(1) According to Method 2.4.4, new porcelain crucibles, used crucibles, and quartz crucibles were separately subjected to the negative control experiment described above to observe the influence of different crucibles on the experiment.

As shown in Figure 1, we found that the negative control experiments using new porcelain crucibles and quartz crucibles were consistent with the blank control group, but we also found that the experimental results of the crucibles that had been used 3 times or more than 5 times showed a color reaction. The stronger the number of times the crucible was used, the more intense the color reaction.

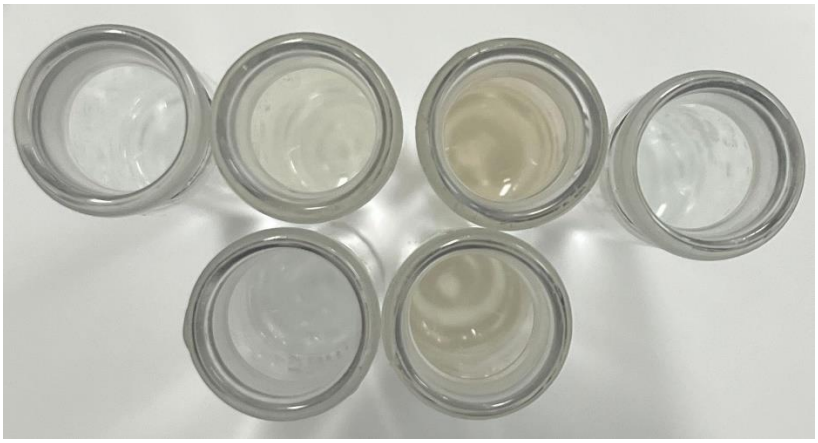

Figure 1. Negative control experiments with different crucibles

Note: The tubes in the top row from left to right are new ceramic crucibles, ceramic crucibles used 3 times, ceramic crucibles used 10 times or more, and a negative control tube for the quartz crucible set; the tubes in the bottom row from left to right are blank tubes without reagents, and positive control tubes (10 mg/kg)

(2) Using quartz crucibles, according to Method 2.4, we selected three batches of samples for the detection of heavy metal content. The test results are shown in Table 1. At the same time, we conducted 6 repetitive tests for each batch to observe the precision of the method using quartz crucibles instead of ceramic crucibles.

Table 1. Repeatability Verification of Different Batch Samples

batch	Test Results (mg/kg)						average (mg/kg)	SD	RSD %
1	4	4	3	3	4	4	3.7	0.516	14
2	8	8	8	9	9	8	8.5	0.548	6
3	15	16	15	·14	17	16	15.8	0.837	5

(3) We conducted different concentration recovery tests on three batches of samples. As shown in Table 2.

Table 2. Validation of recovery rate for samples from different batches

	Background value (mg/kg)	Theoretical Addition Amount (mg/kg)	Actual test values (mg/kg)	Actual recovery rate (mg/kg)	recovery rate %
batch 1	4	2	6	2	100.0%
	4	4	8	4	100.0%
	4	5	9	5	100.0%
batch 2	9	4	13	4	100.0%
	9	9	17	8	88.9%
	9	11	21	12	109.1%
batch 3	16	8	23	7	87.5%
	16	16	31	15	93.8%
	16	19	38	22	115.8%

4. DISCUSSION

Through this study, we found that the use of porcelain crucibles to detect heavy metal limits using the thioglycolic acid method in the Chinese Pharmacopoeia requires evaluation, as the quality of the crucibles can be biased by different manufacturing processes and cost factors, leading to deviations in the detection results. The probability and extent of false positives will increase as the use of porcelain crucibles increases and the degree of corrosion of the crucibles increases. Using quartz crucibles to replace traditional porcelain crucibles for this method of detection will not cause false positive reactions, and quartz crucibles should not have residual impurities after each use and can be soaked in a 5% nitric acid solution for a long time. Due to the inherent properties of the material, quartz crucibles have a smaller coefficient of expansion and are more resistant to temperature changes in practical operation, and are acid-resistant.

This study conducted a preliminary investigation into the precision of using quartz crucibles to replace traditional ceramic crucibles in the measurement of heavy metals using thioglycolic acid method. We used three batches of samples for multiple determinations, with relative standard deviations below 20%, indicating good reproducibility. However, the reproducibility of detection for samples with a background level of less than 5 mg/kg may be slightly lower, possibly due to the low concentration of heavy metals, resulting in a lighter reaction color that is less sensitive to human observation. For accuracy, we used recovery rates to evaluate the method. By adding standardized samples of different concentrations after the steps of carbonization, ashification, dissolution, transfer, and coloration, we recovered the heavy metals and the theoretically added amounts were almost identical, with recovery rates ranging from 80% to 110%, indicating good accuracy.

In summary, using quartz crucibles instead of traditional ceramic crucibles for the detection of heavy metals in raw pharmaceutical and cosmetic ingredients by thioglycolic acid method, has good accuracy and reproducibility, and no false positive reactions occur.

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