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VALIDATION PROCEDURE FOR TOTAL SULPHUR DETERMINATION IN COMPLEX FERTILIZER MATRICES USED IN ORGANIC FARMING

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Abstract

Sulphur plays a vital role in plant nutrition. Its deficiency can have severe effects on crops, being essential in many biological processes. For an optimal supply it is necessary to know the content of elements of both the soil and the inputs used in agriculture. With the conversion from conventional to organic farming, an increasing number of analyses are needed on soil, the used inputs, and the obtained products. In general, sulphur determination methods are tedious and time consuming. This study proposes a dry combustion method for sulphur determination, which can gain ground due to the speed and ease of application. This elemental analysis method is sensitive and accurate, and can be applied for the determination of total S content in many types of sample matrices, including fertilizers. Different validation parameters were measured to ensure that the method can be used successfully applied and the procedure can be standardized, fully validated and nationally accepted.

***Key words**: CHNS, dry combustion, method validation, organic inputs, sulphur.*

INTRODUCTION

Sulphur is one of the essential macronutrients for the good development of plants, having multiple roles in the synthesis of proteins, vitamins, enzymes or chlorophyll (Narayan et al., 2022).

The soil contains many sources of sulphur, a large part of which is present in organic matter. The decomposition of organic matter makes sulphur available for plants. Various soil minerals also contain different forms of sulphur which can be released for plant use. Sulphur deficiency can affect plant growth and development, resistance to various diseases and has a major impact on harvest quality (Kopriva et al., 2019). As a safety factor, the soil sulphur content represents an indicator of available sulphur that must be taken into account at crop establishment, in order to correct the deficiency with appropriate inputs. The growing number of analyzes required for organic crops, as well as the time required to perform them, led to the search for fast, precise and low-cost alternatives.

Although it is one of the essential macrontrients, sulphur analysis is not as popular as the other macronutrients. Until two decades ago sulphur deficiency was almost non-existent due to fertilizers and atmospheric sulphur content (Ibrahim et al., 2019), but nowadays this problem has become more and more visible due to the limitation of the use in ecological agriculture of sulphur-based pesticides, conventional fertilizers with sulphur and the increasingly strict legislation regarding atmospheric emissions in order to make agriculture more sustainable and environmentally friendly. (Jamal et al., 2010; Scherer, 2009; Koufiotis et al., 2016).

Unlike other mineral elements, the determination of sulphur is done by laborious methods. Most of the time, the soluble fraction of sulphur in certain solvents is determined by various analytical methods. A colorimetric method on an acetone extract was described by Maynard and Addison in 1985, but due to high limit of detection it was not widely accepted. Later, a HPLC analysis on a chloroform extraction was tested by Watkinson (1987) with good results. In 2015, this method was optimized by Alberta Environment and Parks, performing tests with more solvents and different equipment parameters.

The development and validation of the total sulphur determination method was successful, but many problems appeared during the tests (such as clogging of the column due to the extraction methods). Although efficient methods and equipments have recently appeared, sulphur analysis is still problematic due to the high costs of this analysis.

Thus, elemental analysis of sulphur can be performed using the ICP-MS technique, which is still problematic using conventional single quadrupole ICP-MS.

With some optimizations, such as the additional oxygen cell or using a triple quadrupole ICP-MS (ICP-MS/MS), the method can generate very good results (McCurdy et al., 2020; Sugiyama et al., 2019).

Another method often used for sulphur determination involve the full conversion of S into sulphate followed by Ion-Chromatography detection (Rahier, 2005)

Studies have shown that the method is superior to gravimetric or colorimetric methods and the results are comparable to other modern methods (Nicolescu et al., 2017; Sapcanin et al., 2013)

The dry combustion method, a much more accessible method, has been tested in recent years with promising results.

In 2014, Bernius et al. conducted a study for the validation of the sulphur determination method by dry combustion (Dumas, 1831) for inorganic fertilizers. The method proved to have a very good accuracy, managing to determine concentrations of 1% S.

The precision of the method was acceptable, and it proved to be efficient in terms of time and resources, allowing the analysis of dozens of samples per day with a single calibration curve and far fewer hazardous reagents than other procedures. Also, the device does not require an operator during the analyses.

The same method was also tested for food, with very good results as well. The only change was that the Flame Photometric Detector (FPD) was used instead of the Thermal Conductivity Detector (TCD) (Krotz & Giazzi, 2017).

