Determination of Heavy Metals in the Plant Sample Pretreatment Methods

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Abstract. In this paper, dry ashing method, mixed acid nitration (H₂SO₄-H₂O₂), three acid digestion (HNO₃-H₂SO₄-HClO₄) and aqua-regia-HClO₄ these four test solution preparation method on the decomposition of plant standards GBW07603 processing, use atomic absorption spectrophotometry Zn, Cu, Pb, Fe, Cd, Cr six kinds of metal elements, and the measured values compared with the standard value. The results showed that after dry ashing recoveries higher than Zn recovery after acid digestion; Cu determination should not use dry ashing; Pb determination should not be used mixed acid nitration (H₂SO₄-H₂O₂) or tri-acid digestion method (HNO₃-H₂SO₄-HClO₄); dry ashing treatment after acid digestion Cr yield higher Cr recoveries. Comprehensive analysis of the low carbon content of the metal elements in plant samples (Zn, Cu, Pb, Fe, Cd, Cr) of the measurement, can be aqua-regia-HClO₄ pretreatment method, and for comparing the carbon content high plant samples should be used as a dry ashing pretreatment method. That is, different pretreatment methods for the determination of different analyte have a certain impact.

1. Preface
In recent years, metal pollution has become more and more serious, and phytoremediation has been widely recognized [1] in the treatment of metal pollution. The accurate determination of plant metal elements is one of the important links in the research of phytoremediation. As a key step in the determination of plant metal elements, the pretreatment of plant samples directly affects the precision and accuracy of the analysis results. Therefore, it is an important method to select the appropriate pretreatment method, shorten the pretreatment time of the sample and digest the plant sample quickly and thoroughly to ensure the determination of metal elements and improve the efficiency of metal elements.

2. Materials and methods
2.1. Instruments and reagents
Experimental instruments: digester, universal electric furnace, muffle furnace, electronic balance, atomic spectrophotometer [2], dry filter; Experimental reagents: HCl, HNO₃, H₂SO₄, HClO₄ (superior purity); H₂O₂ (superior purity); Zn, Cu, Pb, Fe, CD, Cr standard stock solution; Experimental sample:
GBW0763 biological component analysis - shrub branch and leaf component analysis reference material; all experimental reagents are of superior grade, and the experimental water is secondary distilled water.

2.2. Test method

2.2.1. Dry ashing: Accurately weigh 0.1500g of sample and put it into a porcelain crucible, carbonize it completely on the electric furnace, add 1ml of HNO₃ (1+1) after cooling, evaporate it dry at low temperature on the electric furnace, cover it, transfer it to the muffle furnace, keep it for half an hour when the temperature rises to 250 °C, keep it for half an hour every time when the temperature rises to 50 °C, keep it for 4 hours when the temperature rises to 500 °C until the ashing is complete. After natural cooling, add 6ml of existing HCl-HNO₃ (1+1) on the electric furnace When heating to a small volume, add the same amount of water along the wall of the crucible, take it off when steaming to 2ml, transfer it into a 50ml volumetric flask, fix the volume with 1% HNO₃, dry filter and make blank at the same time.

2.2.2. Three acid digestion (HNO₃-H₂SO₄-HClO₄): Accurately weigh 0.1500g of sample and put it into 100ml of Teflon digestion tube, add a small amount of water to moisten it, add 10ml of concentrated HNO₃ and 10 drops of concentrated H₂SO₄, heat it on the electric heating plate at low temperature, when it is nearly dry and dark brown appears, remove it slightly cold, add 1ml of HNO₃-HClO₄ mixed acid (4+1), continue heating, if it still appears brown, add several drops of mixed acid until it does not appear brown, and add 1ml when white smoke is exhausted No3, evaporate to dryness, add 4ml of HCl-HNO₃ (1+1), when it is heated to a small volume on the electric furnace, add equal amount of water along the wall of the flask, when it is evaporated to 2ml, remove it, transfer it into a 50ml volumetric flask, fix the volume with 1% HNO₃, dry filter, and make blank at the same time.

2.2.3. Mixed acid nitrification (H₂SO₄-H₂O₂): Accurately weigh 0.1500g of sample and put it into the hard digestion tube, add 46ml of concentrated H₂SO₄, mix well and soak it, then slowly heat it until it starts to emit white smoke, take it off and cool it a little (about 1 minute), add 30% H₂O₂ 20 drops drop by drop, continue to heat it for a few minutes, then take it down and cool it, add several drops of H₂O₂, repeat this for many times until the digestion solution is completely bright. The last time should be slightly boiling for 5 minutes to remove the remaining H₂O₂. After cooling, add 10ml of water, transfer it into a 50ml volumetric flask, fix the volume with 1% HNO₃, dry filter and make blank at the same time.

