Automated Multielement Analysis of Plant Material by Flame Atomic Absorption Spectroscopy

Application Note

Atomic Absorption

Introduction

In this study an automatic flame atomic absorption system was used for the sequential analysis of sodium, potassium, calcium, magnesium, aluminium, iron, zinc and manganese in Pinus Radiata after a nitric acid perchloric acid digestion [5,7,6,9].

The analysis of biological material presents unique problems for the analyst. Many samples have only a limited lifetime before the onset of decay. Consequently, sample history and pretreatment are important factors in obtaining realistic and useful results. The sample can be freeze dried, or oven dried at 60 – 80 °C depending on the particular method of analysis. The greatest problem with many botanical samples is equating dry sample weight to fresh sample weight. Bowen has shown that if kale leaf is dried at 90 °C (or slightly below), an equilibrium is reached where water remains within the sample irrespective of drying time [6]. At higher temperatures (100 °C for example), the sample shows evidence of decomposition. In this study the samples were oven dried at 80 °C for 24 hours and a moisture factor was calculated.

As a general rule biological and organic samples should be analyzed as soon as possible after collection. Further information about digesting biological and agricultural samples will be found in references 1 through 4.
**Instrumentation**

Agilent Techtron AA-975 Atomic Absorption Spectrophotometer
Agilent Techtron PSC-55 Programmable Sample Changer
Hewlett Packard HP-85 Desktop Computer
Hewlett Packard HP-82905A Printer
Agilent Techtron Data Tape

**Equipment**

Aluminium block or sand bath to accommodate digestion test tubes
Hot plate – to accommodate either sand bath or aluminium block
Test tubes – 19 mm x 150 mm for PSC-55; 27 mL capacity
Standard flask – 25 mL, 250 mL, 500 mL, 1000 mL
Pyrex/plastic filter funnels – 55 mm diameter
Thermometer – for monitoring the temperature in the aluminium block or sand bath
Filter paper – Whatman 541 filter paper

**Reagents**

Nitric acid (Analar 70% W/W SG = 1.41)
Perchloric acid (Analar 70% SG = 1.67)
Dionized distilled water

**Stock Standards**

Stock standards of 1000 µg/mL of Na, K, Ca, Mg, Al, Fe, Zn and Mn were used to prepare a series of composite standards.

**Analytical Standards**

Previous experience with similar digests indicated that the series of standards (µg/mL) listed in Table 1 was adequate for most analytical cases. A blank and three standards containing all elements of analytical interest were prepared in 4% HClO₄.

**Samples**

Pinus radiata, pine needles  Sample nos. 1, 5, 9
Orchard leaves  Sample nos. 2, 6
Pinus radiata, bark  Sample nos. 3, 7
Pinus radiata, wood  Sample nos. 4, 8

**Instrumental Conditions and System Set Up Results**

The AA-975 Atomic Absorption Spectrophotometer, PSC-55 Programmable Flame Autosampler, HP-85 Desktop Computer and HP-82905A Printer were connected as shown in the Analytical System Operation Manual. The hollow cathode lamps for the elements of interest were loaded into the twelve lamp turret and the data tape into the HP-85 desktop computer.

The AA-975 and PSC-55 were programmed for each element through the P`GRM SETUP keys and parameters stored on disc. Figure 1 shows program No. 6 for the determination of zinc. Table 1 lists the instrumental parameters used for the determination of the eight elements.

![Figure 1. Explanation of program parameters used on AA-975 and PSC 55.](image-url)
The system was preprogrammed to provide:

- Automatic sequential analysis for eight elements.
- An automatic rinse between all standards and samples.
- A sequential report plus calibration graphics for all elements.
- Analytical titles, calibration units, values of all standards, and sample labels.

**Sample Preparation Procedure**

Take about 0.25 g of plant material and weigh accurately in a test tube and place in an aluminium block or sand bath containing a thermometer (0 – 400 °C). Add 5 mL of a mixed nitric perchloric digesting acid (1 mL 70% HClO₄ and 4 mL 70% HNO₃). Heat the block for 2 hours at 120 °C, then slowly increase the temperature to 180 °C over a three hour period to drive off the nitric acid. White fumes from the perchloric acid will indicate the end of the digestion procedure. It is important not to allow the digestate to dry out. Carry out the digestion under strict supervision in a protected fume hood (See Note 3).

On completion of the digestion the contents of the test tube are rinsed into a 25 mL volumetric flask and made up to the mark with distilled deionized water.