Following the results obtained in the studies mentioned above, the aim of this paper is to develop and validate a method based on automated Pregl-Dumas technique for the quantification of total sulphur in organic fertilizers.

This method can be also optimised for other types of organic fertilizers based on biomass waste material, soils, sediments and also food.

MATERIALS AND METHODS

Chemicals and reagents

For sulphur determination, the following reagents and consumables were used:

- *CHNS combustion tube* (Elemental Microanalysis Ltd), compatible with the elemental analyzer Eurovector Elemental Analizer EA 3100;
- *Pure O_2 gas* (Messer 5.0, 99,999% purity) for complete sample combustion;
- *Pure He gas* (He 6.0, 99.9999% purity), used as carrier gas;
- *Tin capsules* (8 x 5 mm) for weighing and packing the samples. These capsules also enhance the combustion. Tweezers were used to seal them in order to prevent sample loss and also to exclude atmospheric air.
- *Cystine OAS (Organic Analytical Standard)* (Certif. no. 347115, Elemental Microanalysis Ltd), used as calibration standard (S content % w/w = 26.67).
- *Ethanol 99%* was used for tweezers and spatulas washing after each sample.
- *Chromosorb W 30-60 mesh* (Elemental Microanalysis Ltd), used for liquid samples to prevent sample loss during packing.

Samples

Two types of fertilizers were used as test matrices:

- CF - (Commercial liquid water-soluble fertilizer which contains N (nitrogen), P (phosphorous), K (potassium) and S;
- BIPEA Nitrogen fertilizer (Sample 04-4024)

The samples were stored according to the manufacturer's instructions and were used as such, without any preparation.

Elemental analysis instrument and parameters

Elemental Analyzer CHNS Eurovector EA 3100 equipment was used for the development of the method and its validation. The following components were used:

- 80 positions autosampler;
- Mettler Toledo microbalance (precision 0.000001 g);
- H_2O trap for water retention;
- PTFE GC Column, outer diameter 8 mm, length 2 m;
- Eurovector Weaver.NET 1.8.0.0 software.

The parameters used for sulphur determination method are detailed in the Table 1.

Table 1. Method parameters for S determination using elemental analyzer Eurovector EA3100

Parameter	Unit	Value
Carrier Pressure	kPa	90
Reference Pressure	kPa	20
Furnace #1	°C	950
GC Oven	°C	110
Transfer Line	°C	110
Run Time	s	600
Sample Delay	s	6
O_2 Volume	mL	15
O_2 Injection Rate		slow

The analysis lasts 600 seconds for each sample and is fully automated (sample handling, equipment control, data processing, equipment and gases shutdown).

Method validation

The verification steps for method validations included the checking of some performance indicators:

- linear response domain (drawing calibration lines);
- accuracy (fairness) of the measurement;
- intra-day and inter-day precision ;
- standard and extended uncertainty.

RESULTS AND DISCUSSIONS

Method principles

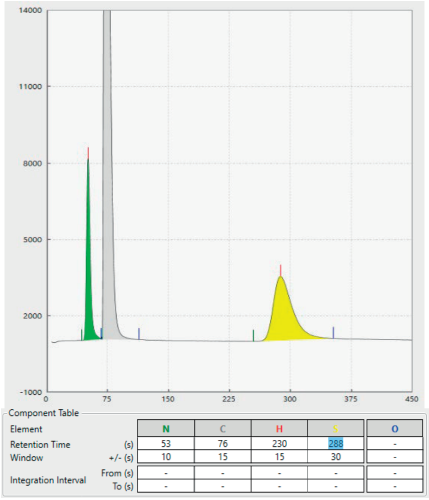
The well-homogenized sample is subjected to combustion in a furnace heated to a high temperature where combustion occurs rapidly at over 900°C in the presence of pure oxygen. The combustion produces a number of gases, mainly water, carbon dioxide, sulphur oxides and nitrogen as several oxides (NyOx).

If other elements, such as chloride, also appear in the sample, they will also be transformed into the corresponding compounds, such as hydrogen chloride (HCl). In order not to interfere with the gases to be determined, these secondary combustion compounds are eliminated using special traps that retain these substances. A certain type of traps can also be used to remove some of the main elements, such as water or carbon dioxide, if their determination is not necessary. This gas mixture passes through a reduction chamber consisting of heated copper. This stage transforms nitrogen oxides into elemental nitrogen and collects the excess of oxygen left after the combustion of the sample. The gases are carried by a carrier gas (usually pure helium) then passed through the absorbent traps that allows only to pass carbon dioxide, water, nitrogen, and sulphur dioxide. The gases are passed through a GC column where they are separated, and the total content of each gas arrives after a certain time at a thermal conductivity detector (TCD), where each amount of gas is quantified.

The interface of Weaver.NET 1.8.0.0 software allows the control of the equipment, the creation of analysis methods by establishing the running parameters, the storage of data and their subsequent processing. Also, it enables a real-time view of the analytical process during the analysis.

Once that a peak has been detected and its area has been calculated using the integration parameters, it labels the element in order to identify the detected peak as N, C, H, S or O, based on time around which the peak is expected to appear at the detector (Retention Time). For the integration parameters used in the development of the method, a chromatogram shows as in Figure 1, where the sulphur retention time is at 288 s.

Quantification of these elements is based on calibration for each element by using high purity standards such as cystine, BBOT, sulphanilic acid, etc. (AMC technical briefs, 2008).



Component Table

Element		N	C	H	S	O
Retention Time	(s)	53	76	230	288	-
Window	+/- (s)	10	15	15	30	-
Integration Interval	From (s)	-	-	-	-	-
	To (s)	-	-	-	-	-

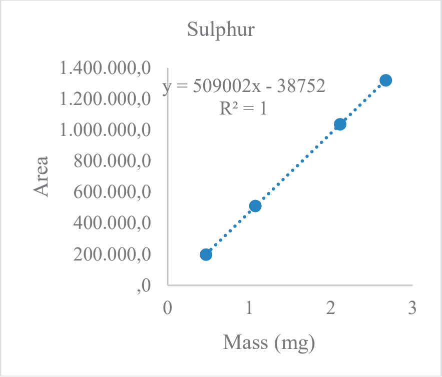
Figure 1. A typical chromatogram for CHNS analysis generated by Weaver.NET software (green peak - Nitrogen; grey peak - Carbon; yellow peak - Sulphur)

Linearity

Linearity represents the ability of the method to provide results that are directly proportional to the amount of analyte in the sample. Linearity was determined using 4 calibration points, whose analyte concentration should range from 80% to 120% of the expected levels. Linearity report should include the slope, linear range and correlation coefficient data. Correlation coefficient must be greater or equal to 0.99 in the working range. A minimum correlation coefficient (R) > 0.999 should be obtained in the working range.

Calibration curve was made with Cystine as a calibration standard and it was obtained from four points that covered a wide range of sulphur masses, corresponding from 0.50 to 2.50 mg of standard material.

The calibration curve was obtained from four points, using cystine (SRM) as a calibration standard. The following parameters were calculated, as shown in Figure 2: slope, linear range, correlation coefficient, residual standard deviation (Sy) and the deviation of the analysis method (Sx0), the coefficient of variation (CV). All acceptability criteria were met, according to the Eurachem Guide: $R \geq 0.995$, CV< 5%.



Equation	ax+b
Slope, a	509002
Corelation coef. R	1
Regression coef. R^2	1
y Intercept, b	-38752
S_y=	21553.64
S_{x0}=	0.04
CV %	3.35

Figure 2. Calibration curve for S determination

Intra-day (RSD_r %) and the inter-day (RSD_R %) precision coefficients

Precision is a determination of the closeness between measurements obtained through a series of experiments under similar conditions. It must be considered that a high precision does not necessarily mean good accuracy. The precision can be considered as three indicators: repeatability, intermediate precision and reproducibility and it can be usually expressed as the variance, standard deviation or as coefficient of variation of a series of determinations. A minimum of five determinations should be carried out for accurate results.

The precision of the analytical method was evaluated based on the relative standard deviation (RSD) of the intra-day precision (RSD_r %) and the inter-day precision (RSD_R %). For determination of repeatability precision, Cystine (Microelemental Analysis), BIPEA and CF was successively analyzed 5 times as 5 different sample weights in the same day, by one analyst. Intra-laboratory reproducibility precision was determined by analyzing the same sample in 8 replicates during 4 days by two different analysts.

The intra-day (repeatability) and inter-day (reproducibility) precision was evaluated using Cystine, BIPEA and CF. The following parameters were calculated: mean (X_m), standard deviation (Sr), repeatability limit (r), reproducibility limit (R), RSD_r, RSD_R (Tables 2 and 3).

