

## **A Comparison of X-Ray Fluorescence Spectrometry and Chemical Methods for Determining Sulphur in Plant Material**

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*(Manuscript received 16 November 1972 accepted 1 February 1973)*

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A rapid method for determining total S in plants by X-ray fluorescence spectrometric analysis of ground pelletised plant material is compared with chemical methods using titrimetric, flame photometric and turbidimetric methods of sulphate determination. Errors in the chemical methods were caused by incomplete oxidation of organic sulphur and by non-reproducibility of the turbidimetric determination of sulphate. A method incorporating the oxidation of the sample with  $\text{HNO}_3$  and  $\text{Mg}(\text{NO}_3)_2$  and titration of the reduced sulphate by mercuric acetate gave recoveries accurate to a mean of 1% when determining the sulphur content of sulphur compounds.

Taking the results for plant materials determined by the titrimetric method as standard, XRF gave sulphur values with a mean difference of 4%; the flame photometric determination of sulphate after  $\text{HNO}_3 + \text{Mg}(\text{NO}_3)_2$  oxidation gave results with a mean difference of 12%. The effect of absorption of S radiation by silicon is noted.

### **1. Introduction**

Because of increasing interest in S as a plant nutrient a quick and accurate method for determining total S in plant materials is desirable, and we have compared XRF analysis with several chemical methods. XRFS provides a rapid and accurate method for determining S in soils;<sup>1</sup> McLachlan and Crawford<sup>2</sup> successfully determined S in subterranean clover and grass with loose powder specimens. Evans<sup>3</sup> tested XRFS on a wider range of species; the plant material was diluted with cellulose before being homogenised and pelleted and the results compared with those obtained turbidimetrically. Souty and Guennelon<sup>4</sup> also analysed a wide range of plants by XRFS, using MgO as a diluent and absorber to lessen matrix variations. The results were compared with those obtained by bomb oxidation followed by turbidimetry. Chemical methods for the determination of total S in plants include those described by Iismaa<sup>5</sup> and Johnson and Nishita.<sup>6</sup>

X-ray fluorescence spectrometry is a comparative method and samples accurately analysed by chemical methods are necessary, either as standards or to check results obtained using artificial standards. Preliminary XRF examination of two sets of plant materials, analysed by two different chemical methods, gave different calibrations and a systematic error in one of the chemical methods was suspected. These methods

differed in both the initial oxidation and final determination procedures and so we compared the results given by several methods with a range of plant materials.

## 2. Experimental

### 2.1. X-ray fluorescence spectrometry

Oven-dry samples of plant material were ground for 5 min in a tungsten carbide disc mill. Pellets were made by pressing between 3 and 5 g of ground sample in a 31 mm die at 10 tonnes although pellets can be made with as little as 0.5 g of sample with some materials. The pellets were analysed in a Philips 1540 Manual Spectrometer.<sup>7</sup>

The instrumental conditions were as follows.

Spectrograph, Philips 1540/10 all vacuum; tube, Cr anode operated at 44 kV, 20 mA; analysing crystal, Ge (111) at  $2\theta = 110.71^\circ$  (sulphur  $K_\alpha$  radiation); collimator, 480  $\mu\text{m}$ ; detector, argon-methane gas flow proportional counter with 1  $\mu\text{m}$  Al-flashed polypropylene window. The background counts were equivalent to 20 parts/million S or less, which was small enough to be negligible for our purposes, and so the measurements were made in vacuum at the peak position only, counting for 20 s; the count rate for sulphur  $K_\alpha$  radiation was about  $5200\text{ s}^{-1}$  for 1 % S and the lower limit of detection about 50 parts/million, although when determining small amounts of S, a correction for background and longer counting times will decrease the lower limit of detection.

Standards were prepared by adding known amounts of L-methionine to cellulose. Because of the absence of the more absorbing elements, the mass absorption coefficient of cellulose for sulphur  $K_\alpha$  radiation is less than that of most plant materials. The addition of 2 %  $\text{SiO}_2$  to standards provided a matrix which simulated average plant material.

### 2.2. Chemical methods

The chemical determination of S involves two stages—the oxidation of organic sulphur compounds to sulphate, then the determination of total sulphate in the sample.

