

# 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.



#### Author

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#### 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  $\mu$ g/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 ( $\mu$ g/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<sub>4</sub>.

#### 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.

AA-975			
PROGRAM 1D INT TIME NAVELENGTH SLIT - LAMP NUMBER LAMP CURRENT EXPN FAC10R STANDARD 1 STANDARD 1 STANDARD 3 A&S BC ON INT HOLD	6. 3.0 213.9 1.0 1. 0.5 1.0 2.0	]	Program identification number Integration time in seconds (s) Wavelength (nm) Spectral bandwidth (nm) Position of lamp in turret in milliamps (mA) Absorbance expansion factor Value of standards in units of operator's choice (µg/mL) Instrument measurement mode Background corrector Reading mode
AIR SET UP ACET SET UP	13.6 2.30	]	Conditions set on automatic gas control in litres/min (L/min)
PSC 55			
NO.STANDARDS RINSE RATE RINSE TIME DELAY TIME	3 1 3 5		Number of standards Frequency of rinsing between samples A rinse rate of 1 = rinsing after every sample Time allowed for the sample to reach the flame before reading.
MULTIPLES			Number of readings taken after delay time
RESLOPE RAIE	0		Reside trequency; U = No reside while 10 = Resident using the blank and standard 2 after 10 samples

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

Element	Na	К	Ca	Mg	Mn	Zn	Fe	AI
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 <sub>2</sub> O Set up								11.0

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

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

- 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.

Moisture factor = Weight sample after 24 hrs. at 80 °C 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.