---

**Table 1**  
*Instrument Conditions Used for Eight Elements (Values of Standards are µg/mL; 45 Degree Burner Rotation Used For Na And K)*

<table>
<thead>
<tr>
<th>Element</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Mn</th>
<th>Zn</th>
<th>Fe</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Program ID</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Int time</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Wavelength</td>
<td>589.0</td>
<td>766.5</td>
<td>422.7</td>
<td>202.5</td>
<td>279.5</td>
<td>213.9</td>
<td>248.3</td>
<td>309.3</td>
</tr>
<tr>
<td>Slit</td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
<td>1.0</td>
<td>0.2</td>
<td>1.0</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Lamp number</td>
<td>4</td>
<td>8</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Lamp current</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Expn factor</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Standard 1</td>
<td>1.0</td>
<td>5.0</td>
<td>10</td>
<td>5</td>
<td>1.0</td>
<td>0.5</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Standard 2</td>
<td>5.0</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>3.0</td>
<td>1.0</td>
<td>5.0</td>
<td>10</td>
</tr>
<tr>
<td>Standard 3</td>
<td>5.0</td>
<td>20</td>
<td>40</td>
<td>20</td>
<td>5.0</td>
<td>2.0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>ABS Int hold</td>
<td>BC on</td>
<td>BC on</td>
<td>BC on</td>
<td>BC on</td>
<td>BC on</td>
<td>BC on</td>
<td>BC on</td>
<td>BC on</td>
</tr>
<tr>
<td>Air set up</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>ACET Set up</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>2.30</td>
<td>3.00</td>
<td>5.00</td>
</tr>
<tr>
<td>N₂O Set up</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

1. The digestate is normally clear and does not require filtering; if a small amount of solid material is present this can be removed by filtering the digestate through a Whatman 541 filter paper with some distilled deionized water.

2. A moisture factor is determined on the sample and considered in the final calculation. The moisture factor is determined by weighing 2 g of sample and placing the sample in an oven at 80 °C for 24 hours and then reweighing.

   \[
   \text{Moisture factor} = \frac{\text{Weight sample after 24 hrs. at 80 °C}}{\text{Weight of sample on entry to oven}}
   \]

3. Safety procedures for the use of perchloric acid are well documented and should be strictly observed [10,11].

**Results**

The results are presented in Tables 3 and 4. Figures 2 and 3 and Table 2 show an example of the sequential report and calibration graphics for manganese and zinc, while Table 3 gives the full multielement report. The samples indicated as overrange were re-analyzed and the results listed in Table 4.
Figure 2.

Table 2.

Table 3.

Table 4.
Summary

The system provided fully automatic analysis of ten samples for eight elements using three calibration standards, duplicate readings, and a 5-second delay to allow each solution to reach the flame. The only manual intervention required was occasioned by the need to rotate the burner between programs 2 and 3. The entire program was completed in an hour and forty-five minutes.

The overrange samples were subsequently diluted by a factor of ten and automatically re-analyzed by programming the system to repeat programs 1, 2 and 3. This repeat analysis was completed in about thirty minutes. The need to dilute and re-analyze the overrange samples could have been avoided by a better initial choice of standard values for calcium, magnesium and potassium.

Three standards were used for each element. It was subsequently noted that aluminium could have been determined against only one standard. The lowest aluminium standard (5 µg/mL) gave less than 0.1. Absorbance and the calibration was linear up to 20 µg/mL. A single standard is generally adequate when sample concentration is greater than ten times the detection limit and absorbance is about 0.1 or lower.

The system was programmed to generate calibration graphics, a sequential report, and a multi-element report. Examples of calibration graphics are shown in Figures 2 and 3 and results associated with these curves are shown in Table 2. The multi-element report is presented as Table 3. This gives the analysis title, elements analyzed, concentration units, sample identification, date, batch identification and operator’s name.

Calculation of percentage weight in the sample

\[
\% \text{ Weight in sample} = \frac{\text{Final digest volume} \times \frac{\text{Conc’n of element in diluted sample}}{(\mu g/mL)} \times 10^{-4}}{\text{Weight sample} \times \text{Moisture factor}}
\]

for example, sample V1 Pinus Radiata pine needles.

The final digest volume (mL), weight of sample and moisture factor are all the same for each particular sample and are used as a constant factor for the calculation of the percentage weight of element within a sample.

<table>
<thead>
<tr>
<th>V1</th>
<th>Final digest volume (mL)</th>
<th>25 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight sample</td>
<td>0.255 g</td>
</tr>
<tr>
<td></td>
<td>Moisture factor</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Constant factor for V1(K) = \( \frac{25}{0.255} \times 10^{-4} \times 0.94 \)

\( K_{V1} = 0.0104 \) (all elements except potassium)

\( K_{V1} \) potassium = 0.104

% Element by weight in V1 = \( K_{V1} \times \) Concentration of diluted element in diluted sample (µg/mL)

% Mn by weight V1 = 0.0104 \times 1.796 (Table 3) = 0.019

<table>
<thead>
<tr>
<th>Element</th>
<th>% Element in V1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>0.013</td>
</tr>
<tr>
<td>K</td>
<td>1.41</td>
</tr>
<tr>
<td>Ca</td>
<td>0.19</td>
</tr>
<tr>
<td>Mg</td>
<td>0.10</td>
</tr>
<tr>
<td>Mn</td>
<td>0.019</td>
</tr>
<tr>
<td>Zn</td>
<td>0.005</td>
</tr>
<tr>
<td>Fe</td>
<td>0.042</td>
</tr>
<tr>
<td>Al</td>
<td>0.072</td>
</tr>
</tbody>
</table>

Where there is a constant factor relating to the concentration in µg/mL to the percentage weight in the sample, the values of the standard entered can incorporate this factor, permitting direct readout in percent by weight.
References


5. T. H. Risby, Ultratrace Metal Analysis in Biological Sciences and Environment, Advances in Chemistry Series 172, American Chemical Society.


For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem