Automated Multielement Analysis of Plant Material by Flame Atomic Absorption Spectroscopy

Application Note

Atomic Absorption

Author

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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.

Agilent Technologies

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% $HClO_4$.

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.

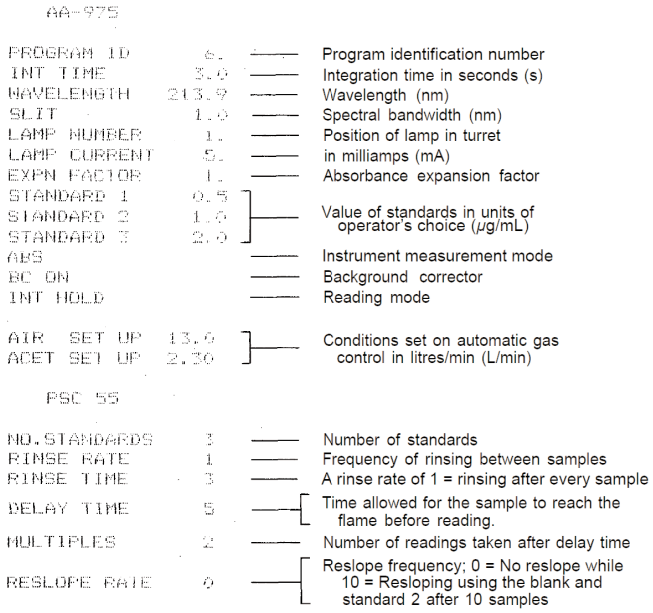

Figure 1. Explanation of program parameters used on AA-975 and PSC 55.

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

Element	Na	K	Ca	Mg	Mn	Zn	Fe	Al
Program ID	1	2	3	4	5	6	7	8
Int time	3.0	3.0	3.0	3.0	3.0	3.0	3.0	4.0
Wavelength	589.0	766.5	422.7	202.5	279.5	213.9	248.3	309.3
Slit	0.5	1.0	0.5	1.0	0.2	1.0	0.2	0.5
Lamp number	4	8	9	3	5	1	2	6
Lamp current	5	5	4	5	5	5	5	8
Expn factor	1	1	1	1	1	1	1	1
Standard 1	1.0	5.	10	5	1.0	0.5	2.0	5.0
Standard 2	5.0	10	20	20	3.0	1.0	5.0	10
Standard 3	5.0	20	40	20	5.0	2.0	10	20
ABS Int hold				BC on		BC on		
Air set up	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
ACET Set up	2.50	2.50	2.50	2.50	2.50	2.30	3.00	5.00
N_2O Set up								11.0

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% $HClO_4$ and 4 mL 70% HNO_3). 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.

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.

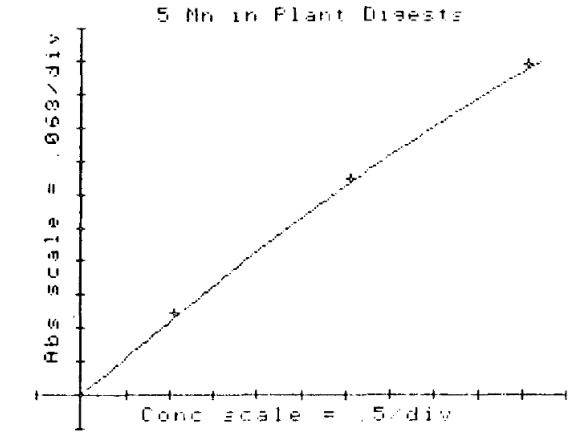

Figure 2.

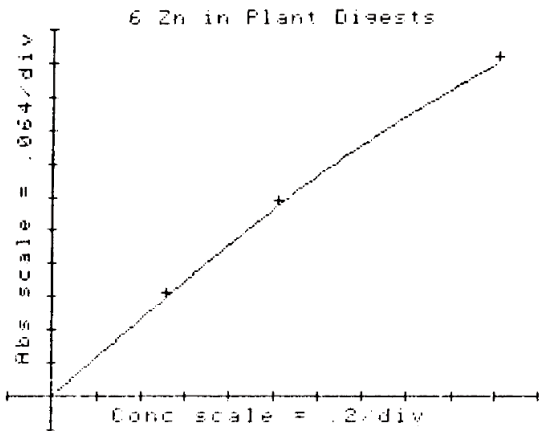

Figure 3.

Table 2.

AUTO-PROGRAM 5 Mn in Plant Digests

SOLUTION	CONC ug/mL	RSD	MEAN ABS	ABSORBANCE READINGS
BLANK	0.000	0.0%	0.000	-0.001 0.001
STANDARD 1	1.000	0.7%	0.150	0.150 0.151
STANDARD 2	3.000	0.2%	0.425	0.426 0.424
STANDARD 3	5.000	0.8%	0.660	0.657 0.664
V1	1.796	0.8%	0.264	0.263 0.266
V2	0.820	0.8%	0.123	0.122 0.124
V3	0.386	1.7%	0.058	0.057 0.059
V4	0.146	9.1%	0.022	0.021 0.024
V5	1.955	1.0%	0.286	0.284 0.289
V6	0.886	0.8%	0.133	0.134 0.132
V7	0.360	5.6%	0.054	0.057 0.052
V8	0.186	0.0%	0.028	0.028 0.028
V9	1.962	0.3%	0.287	0.286 0.288
V10	0.006	100.0%	0.001	0.002 0.000

AUTO-PROGRAM 6 Zn in Plant Digests

SOLUTION	CONC ug/mL	RSD	MEAN ABS	ABSORBANCE READINGS
BLANK	0.000	0.0%	0.001	0.001 0.001
STANDARD 1	0.500	1.6%	0.185	0.183 0.188
STANDARD 2	1.000	0.0%	0.361	0.361 0.361
STANDARD 3	2.000	0.6%	0.639	0.636 0.642
V1	0.519	0.0%	0.192	0.192 0.192
V2	0.232	1.2%	0.086	0.087 0.086
V3	0.175	1.5%	0.065	0.064 0.066
V4	0.075	0.0%	0.028	0.028 0.028
V5	0.550	0.5%	0.199	0.199 0.200
V6	0.310	0.9%	0.115	0.116 0.115
V7	0.156	3.4%	0.058	0.060 0.056
V8	0.108	2.5%	0.040	0.039 0.041
V9	0.584	2.3%	0.218	0.212 0.220
V10	0.021	25.0%	0.008	0.010 0.006

Table 3.

VARIAN AA-975

OPERATOR: Trevor McKenzie
DATE: 22.4.1982
BATCH: PLANT DIGESTS

SOLUTION	Na ug/mL	K ug/mL	Ca ug/mL	Mg ug/mL	Mn ug/mL	Zn ug/mL
V1	1.260	Overrange	18.19	9.631	1.796	0.519
V2	0.392	Overrange	Overrange	Overrange	0.820	0.232
V3	2.956	Overrange	22.08	17.17	0.386	0.175
V4	0.267	11.91	9.536	2.932	0.146	0.075
V5	1.111	Overrange	14.73	10.27	1.955	0.538
V6	0.946	Overrange	Overrange	Overrange	0.886	0.310
V7	2.574	Overrange	19.55	15.69	0.360	0.156
V8	0.357	9.091	7.317	2.905	0.186	0.108
V9	1.429	Overrange	17.85	9.723	1.962	0.584
V10	0.000	0.080	0.066	0.083	0.006	0.021

SOLUTION	Fe ug/mL	Al ug/mL
V1	4.026	6.889
V2	2.240	2.000
V3	0.651	4.333
V4	0.118	1.583
V5	4.097	8.898
V6	2.631	3.750
V7	0.503	5.000
V8	0.133	2.333
V9	4.115	9.377
V10	0.103	2.500

Table 4.

AUTO-PROGRAM 2 K in Plant Digests

SOLUTION	CONC ug/mL	RSD	MEAN ABS	ABSORBANCE READINGS
BLANK	0.000	40.0%	0.005	0.004 0.007
STANDARD 1	5.000	1.6%	0.125	0.123 0.127
STANDARD 2	10.00	1.2%	0.251	0.249 0.254
STANDARD 3	20.00	1.4%	0.488	0.483 0.494
V1	13.57	0.6%	0.339	0.341 0.337
V2	17.94	1.4%	0.442	0.438 0.447
V3	7.968	0.5%	0.200	0.201 0.200
V5	18.21	3.6%	0.448	0.460 0.437
V6	18.87	0.6%	0.463	0.466 0.461
V7	7.315	1.1%	0.181	0.180 0.183
V9	18.74	0.9%	0.460	0.463 0.457

SOLUTION	Ca ug/mL	Mg ug/mL
V2	25.22	5.652
V6	25.61	5.621

Calculation of percentage weight in the sample

$$\% \text{ Weight in sample} = \frac{\text{Final digest volume (mL)}}{\text{Weight sample}} \times \frac{\text{Conc'n of element in diluted sample (µg/mL)}}{\text{Moisture factor}} \times 10^{-4}$$

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.

V1	Final digest volume	25 mL
	Weight sample	0.255 g
	Moisture factor	0.94

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

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

$$K_{V1} \text{ potassium} = 0.104$$

$$\% \text{ Element by weight in V1} = K_{V1} \times \left[\text{Concentration of diluted element in diluted sample (µg/mL)}\right]$$

$$\% \text{ Mn by weight V1} = 0.0104 \times 1.796 \text{ (Table 3)} = 0.019$$

Element	% Element in V1
Na	0.013
K	1.41
Ca	0.19
Mg	0.10
Mn	0.019
Zn	0.005
Fe	0.042
Al	0.072

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.

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.

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