# Metal Analysis by Inductively Coupled Plasma/Microwave Digestion versus Traditional Heavy Metals Limit USP <231>


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## INTRODUCTION

The original USP method, chapter <231>, for a limit test of heavy metals is based on the sulfide precipitation of copper arsenic metals. The test demonstrates that the content of metallic impurities that are colored by sulfide ion does not exceed the specified limit. The following metals respond to this test: lead (Pb), mercury (Hg), bismuth (Bi), arsenic (As), antimony (Sb), tin (Sn), cadmium (Cd), silver (Ag), copper (Co), and molybdenum (Mo). There are significant problems associated with the reliability of this method. The most problematic is the procedure (Method II) for the analysis of samples that does not produce a clear solution. Method II involves carbonization using sulfuric acid followed by ashing in a furnace at 500-600 ºC. The remainder is taken up in a solution and treated with a sulfide reagent. The color produced is compared to the color of a standard solution to demonstrate that the heavy metals in the sample is under a specified limit. This method is not specific. It also has been known to be highly unreliable. There can be loss of analyte during the sample preparation. The color density is not stable. Comparison of the color is subjective.

New USP chapters <1232>, <232> and <233> under development will provide information on the safety profile of a range of elemental impurities; safety limits and approaches to establish their threshold concentrations in materials; and methodologies to determine concentrations of elemental impurities

Irvine Pharmaceutical Services participated in a collaboration between the Expert Committee for Heavy Metals at USP and independent laboratories to evaluate the original test for heavy metals versus new approaches to establishing limits of elemental impurities in materials used in the manufacturing of pharmaceutical products. The study described here was aimed at providing a systematic comparison of the performance of Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and the USP Chapter <231> Method II limit test for the quantitation of elemental impurities corresponding to nine of the ten elements corresponding to the copper arsenic metals group.

## METHODOLOGY

**Sample Preparation**

Microwave Digestion:
Approximately one gram of sample is accurately weighed into a microwave digestion vessel. 10 mL of aqua regia is added and the sample is digested in a microwave oven. The microwave settings is shown in Table 1.

**Table 1. Microwave Oven Settings**

| Cycle | %Power | Pressure (bar) | Time (minute) |
|---|---|---|---|
| 1 | 30 | 100 | 10 |
| 2 | 50 | 100 | 12 |

The sample is cooled and transferred into a 25 mL volumetric flask and diluted to volume with 1% nitric acid.

USP <231> Method II:
Approximately 2 gram of sample is accurately weighed. Sufficient amount of sulfuric acid was added to wet the sample and ignited on a hot plate until thoroughly charred. To the carbonized mass 2 mL of nitric acids and 5 drops of a sulfuric acid was added and heated until white fumes no longer evolved. Heated in furnace at 500 ºC until the carbon was completely burned off. Cooled and added 4 mL of 6N HCl. Covered and digested for 15 minutes on a steam bath followed by evaporation dryness after uncovering sample. The residue was moistened with 1 drop of HCl and added 10 mL water and digested for 10 minutes. Diluted to 50 mL with 1% nitric acid.

Spiked samples were prepared in a similar fashion as above, except that appropriate solutions containing the elements studied were added before microwave digestion.

Standard preparation:
Standards were prepared in 1% nitric acid containing the nine metals studied in the 0.5 to 20.0 ppm concentration range. Six point calibration curves were obtained.

**ICP-OES Method**

Samples and standards were analyzed using ICP-OES instrumentation. Table 2 shows the elements studied and the selected wavelengths for each.

**Table 2. ICP-OES Wavelengths for Elements Studied**

| Element | Ag | Cd | Cu | Mo | Pb | Sb | Sn | Bi | As |
|---|---|---|---|---|---|---|---|---|---|
| Wavelength (nm) | 328.068 | 228.802 | 327.393 | 202.031 | 220.353 | 206.836 | 189.927 | 223.061 | 188.979 |

## RESULTS

Samples of caffeine and microcrystalline cellulose was spiked at concentrations of 2, 5 and 10 ppm with the following elements: Ag, Cd, Cu, Mo, Pb, Sb, Sn, Bi and As. The resulting samples were prepared for ICP-OES analysis by following two different procedures as described in the Methodology section of this poster. One used concentrated oxidizing acid solution (aqua regia) and microwave digestion the other followed the sample preparation procedure for USP Chapter <231> Method II. Overall, two sets of spiked samples were generated both with caffeine and microcrystalline cellulose for ICP-OES analysis. All samples were prepared and analyzed in triplicate. The values reported are the average of three determinations. The objective of this study design was to evaluate the recoveries achieved for individual elements after undergoing these sample preparation procedures.