2.2.4. Aqua regia +HClO₄ [3]: Accurately weigh 0.1500g of sample and put it into the digestive tube, drop a few drops of distilled water to disperse and wet it, add 20ml of aqua regia (15ml HCl and 5ml HNO₃), shake it well, and then start cooking. Decoct for 0.5-1h at 80-90 °C and remove for cooling. After cooling to room temperature, add 4ml HClO₄, and then decoct to clear under the temperature of 150-220 °C. After cooling, transfer to a 50ml volumetric flask, fix the volume with 1% HNO₃, and finally conduct dry filtration and blank.

2.3. Determination of element content

The principle of FAAS is that the digested sample solution is directly inhaled into the air acetylene flame [4], and the ground state atomic vapor of Zn, Cu, Pb, Fe, Cd and Cr formed in the flame absorbs the characteristic electromagnetic radiation emitted by the light source. Deduct the blank absorbance of the whole procedure reagent from the measured sample solution absorbance, and compare it with the absorbance of the standard solution to determine the content of the elements to be measured.

3. Results and discussion

In this study, three plant reference materials GBW0763 were processed in parallel according to four plant sample processing methods. Six elements of Zn, Cu, Pb, Fe, Cd and Cr were determined by FAAS.
The comprehensive analysis was carried out by calculating the average content (x), recovery rate (RR), relative standard deviation (RSD) and relative error (RE). See Table 4 for the specific experimental measurement results.

**Table 1.** Determination results of elements to be tested under different pretreatment methods

| Pretreatment method       | Zn (mg/kg) | Cu (mg/kg) | Pb (mg/kg) | Fe (mg/kg) | Cd (mg/kg) | Cr (mg/kg) |
|---------------------------|------------|------------|------------|------------|------------|------------|
| Dry Ashing                | 56.21      | 18.9       | 47.28      | 857        | 0.36       | 1.88       |
| RR (%)                    | 89-115     | 269-304    | 98-103     | 76-83      | 88-101     | 56-89      |
| RSD (%)                   | 7.2        | 1.2        | 1.0        | 39.2       | 0.02       | 0.43       |
| RE%                       | 2.2        | 186        | 0.6        | 19.8       | 4.8        | 27.6       |
| Three Acid Digestion      | X (mg/kg)  |            |            |            |            |            |
| RR (%)                    | 84-104     | 72-99      | 122-166    | 57-64      | 65-98      | 56-108     |
| RSD (%)                   | 5.4        | 0.9        | 10.3       | 37.3       | 0.06       | 0.68       |
| RE%                       | 5.4        | 14         | 44.5       | 38.9       | 18.3       | 17.8       |
| Mixed Acid Nitrification  | X (mg/kg)  |            |            |            |            |            |
| RR (%)                    | 81-89      | 68-99      | 91-103     | 57-59      | 83-95      | 77-102     |
| RSD (%)                   | 2.1        | 1.0        | 2.8        | 14.8       | 0.02       | 0.32       |
| RE%                       | 14.4       | 16         | 2.8        | 41.5       | 10.6       | 10.2       |
| Aqua Regia-HClO₄          | X (mg/kg)  |            |            |            |            |            |
| RR (%)                    | 77-86      | 104-131    | 86-135     | 29-36      | 32-83      | 60-95      |
| RSD (%)                   | 2.6        | 0.9        | 6.4        | 39.4       | 0.1        | 0.5        |
| RE%                       | 18.2       | 0.2        | 1.0        | 67.1       | 42.4       | 22.7       |
| μ (mg/kg)                 | 55         | 6.6        | 47         | 1070       | 0.38       | 2.6        |

Table 1. Shows that there is a certain relationship between the pre-treatment method and the elements to be tested, that is, not only the same pre-treatment method has an impact on different elements to be tested, but also different pre-treatment methods have an impact on the determination of the same element. The specific analysis is as follows.

3.1. Analysis of the influence of the same pretreatment method on different elements

Determination of plant samples treated by dry ashing, the recovery of Zn, Pb, Cd is higher than that of Fe and Cr, only 76% ~ 83% and 56% ~ 89%. In the determination of Cu, when the relative standard deviation is only 1.2, the relative error is as high as 186.6. These data show that the pre-treatment method of dry ashing is suitable for the determination of most elements in plant standard samples, for example, Zn, Pb and Cd all reach good recovery. The content of Fe and Cr is low, which may be due to the loss of elements in plant samples due to the rapid carbonization rate. The determination of Cu in plants is not suitable for dry ashing. This may be related to the condition factors of dry ashing, that is, the dry ashing is affected by the ashing temperature and time, and the ashing time should not be too long, otherwise it will cause the loss of some elements [5], while when the dry ashing temperature is too high, elements such as Zn, Cd, Cr are easy to lose [6]. In addition, dry ashing is prone to volatilization, loss and adsorption effect [7].

Because the Si containing substances in plant samples were not removed by HF in the three acid digestion method HNO₃-H₂SO₄-HClO₄, some easily adsorbed elements such as Cu and Fe could not be completely released during digestion, resulting in the relatively low recovery of Fe measured by the standard plant samples after HNO₃-H₂SO₄-HClO₄ treatment, and the recovery was only 57%~64%. Although the recovery rate of other elements is relatively ideal, its range is large, and the relative error of this method is also large, for example, the relative error of Pb is as high as 44.5. This is because when HNO₃-H₂SO₄-HClO₄ is used for digestion and decomposition, it is not easy to grasp the exact amount, resulting in low accuracy. Some elements, such as Cu, Zn and so on, may also precipitate with ClO₄⁻, thus affecting the determination of their contents.
The determination value of the same plant standard sample after digestion and decomposition by mixed acid nitrification (HNO$_3$-H$_2$SO$_4$-HClO$_4$) can be seen from the table that under this digestion method, the recovery rate of Pb reaches 91% – 103%, and the relative error is only 2.8, that is to say, the mixed acid digestion method of H$_2$SO$_4$-H$_2$O$_2$ is suitable for the determination of Pb content in plants. The recoveries of Zn and Fe are 81% – 89% and 57% – 59%, respectively. The reason for the low recoveries may be that these metal elements react with digestive solution to form insoluble substances.

The recoveries of Cr digested by aqua regia and HClO$_4$ were 28% – 36%, 32% – 83% and 60% – 95%, respectively. This is because the plant standard gbw0763 selected in this study has a high carbon content. When it is digested and decomposed with aqua regia-HClO$_4$, it reacts violently and produces a lot of bubbles. Even after it is immersed in aqua regia overnight, it reacts violently during digestion and overflows the digestion tube, which leads to the low measured value.

3.2. Analysis of the influence of different pretreatment methods on the same element

According to the recoveries of different pretreatment methods in Table 4, the recoveries of Zn and Pb are the highest under dry ashing conditions, 89%~115% and 98%~103% respectively. The recoveries of Zn under other three acid digestion conditions are lower than those of dry ashing treatment. The reason for this phenomenon is that Zn reacts with some acids, such as precipitation with ClO$_4^-$, thus affecting the recoveries. However, the recovery of Pb after HNO$_3$-H$_2$SO$_4$-HClO$_4$ decomposition is 122%~166%, but the relative error is as high as 44.5, so it can not be determined whether HNO$_3$-H$_2$SO$_4$-HClO$_4$ digestion method is suitable for the determination of Pb content in plant samples. The recovery of Cu is the highest at aqua regia-HClO$_4$, which is 104%~131%, and the relative error is only 0.2. The recovery rate of Fe in four pretreatment conditions is not ideal, but the recovery rate of dry ashing decomposition is slightly higher than other three digestion methods, and the value is 76%~83%. The recovery and relative error of Cd element in the other three pretreatment conditions are almost the same except for the big error caused by the violent reaction of the fourth digestion method. The recovery of Cr in four pretreatment methods is 56%~89%, 56%~108%, 72%~102% and 60%~95% respectively, that is to say, the recovery of Cr in dry ashing is slightly lower than that in other three acid digestion methods, which may be caused by the presence of Si in the plant standard sample, which can adsorb Cr and increase the residual amount of Cr on the crucible wall.

3.3. Discussion

According to the above-mentioned factors, such as the difficulty, recovery, relative standard deviation and relative error of the four digestion methods, it shows that different digestion methods have certain influence on the determination of a certain element, and the same digestion method has certain influence on the determination of each element content. That is, each method has its own advantages and disadvantages. The dry ashing method is suitable for the determination of Zn and Fe; the aqua regia-HClO$_4$ method is suitable for the determination of Cu, and the dry ashing method is not suitable; the dry ashing method and H$_2$SO$_4$-H$_2$O$_2$ mixed acid digestion method can be used for the determination of Pb; during the dry ashing process, the probability of Cd loss is small at 500°C, so the four methods are all used, but compared with the dry ashing method and H$_2$SO$_4$-H$_2$O$_2$ mixed acid digestion method, the accuracy is higher; the Cr in acid digestion. The recovery is higher than that of dry ashing. At the same time, the dry ashing method or H$_2$SO$_4$-H$_2$O$_2$ mixed acid digestion method should be used in the determination of six elements Zn, Cu, Pb, Fe, Cd and Cr.

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