Determination of trace Mercury in Amylase by Atomic Fluorescence Spectrometry

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Abstract: An atomic fluorescence method was established for the determination of mercury in amylase. After sample digestion, mercury reacts with potassium borohydride in a specific medium to produce volatile hydride. The fluorescence of characteristic wavelength is emitted under the irradiation of argon loaded quartz atomizer light source, and the fluorescence intensity is proportional to the content of elements.

Keywords: Microwave digestion; atomic fluorescence; amylase; mercury.

1. Introduction

Enzyme is a kind of biocatalyzer, which can improve the processing performance of apple raw materials and improve the quality of its products. Amylase is a kind of hydrolytic enzyme, which is widely used in fermentation industry at present.

Mercury is a toxic element with strong accumulation. There are silver salt colorimetric method, spectrophotometry, atomic absorption spectrometry, atomic fluorescence spectrometry and other atomic fluorescence techniques[1-3] are developing rapidly in recent years. Hg is a toxic element commonly contained in many herbal foods. Both inorganic and organic Hg compounds are accumulated in kidney and brain and exhibit serious of the poisonousness [4]. Hg also is genotoxic which will increase the risk of chromosomal aberrations [5, 6].

It is well known that As at trace and ultra-trace level can be determined by AAS, ICP-

AES , ICP-MS , ICP-OES and CVG-AFS . AFS was the most frequently-used analytical method in China because it had a wide linear range, high sensitivity, low background noise, convenient operation, and low cost. Chemical vapor generation is an efficient method to separate ultra-trace species from sample matrix of Chinese herbs, which can improve the detection limit by utilizing an optimized flow system[7-15].

2. Experimence

2.1 Instrument and Reagents

Atomic fluorescence spectrophotometer; Merury hollow cathode lamp; CEM microwave digester, The used reagents were analytical-reagent grade. The carrier gas and shield gas were N-50 purity compressed argon (99.999%) of BOC GAZY (China). 18.2MΩ.cm de-ionized water from a Mille-Q system (Millipore, Milford, MA, USA). The commercial standard stock solutions were purchased from the National Standard Material Center of China(1.0 g L-1 of As, Sb, Se and Hg) and stored in the refrigerator at 4℃. The As working standard solutions were in 5% (v/v) HCl, diluted by the stock solutions. Potassium tetrahydroborate solution was in 1% (w/w) KOH solution, and dissolving the solid KBH4 of Xi'an Chemicals Co., China. The HCl solution was prepared by GR grade HCl from Xi'an Chemicals Co., China.

2.2 Sample pretreatment

Microwave digestion program: the amylase sample was weighed about 1.0g，and added 2.5ml nitric acid,5ml water, 5min at 0-120℃, 20min at 120-170℃. 2.5 mL hydrochloric acid 2.5ml thiosara-ascorbic acid mixed solution was added in turn. The solution was volumetric and shaken well, and placed for at 20 min.

3. Results and Discussion

The selective fluorescence intensity of the lamp current and negative high voltage increases with the increase of the lamp current and negative high voltage of the photomultiplier tube. The increase of negative high voltage is beneficial to the improvement of sensitivity, but the reduction of linear range is considered comprehensively. The lamp current is 20 mA and negative high voltage is 270V.

Observation height was very important to the fluorescence signals of Hg, which is the distance from the top of the atomizer to the point where the atomic fluorescence signal is generated. For Hg element, the intensities were decreased slowly. Take signal-to-noise ratio into consideration of this method and signal intensities, the observation height was selected at 8 mm.

The results of potassium borocyanide dosage show that too low or too high concentration of potassium borocyanide will lead to the decrease of sensitivity, the concentration of potassium borocyanide at 0.05%~0.5% is more stable, so the

concentration of potassium borocyanide at 0.1% is used as a reducing agent.

In this method, hydrochloric acid was selected as the carrier. Since the generation efficiency of chemical vapors extremely depended on the acidity of the reaction solution, the intensity of analyzed elements can be affected by the concentration of HCl. Moreover, the stability of the reaction system is related to the concentration of HCl as well. When the concentration of hydrochloric acid was 1% and 10%, the fluorescence intensity was stable, and 5% hydrochloric acid was used as the medium.

Standard Curve

There was a linear relationship between fluorescence intensity and concentration, when Hg was 1～10.00μg/L. Linear equation was Y=156.28x-22.45, r=0.999.

Precision test

The relative standard deviation (RSD%) of amylase samples was calculated for 7 times, and the relative standard deviation (RSD%) of mercury was 3.3%.

Standard addition and recycling experiment

The mercury standard solution was added to amylase samples, and the mercury standard recovery ranged from 91.4%to 105.1%, which indicated high method accuracy.

Interferences

The potential influencing factors of 14 metallic elements were tested in the method. The interferences were studied in a mixed standard solution with the concentration 1 ng mL −1 of Hg, 100 mg L−1 of Fe^{3+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, 50mg L −1 of Cu^{2+}, Ni^{2+}, Cr^{6+}, Mo^{6+}, Sr^{2+} and 5mg L −1 of Pb^{2+}, Co^{2+}, Ag^+, Au^{3+}. The error range of determination results in the optimal chemical conditions was ±5%. The aforementioned metal contents in Chinese herb samples were well within the tolerant concentration range. So there were no interferences in the determination of Hg.

4. Conclusion

This method is suitable for the determination of mercury by atomic fluorescence method. The results are in line with the national standard limit. This method has the advantages of simple, rapid and accurate operation, less interference, high sensitivity, precision and recovery tests, and is suitable for the determination of mercury in amylase.

Acknowledgements

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