Butters and Chenery<sup>8</sup> followed Swanson and Latshaw<sup>9</sup> in igniting their samples with added  $\text{Mg}(\text{NO}_3)_2$  after digesting with  $\text{HNO}_3$ , but give no reason for the necessity of the  $\text{Mg}(\text{NO}_3)_2$  treatment and we therefore determined the effect of this on the recovery of S, using the titrimetric estimation described below.

#### 2.2.1. Digestion with $\text{HNO}_3$

The samples were treated with fuming  $\text{HNO}_3$  and allowed to stand overnight. Approx. 0.1 g of NaCl was added to prevent loss of  $\text{SO}_3$  and the solutions evaporated to dryness on a hot plate. The last traces of  $\text{HNO}_3$  were eliminated by the addition of a few drops of concentrated HCl and re-evaporating the solution. The residue was dissolved in water and diluted to 50 ml.

#### 2.2.2. Digestion with $\text{HNO}_3 + \text{Mg}(\text{NO}_3)_2$

1 ml of water and then 5 ml of fuming  $\text{HNO}_3$  were added to a beaker containing 200 mg of plant material. The beaker was covered and left overnight. After adding 1 ml of 2 M- $\text{Mg}(\text{NO}_3)_2$  solution the mixture was evaporated to dryness on a water bath and

heated for 2 h at 450 °C in a muffle furnace. After cooling, the residue was dissolved in 5 ml of 6 M-HCl and diluted to 50 ml with water, ignoring any small amounts of silica in the digest solution.

### 2.2.3. Determination of sulphate

#### 2.2.3.1. Flame photometric determination of sulphate

A suitable aliquot of the digest was treated with BaCl<sub>2</sub> solution and the precipitated BaSO<sub>4</sub> collected by centrifugation, washed with water and dissolved in 10% ammonium EDTA. The Ba content of the resulting solution was determined according to Cunningham<sup>10</sup> on a UNICAM SP900 flame spectrophotometer 493 nm.

#### 2.2.3.2. Turbidimetric determination of sulphate

The sulphate was precipitated by adding BaCl<sub>2</sub> in the presence of gelatine; the turbidity of the suspension was measured at 557 μm on a Technicon AutoAnalyzer.<sup>11</sup>

#### 2.2.3.3. Titrimetric determination of sulphate

The sulphate was reduced to H<sub>2</sub>S with the modified St Larent hydriodic reagent of Luke<sup>12</sup> and the H<sub>2</sub>S absorbed in 2 N-NaOH solution and titrated with 0.002 M-mercuric acetate, using dithizone in acetone as indicator.<sup>13</sup>

## 3. Comparison of methods for determining sulphate

Tables 1 and 2 show that oxidation with HNO<sub>3</sub> alone is less effective than HNO<sub>3</sub>-Mg(NO<sub>3</sub>)<sub>2</sub>, particularly with L-methionine; the HNO<sub>3</sub>-Mg(NO<sub>3</sub>)<sub>2</sub> attack gave between 98.6 and 100% recovery of S from S compounds. With the plant materials, oxidation with HNO<sub>3</sub>-Mg(NO<sub>3</sub>)<sub>2</sub> gave consistently higher results, the difference probably reflecting the methionine content. The Mg(NO<sub>3</sub>)<sub>2</sub>-HNO<sub>3</sub> procedure was, therefore, used when comparing the different methods of determining sulphur.

TABLE 1. A comparison of oxidation methods using sulphur compounds

| Compound                    | % Sulphur   |                                 |  |
|-----------------------------|-------------|---------------------------------|--|
|                             | Theoretical | Oxidation with HNO <sub>3</sub> | Oxidation with HNO <sub>3</sub> and Mg(NO <sub>3</sub> ) <sub>2</sub> followed by ignition |
| Sulphanilic acid            | 18.51       | 18.41                           | 18.38  |
| Sulphamic acid              | 33.03       | 32.01                           | 33.08  |
| S-Benzylthiuronium chloride | 15.82       | 15.21                           | 15.62  |
| Methionine                  | 21.49       | 0.79                            | 21.32  |
| Cystine                     | 26.68       | 25.62                           | 26.29  |
| Dibenzyl disulphide         | 26.03       | 25.46                           | 25.66  |
| Potassium sulphate          | 18.40       | 18.30                           | 18.29  |