Table 2. Intra-day precision

Analyte	**Mass (mg)**	**X_m (%)**	**Sr (%)**	**r (%)**	**RSD_r (%)**
Cystine	0.50-2.50	26.65	0.80	2.24	3.01
BIPEA	0.50-2.50	5.21	0.29	0.81	5.52
CF	2.50-5.00	62.60	3.13	8.77	8.77

Table 3. Inter-day precision

Analyte	**Mass (mg)**	**X_m (%)**	**Sr (%)**	**R (%)**	**RSD_R (%)**
Cystine	0.50-2.50	26.82	0.76	3.39	4.52
BIPEA	0.50-2.50	5.14	0.32	1.43	9.93
CF	2.50-5.00	64.84	4.57	20.46	11.27

The method has good results for the sample quantities used for tests. Smaller amounts will either suffer from sample heterogeneity or will not be detected by TCD. Larger quantities can lead to superposition of the peaks and the wrong integration of the areas. Also, large amounts can lead to incomplete combustion or oversaturation of the TCD.

The obtained RSD_r values for standard materials are situated under 10.00%, from 3.01% for Cystine SRM to 5.52% for BIPEA, which indicated that the equipment method is repeatable (Table 2). Also, the RSD_r value for tested fertilizer was below the value indicated in the validation guide (Magnusson & Örnemark, 2014).

The RSD_R% (inter-day precision) values for all three types of analytes were less than 20% which means that the method can be used in different days and by different analysts.

Accuracy and Bias

Accuracy is one of the most critical parameter in method validation, and it shows the degree of closeness between the 'true' value of analytes in the sample (as mentioned by the manufacturer in the certificate of conformity) and the value determined by the method to be validated.

For accuracy and bias determination, 10 samples of cystine were successively analyzed and the obtained values were compared with the values from the certificate issued by the manufacturer (Table 4). The obtained results were compared with the certificate value. Bias was expressed as the difference between the mean of the 10 samples and the real value from certificate.

$$Accuracy\ (\%) = \frac{\bar{x}}{\mu} \times 100 \quad (1)$$

where:

$\bar{x}$ = the mean of SRM samples;

μ = "real" value from SRM certificate.

$$Bias\ (\%) = \frac{\bar{x}-\mu}{\mu} \times 100 \quad (2)$$

Table 4. Accuracy and BIAS of Cystine SRM

Material	Cystine
Mass (mg)	0.50 – 2.50
Mean value (% S)	26.65
Certificate value (% S)	26.67 ± 0.12
Accuracy (%)	99.91
BIAS (%)	-0.09

The standard material obtained a very good accuracy, in the accordance with EURACHEM Guide (Magnusson & Örnemark, 2014), which recommends that the accuracy should fall within the range 70-110%. BIAS (trueness) also obtainned good values, -0.09%, which is below 15% (the value mentioned in the above guide) and very close to zero, which is the optimal value.

Measurement Uncertainty

To perform measurement uncertainty, several sources of uncertainty were taken into account, considered to contribute the most when the extended uncertainty is calculated:

- The standard uncertainty compounded from weighing;
- Uncertainty derived from method calibration data (CV % value);
- The uncertainty derived from the internal repeatability test (RSD_r value) on the standard solution;
- The uncertainty derived from the internal reproducibility test (RSD_R value) on the matrix;
- Uncertainty derived from accuracy and bias tests.

Using the dry combustion method (Dumas) good results were obtained for the analysis of SRM and organic fertilizer (Table 5). Although the extended uncertainty had high values, the method suited below the limits accepted by the validation guide.

These values can be improved using some optimizations such as the homogeneity of the sample or using different calibration standards depending on the analyzed sample.

Table 5. Uncertainty results for reference material and CF fertilizer

Sample	Mean Conc (%)	Ustd (%)	Uext (%)
BIPEA	5.14	10.90	21.80
CF	64.84	12.14	24.28

CONCLUSIONS

The method development and validation revealed good values for:

- linearity,
- accuracy and bias,
- intra-day precision
- inter-day precision

The method was successfully applied to organic fertilizers, obtaining fast results, in accordance with the certificates of used materials. A variation of this method can be used also for other type of samples, as soils, to establish the level of sulphur fertilization. It must be taken into account that certain samples may require a prior preparation.

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REFERENCES

Alberta Environment and Parks. (2015). Development and Validation of Analytical methods for Elemental Sulphur in Alberta Soils. Edmonton, Alberta.

AMC Technical Briefs. (2008). General and specific fitness functions for proficiency tests and other purposes-clarifying an old idea. *Analytical Methods Committee AMCTB, 29.*

Bernius, J., Kraus, S., Hughes, S., Margraf, D., Bartos, J., Newlon, N., & Sieper, H.-P. (2014). Determination of total sulfur in fertilizers by high temperature combustion: Single-laboratory validation. *Journal of AOAC INTERNATIONAL, 97*(3), 731–735.

Dumas, J.B.A. (1831) Procedes de l'analyse organic. *Ann. Chim. Phys*. *247*,198–213.

Eurovector, (2019). EA 3100 Elemental Analyzer Operating manual. *EuroVector srl Pavia*, Italy

Ibrahim, F., Bemgba, A., & Okoh, G. O. (2019). Determination of the optimum sulphur fertilizer rate for groundnut production in selected soils of Benue State using sorption indices. *International Journal of Horticulture, Agriculture and Food Science, 3*(6), 312–319.

Jamal, A., Moon, Y. S., & Abdin, M. Z. (2010). Enzyme Activity Assessment of Peanut (Arachis hypogea L.) Under Slow-release Sulphur *Fertilization.Australian Journal of Crop Science, 4*(3), 169–174.

Kopriva, S., Malagoli, M., & Takahashi, H. (2019). Sulfur Nutrition: Impacts on plant development, metabolism, and stress responses. *Journal of Experimental Botany, 70*(16), 4069–4073.

Koufiotis, I., Gyftopoulos, N., Andreou, A., Pisimisis, F., Travlos, I., Bilalis, D. & Vlahos, G. (2016). Management of production and distribution of organic products. The case of organic farm managers and distributors in athens' organic open farmers' markets. *AgroLife Scientific Journal 5*(2), 91–94.

Krotz, L.& Giazzi, G. (2017). Sulfur determination in food by the Thermo Scientific FlashSmart Elemental Analyzer coupled with FPD Detector. *Thermo Fisher Scientific Application Note*, Milan, Italy.

Magnusson, B. & Örnemark, U. (eds.) (2014). EURACHEM Guide: The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics, *2nd edition*.

McCurdy, E., Woods, G. & Sugiyama, N. (2020). Accurate, Low-Level Sulfur Analysis by ICP-MS Using MS/MS with Oxygen Reaction Cell Gas. *Spectroscopy, Special Issues 09-01-20, 35*(S4), 27–34.

Maynard, D. G., & Addison, P. A. (1985). Extraction and colorimetric determination of elemental sulfur in organic horizons of forest soils. *Canadian Journal of Soil Science*, 65(4), 811–813.

Narayan, O. P., Kumar, P., Yadav, B., Dua, M., & Johri, A. K. (2022). Sulfur Nutrition and its role in plant growth and development. *Plant Signaling & Behavior, e2030082-2,* 1–12.

Nicolescu, I. C., Bucur, E., Serbanescu, A., Diodiu, R., & Barbu, M. (2017). Determination of sulfur content in waste materials by Ion Chromatography. *SIMI 2017*. 1–6.

Rahier, A. (2005). Analytical Determination of Total Carbon and Sulphur in solid Samples by Full Combustion and Integrated NDIR Detection.S*CK-CEN*, Mol, Belgium.

Sapcanin, A., Jancan, G., Pazalja, M., Kresic, D., Ekrem, P. & Uzunovic, A. (2013). Determination of Total Sulphur Content in Biological Samples by Using High Performance Ion Chromatography and Elemental Analysis, *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina 41*,11–14.

Scherer, H. W. (2009) Sulphur in Soils. *Journal of Plant Nutrition and Soil Science, 172*, 326–335.

Sugiyama, N., McCurdy, E. & Woods, G. (2019). Method Development with ICP-MS/MS: Tools and Techniques to Ensure Accurate Results in Reaction Mode. *Spectroscopy, Special Issues 09-01-2019, 34*(9). 20–27.

Watkinson, J.H. Lee, A. & Lauren, D.R. (1987). Measurement of elemental sulfur in soil and sediments: field sampling, sample storage, pretreatment, extraction and analysis by high-performance liquid chromatography. *Australian Journal of Soil Research, 25*(2), 167–178.