#### Table 2.

AUTO-PROGRAM 5	Mn	in Plar	nt Digests	5
SOLUTION	C014C	RSD	MEAN ABS	ABSORBANCE READINGS
	աց Հովե			
BLODE	0.000	0.0%	0.000	-0.001 0.001
STANDOED 1	1.000	0.7%	0.150	0.150 0.151
STADOARD 2	3.000	0.2%	0.425	0.426 0.424
STANDARD 3	5.000	0.8%	0.660	0.657 0.664
V1	1.795	0.8%	0.264	0.263 0.266
V2	0.820	0.8%	0.123	0.122 0.124
V3	0.396	1.7%	0.058	0.057 0.059
V4	0.146	9.1%	0.022	0.021 0.024
95	1.955	1.0%	0.286	0.284 0.289
98	0.886	0.8%	0.133	0.134 0.132
₩7	0.3a0	5.6%	0.054	0.057 0.052
va	0.186	0.07	0.028	0.028 0.028
V9	1.962	0.37	0.287	0.285 0.298
V10	0.005	100.0%	0.001	0.002 0.000
AUTO-PROGRAM 5	Za	in Flar	nt Digests	
AUTO-PROGRAM 6 SOLUTION	Zn CONC	in Plar RSD	t Digests MEAN ABS	ABSORBANCE READINGS
AUTO-FROGRAM & SOLUTION	Zn CONC ug/mL	in Plar RSD	nt Digests MEAN ABS	ABSORBANCE READINGS
AUTO-FROGRAM & SOLUTION	Zn CONC ແລຼ/mL	in Plan RSD	nt Digests MEAN ABS	ABSORBANCE READINGS
AUTO-PROGRAM 5 SOLUTION BLANK:	2n CONC และ/mL 0.000	in Flar KSD 0.0%	nt Digests MEAN ABS 0.001	ABSORBANCE READINGS
AUTO-FROGRAM 5 SOLUTION BLANK STANDARD 1	2n CONC ug/mL 0.000 0.500	in Flar RSD 0.0% 1.5%	nt Digests MEAN ABS 0.001 0.185	ABSORBANCE READINGS 0.001 0.001 0.183 0.188
AUTO-FROGRAM 5 SOLUTION BLANE STANDARD 1 STANDARD 2	Zn ECNC ug/mL 0.000 0.500 1.600	in Flan RSD 0.0% 1.5% 0.0%	nt Digests MEAN ABS 0.001 0.185 0.351	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.351 0.351
AUTO-FROORAM 6 SOLUTION BLANK STANDARD 1 STANDARD 2 STANDARD 3	Zn CONC ug/mL 0.000 0.500 1.000 2.000	in Plan ESD 0.0% 1.5% 0.0% 0.5%	0.001 0.001 0.185 0.351 0.351	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.361 0.361 0.636 0.642
AUTO-FROGRAM & SOLUTION BLANE STANDARD 1 STANDARD 2 STANDARD 3 V1	Zn CONC ug/mL 0.000 0.500 1.000 2.000 0.519	in Plan RSD 0.0% 1.6% 0.0% 0.6% 0.0%	0.001 0.001 0.185 0.351 0.437 0.437	ABSORBAHCE READINGS 0.001 0.001 0.183 0.188 0.351 0.351 0.635 0.642 0.172 0.172
AUTO-FROGRAM & SOLUTIOH BLANN: STANDARD 1 STANDARD 2 STANDARD 3 V1 V2	Zn CONC ug/mL 0.000 0.500 1.000 2.000 0.519 0.232	in Plan RSD 0.0% 1.6% 0.0% 0.6% 0.0% 1.2%	0.001 0.001 0.185 0.351 0.539 0.192 0.086	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.351 0.351 0.635 0.642 0.192 0.192 0.087 0.086
AUTO-FROCRAM S SOLUTION BLANN: STANDARD 1 STANDARD 2 STANDARD 2 V1 V2 V2 V3	Zn CONC ug/mL 0.000 0.500 1.000 2.000 0.519 0.232 0.175	in Plan RSD 0.0% 1.6% 0.0% 0.6% 0.0% 1.2% 1.5%	0.001 0.001 0.185 0.351 0.537 0.192 0.086 0.065	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.361 0.361 0.436 0.442 0.192 0.192 0.087 0.086 0.064 0.086
AUTO-FROGRAM 4 SOLUTION BLANK: STANDARD 1 STANDARD 2 STANDARD 3 V1 V2 V3 V3 V3 V4	Zn CONC ug / mL 0.000 0.500 1.000 2.000 0.519 0.232 0.175 0.075	in Plan RSD 0.0% 1.6% 0.0% 0.6% 1.2% 1.5% 0.0%	0.001 0.185 0.351 0.439 0.439 0.192 0.086 0.065 0.028	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.361 0.361 0.635 0.642 0.192 0.192 0.087 0.086 0.064 0.066 0.028 0.028
AUTO-FROGRAM 5 SOLUTION BLAND: STANDARD 1 STANDARD 2 STANDARD 3 V1 V2 V3 V3 V4 V5	Zn CDNC ug/mL 0.000 0.500 1.000 2.000 0.519 0.232 0.175 0.075 0.538	in Plan RSD 0.0% 1.6% 0.0% 0.6% 0.0% 1.2% 1.5% 0.0% 0.5%	t Digests MEAN ABS 0.001 0.185 0.351 0.439 0.192 0.086 0.065 0.055 0.028 0.199	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.351 0.351 0.635 0.342 0.192 0.192 0.087 0.086 0.064 0.066 0.028 0.028 0.199 0.200
AUTO-FROGRAM & SOLUTION BLANE STANDARD 1 STANDARD 2 STANDARD 3 V1 V2 V3 V3 V4 V3 V4 V5 V2 V3 V4	Zn CDNC ug/mL 0.000 0.500 1.000 2.000 0.519 0.232 0.175 0.232 0.175 0.558 0.510	in Plan RSD 0.0% 1.6% 0.0% 0.6% 0.0% 1.2% 1.2% 1.5% 0.0% 0.5% 0.5% 0.9%	t Digests MEAN ABS 0.001 0.185 0.351 0.439 0.192 0.086 0.045 0.028 0.199 0.115	ABSORBAHCE READINGS 0.001 0.001 0.183 0.188 0.361 0.361 0.636 0.642 0.192 0.192 0.087 0.086 0.028 0.028 0.199 0.208 0.199 0.200 0.116 0.115
AUTO-FROGRAM 5 SOLUTION BLANE STANDARD 1 STANDARD 2 STANDARD 3 V1 V2 V3 V3 V4 V3 V4 V5 V5 V7	Zn CONC ug/mL 0.000 0.500 1.000 2.000 0.519 0.232 0.175 0.075 0.558 0.558 0.510 0.155	in Plan RSD 0.0% 1.6% 0.0% 0.0% 1.2% 1.2% 1.5% 0.0% 0.5% 0.9% 3.4%	t Digests NEAN ABS 0.001 0.185 0.351 0.425 0.085 0.085 0.028 0.199 0.115 0.059	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.351 0.351 0.635 0.642 0.192 0.192 0.087 0.086 0.044 0.066 0.028 0.028 0.199 0.200 0.116 0.115 0.060 0.055
AUTO-FROGRAM 3 SOLUTIOH BLAND: STANDARD 1 STANDARD 2 STANDARD 3 V1 V2 V3 V3 V4 V5 V5 V5 V5 V6	Zn CDNC ug/mL 0.000 0.500 1.000 2.000 0.519 0.232 0.175 0.075 0.558 0.578 0.558 0.558 0.155	in Plan RSD 0.0% 1.6% 0.0% 0.0% 1.2% 1.5% 0.0% 0.5% 0.9% 2.5%	t Digests MEAN ABS 0.001 0.185 0.351 0.439 0.085 0.098 0.098 0.199 0.115 0.058 0.040	ABSORBANCE READINGS 0.001 0.001 0.163 0.188 0.361 0.361 0.636 0.364 0.192 0.192 0.087 0.086 0.044 0.066 0.028 0.028 0.028 0.028 0.199 0.200 0.115 0.115 0.060 0.055 0.039 0.041
AUTO-FROGRAM 5 SOLUTION BLANK: STANDARD 1 STANDARD 2 STANDARD 2 V1 V2 V3 V4 V3 V4 V5 V4 V5 V4 V7 V7 V7 V7 V7 V7	Zn CONC ug/mL 0.000 0.550 1.000 2.000 0.519 0.232 0.175 0.075 0.519 0.232 0.175 0.519 0.175 0.519 0.510 0.510 0.519 0.5100 0.5100 0.510000000000	in Plan RSD 0.0% 1.6% 0.0% 0.0% 1.2% 1.5% 0.0% 0.5% 0.9% 2.5% 2.5%	t Digests MEAN ABS 0.001 0.185 0.351 0.439 0.439 0.086 0.086 0.028 0.199 0.115 0.058 0.058 0.040	ABSORBANCE READINGS 0.001 0.001 0.183 0.188 0.361 0.361 0.635 0.642 0.172 0.172 0.087 0.086 0.064 0.066 0.028 0.028 0.199 0.200 0.118 0.115 0.060 0.055 0.639 0.041 0.212 0.220