**Tables 3** and **4** show recoveries of the elements studied for spiked caffeine samples after undergoing microwave digestion and Method II sample preparation procedures, respectively. The microwave digestion process, see **Table 3**, resulted in better than 80% recoveries for all the elements and at all the spiking levels. The slope and correlation coefficient were determined for spiking level versus measured value assuming a zero intercept for the correlation. These also indicate a close to complete recovery for all the elements when using this approach over the concentration range studied. On the other hand, the results corresponding to the sample treatment process associated with Method II, see **Table 4**, reflect a very poor performance regarding the recovery of the majority of the elements studied. For Ag, Sn, Bi no recovery was achieved, and for Sb the recovery is also close to zero. For As and Mo the recoveries were consistently in the 30% range. For Pb there was actually a negative slope and correlation obtained between spiking level and measured value, that is the higher the spiking the lower the percent recoveries were. Only for Cd and Cu were any reasonable recoveries obtained.

**Table 3. Results for Spiked Recoveries for Caffeine Samples Prepared Using Microwave Digestion.**

| Element | Spiking level (ppm) | Measured (ppm) | %Recovery | Slope | Correlation coefficient |
|---|---|---|---|---|---|
| Ag | 2 | 1.55 | 77 | 0.979 | 0.9957 |
| | 5 | 4.30 | 86 | | |
| | 10 | 9.71 | 97 | | |
| Cd | 2 | 1.79 | 90 | 0.925 | 1.000 |
| | 5 | 4.58 | 92 | | |
| | 10 | 9.24 | 92 | | |
| Cu | 2 | 1.73 | 86 | 0.915 | 0.9999 |
| | 5 | 4.50 | 90 | | |
| | 10 | 9.12 | 91 | | |
| Mo | 2 | 1.75 | 87 | 0.915 | 0.9999 |
| | 5 | 4.54 | 91 | | |
| | 10 | 9.12 | 91 | | |
| Pb | 2 | 1.72 | 86 | 0.919 | 0.9999 |
| | 5 | 4.53 | 91 | | |
| | 10 | 9.15 | 91 | | |
| Sb | 2 | 1.72 | 86 | 0.917 | 0.9999 |
| | 5 | 4.55 | 91 | | |
| | 10 | 9.13 | 91 | | |
| Sn | 2 | 1.93 | 96 | 0.903 | 0.9993 |
| | 5 | 4.74 | 95 | | |
| | 10 | 9.05 | 90 | | |
| Bi | 2 | 1.78 | 89 | 0.906 | 1.000 |
| | 5 | 4.55 | 91 | | |
| | 10 | 9.05 | 90 | | |
| As | 2 | 1.84 | 92 | 0.913 | 0.9996 |
| | 5 | 4.72 | 94 | | |
| | 10 | 9.12 | 91 | | |

**Table 4. Results for Spiked Recoveries for Caffeine Samples Prepared According to USP <231> Method II.**

| Element | Spiking level (ppm) | Measured (ppm) | %Recovery | Slope | Correlation coefficient |
|---|---|---|---|---|---|
| Ag | 2 | 0.00 | 0 | NA | NA |
| | 5 | 0.00 | 0 | | |
| | 10 | 0.00 | 0 | | |
| Cd | 2 | 1.31 | 66 | 0.695 | 0.9997 |
| | 5 | 3.27 | 65 | | |
| | 10 | 6.85 | 69 | | |
| Cu | 2 | 1.50 | 75 | 0.829 | 0.9994 |
| | 5 | 3.78 | 76 | | |
| | 10 | 8.10 | 81 | | |
| Mo | 2 | 0.95 | 47 | 0.230 | 0.9940 |
| | 5 | 1.83 | 37 | | |
| | 10 | 2.82 | 28 | | |
| Pb | 2 | 1.15 | 57 | -0.081 | -0.7645 |
| | 5 | 1.42 | 28 | | |
| | 10 | 0.58 | 6 | | |
| Sb | 2 | 0.08 | 4 | 0.036 | 0.9924 |
| | 5 | 0.22 | 4 | | |
| | 10 | 0.37 | 4 | | |
| Sn | 2 | 0.00 | 0 | NA | NA |
| | 5 | 0.00 | 0 | | |
| | 10 | 0.00 | 0 | | |
| Bi | 2 | 0.00 | 0 | NA | NA |
| | 5 | 0.00 | 0 | | |
| | 10 | 0.00 | 0 | | |
| As | 2 | 0.521 | 26 | 0.306 | 0.9925 |
| | 5 | 1.72 | 34 | | |
| | 10 | 3.01 | 30 | | |

**Tables 5** and **6** show recoveries of the elements studied for spiked microcrystalline cellulose samples after undergoing microwave digestion and Method II sample preparation procedures, respectively. The results observed for this material are closely similar to those obtained for caffeine samples. The microwave digestion process, see **Table 5**, resulted in better than 80% recoveries for all the elements and at all the spiking levels. These also indicate a close to complete recovery for all the elements when using this approach over the concentration range studied.

On the other hand, the results corresponding to the sample treatment process associated with Method II, see **Table 6**, reflect a very poor performance regarding the recovery of the majority of the elements studied. For Ag, Sn, Bi no recovery was achieved, and for Sb the recovery is also close to zero. For As and Mo the recoveries were consistently in the 30% range. For Pb there was actually a negative slope and correlation obtained between spiking level and measured value, that is the higher the spiking the lower the percent recoveries were. Only for Cd and Cu were any reasonable recoveries obtained.

**Table 5. Results for Spiked Recoveries for Microcrystalline Cellulose Samples Prepared Using Microwave Digestion.**

| Element | Spiking level (ppm) | Measured (ppm) | %Recovery | Slope | Correlation coefficient |
|---|---|---|---|---|---|
| Ag | 2 | 2.58 | 129 | 1.430 | 0.9981 |
| | 5 | 6.57 | 131 | | |
| | 10 | 14.27 | 143 | | |
| Cd | 2 | 1.68 | 84 | 0.884 | 0.9998 |
| | 5 | 4.32 | 86 | | |
| | 10 | 8.81 | 88 | | |
| Cu | 2 | 1.71 | 86 | 0.871 | 0.9999 |
| | 5 | 4.27 | 85 | | |
| | 10 | 8.71 | 87 | | |
| Mo | 2 | 1.74 | 87 | 0.879 | 0.9999 |
| | 5 | 4.33 | 87 | | |
| | 10 | 8.79 | 88 | | |
| Pb | 2 | 1.73 | 86 | 0.880 | 0.9999 |
| | 5 | 4.27 | 85 | | |
| | 10 | 8.80 | 88 | | |
| Sb | 2 | 1.71 | 86 | 0.880 | 0.9998 |
| | 5 | 4.30 | 86 | | |
| | 10 | 8.81 | 88 | | |
| Sn | 2 | 1.93 | 96 | 0.880 | 0.9998 |
| | 5 | 4.60 | 92 | | |
| | 10 | 9.15 | 91 | | |
| Bi | 2 | 1.77 | 89 | 0.880 | 0.9998 |
| | 5 | 4.34 | 87 | | |
| | 10 | 8.79 | 88 | | |
| As | 2 | 1.78 | 89 | 0.880 | 0.9998 |
| | 5 | 4.50 | 90 | | |
| | 10 | 9.00 | 90 | | |

**Table 6. Results for Spiked Recoveries for Microcrystalline Cellulose Samples Prepared According to USP <231> Method II.**

| Element | Spiking level (ppm) | Measured (ppm) | %Recovery | Slope | Correlation coefficient |
|---|---|---|---|---|---|
| Ag | 2 | 0.00 | 0 | NA | NA |
| | 5 | 0.00 | 0 | | |
| | 10 | 0.00 | 0 | | |
| Cd | 2 | 1.30 | 65 | 0.678 | 0.9999 |
| | 5 | 3.29 | 66 | | |
| | 10 | 6.72 | 67 | | |
| Cu | 2 | 1.49 | 74 | 0.812 | 0.9998 |
| | 5 | 3.82 | 76 | | |
| | 10 | 7.97 | 80 | | |
| Mo | 2 | 0.93 | 47 | 0.219 | 0.9902 |
| | 5 | 1.82 | 36 | | |
| | 10 | 2.72 | 27 | | |
| Pb | 2 | 1.15 | 58 | -0.084 | -0.7755 |
| | 5 | 1.41 | 28 | | |
| | 10 | 0.56 | 6 | | |
| Sb | 2 | 0.06 | 3 | 0.036 | 0.9965 |
| | 5 | 0.19 | 4 | | |
| | 10 | 0.35 | 3 | | |
| Sn | 2 | 0.00 | 0 | NA | NA |
| | 5 | 0.00 | 0 | | |
| | 10 | 0.00 | 0 | | |
| Bi | 2 | 0.00 | 0 | NA | NA |
| | 5 | 0.00 | 0 | | |
| | 10 | 0.00 | 0 | | |
| As | 2 | 0.53 | 26 | 0.299 | 0.9931 |
| | 5 | 1.69 | 34 | | |
| | 10 | 2.96 | 30 | | |

## CONCLUSION

Comparison of the microwave digestion sample preparation method normally applied in ICP based elemental analysis to the sample treatment associated with current USP <231> Method II demonstrated the superior performance of ICP technique based elemental analysis approach relative to the Heavy Metal testing methodology currently in effect. The new USP chapters <1232>, <232> and <233> under development, and expected to become effective in 2010, will provide a much needed improvement in the approaches used to assess elemental impurities in materials used in the manufacturing of pharmaceutical products.


AAPS 2009 Annual Meeting , November 8-13, 2009 Poster: T2177