TABLE 2. A comparison of chemical and X-ray methods, % sulphur

|             | XRF<br>using<br>synthetic<br>standards | Oxidation:<br><br>Determination: | Chemical         |  |                      |                    |
|-------------|--|----------------------------------|------------------|--|----------------------|--------------------|
|             |  |                                  | HNO <sub>3</sub> | HNO <sub>3</sub> + Mg(NO <sub>3</sub> ) <sub>2</sub> |                      |                    |
|             |  |                                  | Titrimetric      | Titrimetric  | Flame<br>photometric | Turbidi-<br>metric |
| Flour       | 0.13                                   |                                  | 0.091            | 0.109  | 0.101                | 0.144              |
| Wheat straw | 0.22                                   |                                  | 0.217            | 0.219  | 0.219                | 0.287              |
| Cabbage     | 1.07                                   |                                  | 0.747            | 1.081  | 1.07                 | 1.21               |
| Sugar beet  | 0.62                                   |                                  | 0.594            | 0.625  | 0.556                | 0.578              |
| Barley      | 0.63                                   |                                  | 0.454            | 0.611  | 0.532                | 0.470              |
| Swede       | 1.74                                   |                                  | 1.497            | 1.833  | 1.42                 | 1.83               |
| Beans       | 0.23                                   |                                  | —                | 0.261  | 0.192                | 0.227              |
| Maize       | 0.42                                   |                                  | —                | 0.404  | 0.346                | 0.300              |
| Wheat       | 0.36                                   |                                  | —                | 0.369  | 0.274                | 0.287              |
| Lucerne     | 0.49                                   |                                  | —                | 0.468  | 0.432                | —                  |
| Oats        | 0.41                                   |                                  | —                | 0.400  | 0.334                | —                  |
| Mangold     | 0.35                                   |                                  | —                | 0.428  | 0.420                | —                  |
| Radish 1    | 0.96                                   |                                  | —                | 0.955  | 0.855                | —                  |
| 2           | 0.47                                   |                                  | —                | 0.468  | 0.510                | —                  |
| 3           | 0.87                                   |                                  | —                | 0.873  | 0.855                | —                  |
| 4           | 0.48                                   |                                  | —                | 0.484  | 0.460                | —                  |
| Kale 1      | 1.71                                   |                                  | 1.500            | 1.741  | 1.82                 | 1.90               |
| 2           | 0.89                                   |                                  | 0.639            | 0.875  | —                    | —                  |
| 3           | 0.74                                   |                                  | 0.524            | 0.709  | —                    | —                  |
| 4           | 0.88                                   |                                  | 0.695            | 0.856  | —                    | —                  |
| 5           | 0.97                                   |                                  | 0.696            | 0.942  | —                    | —                  |
| 6           | 1.21                                   |                                  | 0.865            | 1.118  | —                    | —                  |

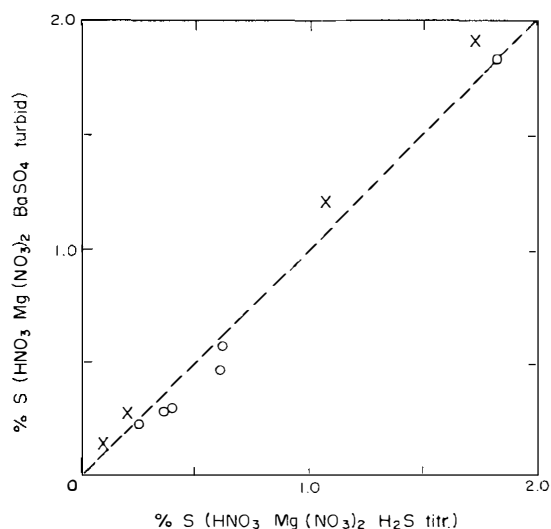


Figure 1. A comparison of turbidimetric and titrimetric methods of determining sulphate. (x), Calibration of December 1970; (o), calibration of January 1972.

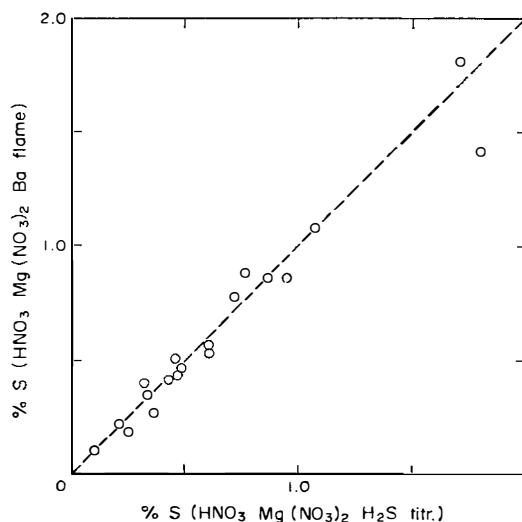


Figure 2. A comparison of flame photometric and titrimetric methods of determining sulphate.

The results given by the chemical and X-ray methods are compared in Table 2. The results obtained with the AutoAnalyzer were not consistent with the results from the other methods and the calibration appeared to vary with different batches (Figure 1). Theoretically, if the results determined by two different, but equally accurate, methods are plotted against each other, a line of 45° slope should be the line of best fit between the points. Plotting the results of the titrimetric finish against those of the turbidimetric finish (Figure 1) shows that there is a large variation between results. Figure 2 shows that the agreement between the flame photometric and titrimetric methods is close, but there is a bias towards low results with flame photometry.

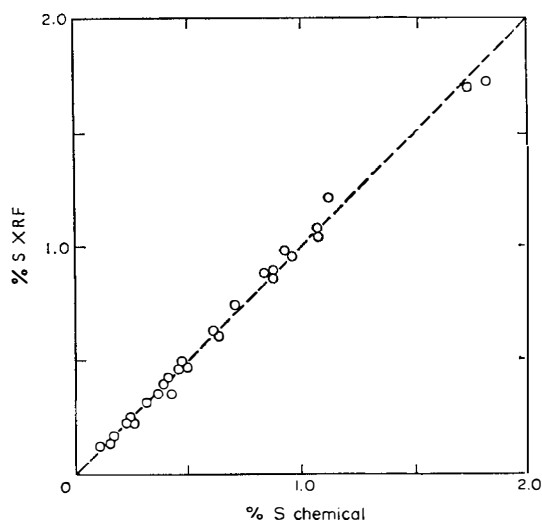


Figure 3. A comparison of sulphur determination by XRF and chemical analysis.

Comparison of the titrimetric and XRF methods (Figure 3) shows that the agreement between these two methods is good, although matrix variations in the samples analysed by XRF have not been taken into account. Some of the disagreement is certainly caused by differences in matrix absorption, for example, the points that lie substantially below the 45° line in Figure 3 are for beans and mangolds which contained 3 and 6% SiO<sub>2</sub>, respectively. Si is very absorbent for sulphur  $K_{\alpha}$  radiation and further investigation showed that the addition of 5% SiO<sub>2</sub> to a standard decreased the count rate for S by 15%.

#### 4. Discussion

X-ray fluorescence analysis offers a rapid simple method for determining S in plant material. The analysis of standard kale (Table 3) gives results very near those of Bowen<sup>14</sup> who gives the mean and standard deviation of 21 determinations of the S content of the

TABLE 3. The sulphur content of standard kale

| % Sulphur       | Method  |
|-----------------|---|
| 1.6010 ± 0.2648 | Various chemical <sup>14</sup>                        |
| 1.741           | HNO <sub>3</sub> /MgNO <sub>3</sub> oxid. tir. finish |
| 1.823           | HNO <sub>3</sub> /MgNO <sub>3</sub> Ba flame finish   |
| 1.904           | HNO <sub>3</sub> /MgNO <sub>3</sub> turbid. finish    |
| 1.500           | HNO <sub>3</sub> oxid. titr. finish                   |
| 1.80            | XRF-solution <sup>15</sup>                            |
| 1.6296          | XRF-pressed powder <sup>3</sup>                       |
| 1.716           | XRF-pressed powder <sup>16</sup>                      |
| 1.71            | XRF-pressed powder                                    |

standard kale by various methods; turbidimetric results were omitted from the published average as they were unsatisfactory. If speed is more important than great accuracy, the intensity of sulphur  $K_{\alpha}$  radiation can be related directly to standards of known S content, or to suitable synthetic standards, but matrix corrections must be made if great accuracy is required; these require the determination of all the major elements in the sample. Of the chemical methods, digestion with HNO<sub>3</sub> and Mg(NO<sub>3</sub>)<sub>2</sub> and determination as H<sub>2</sub>S gives results most consistent with those obtained by XRF.

#### Acknowledgements

We are grateful to B. Messer and V. Cosimini for the help with the analyses and to Dr C. J. Bloomfield for advice and helpful criticism.

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