Tabla	2	
Iavic	J.	

VARIAN AA-	-975
OPERATOR:	Trevor McKenzie
DATE:	22.4.1982

BATCH:	PLA	NT DIGESTS				
SOLUTION	Na ug∕mL	K ug∕mL	Ca ug∕mL	Mg ug∕mL	Mn ug∕mL	Zn ug∕mL
V1 V2 V3 V4 V5	1.260 0.392 2.956 0.267 1.111 0.946	Overrange Overrange Overrange 11.91 Overrange Overrange	18.19 Overrange 22.08 9.536 14.73 Overcanne	9.631 Overrange 17.17 2.932 10.27 Overrange	1.796 0.820 0.386 0.146 1.955 0.886	0.519 0.232 0.175 0.075 0.538 0.538
V7 V8 V9 V10	2.574 0.357 1.429 0.000	Overrange 9.091 Overrange 0.080	19.55 7.317 17.85 0.066	15.69 2.905 9.723 0.083	0.360 0.186 1.962 0.006	0.156 0.108 0.584 0.021
SOLUTION	Fe ug∕mL	Al ug∕mL				
V1 V2 V3 V4 V5 V6 V7 V8 V9 V9 V10	4.026 2.240 0.651 0.118 4.097 2.631 0.503 0.133 4.115 0.103	6.889 2.000 4.333 1.583 8.898 3.750 5.000 2.333 9.377 2.500				

Table 4.

AUTO-PROGRAM 2	ĸ	in Plan	nt Digests	
SOLUTION	CONC	RSD	MEAN ABS	ABSORBANCE READINGS
	ug∕mL			
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	1.47	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
<b>V</b> 3	7.968	0.5%	0.200	0.201 0.200
V5	18.21	3.4%	0.449	0.460 0.437
V6	18.87	0.6%	0.463	0.465 0.461
V7	7.215	1.1%	0.181	0.180 0.183
V9	18.74	0.9%	0.460	0.463 0.457
	Ca	Mg		
SULUTION	cig / mc	սց/ու		
102	75 77	E 150		
V2.	23.22	5,002		
vo .	10.01	5.621		

Calculation of percentage weight in the sample

% Weight in sample =

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 Weight sample			25 mL 0.255 g			
	Moistu	re facto	or		0.94	
Constant fa	ctor for	V1(K)	=	$\frac{25}{0.255}$	×	<u>10<sup>-4</sup></u> 0.94
		K <sub>V1</sub>	=	0.0104 except	(all el t potas	ements sium)
	K <sub>V1</sub> pota	assium	=	0.104		
% Element	by weigh	it in V1	=	K <sub>V1</sub> ×	Con dilu dilu (µg,	icentration of ted element in ted sample /mL)
% N	In by we	ight V1	= =	0.0104 0.019	× 1.79	96 (Table 3)
	Eleme	nt	% E	Element	t in V1	
	Na		0.0	13		
K Ca Ma			1.4	1		
			0.1	9		
			0.1	0		
	Mn		0.0	19		
	Zn		0.0	05		
	Fe		0.04	42		
AI			0.0	72		

Where there is a constant factor relating to the concentration in  $\mu$ g/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  $\mu$ g/mL) gave less than 0.1. Absorbance and the calibration was linear up to 20  $\mu$ g/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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