



# Proceeding Paper The Optimization and Validation of the Method for the Determination of Micronutrients in Organic Fertilizers by Inductively Coupled Plasma Optical Emission Spectrometry <sup>+</sup>

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**Abstract:** Inductively coupled plasma optical emission spectrometry (ICP-OES) analytical method was optimized to determine the micronutrient concentrations from the fertilizers used as inputs in organic farming. The calibration curves showed good linearity for all elements ( $R^2 > 0.999$ ). Recoveries were in the range of 94.9–97.9%. The values of extended uncertainty with a probability of 95% (k = 2) for the liquid organic fertilizer samples were between 0.004% for Mo and 0.12% for Fe. The results have shown good accuracy and reproducibility, and the optimized method was validated.

Keywords: method linearity; detection and quantification limits; repeatability; measurement uncertainty

# 1. Introduction

Consumers' growing interest in products obtained from organic agriculture is the major driver for the increased adoption of organic farming. In Europe, the European Green Deal policy and the Farm2Fork strategy create a framework for the sustainable development of organic farming [1,2]. It is considered that organic farming can ensure sustainability and the transition to a green economy, with a positive impact on the environment and food security, as it prohibits the use of synthetic chemicals in agriculture and in the food industry [3].

Organic agriculture relies on crop rotation, animal and green manures, and green plant protection products to maintain soil health and crop productivity without adversely affecting the environment [4]. Decreasing the use of synthetic fertilizers and plant protection products involves an increased need for organic certified inputs, that the European Union has to comply with Regulation (EU) 2018/848, EU 2021/1165 and EU 2019/1009 [5].

Small amounts of nutrients such as boron, cobalt, copper, iron, manganese, molybdenum, and zinc are essential for plant growth-micro/oligonutrient [6]. Micro/oligonutrients functions in plants are complex: (i) most micronutrients are part of the enzyme systems of plants; (ii) play significant roles in photosynthesis, maintaining reactive oxygen species at the physiological level and mitigation of the abiotic stress effects; (iii) are important in reactions such as N fixation or protein synthesis [7,8].

Considering the increased interest in fertilizing products certified for organic farming and the need to control their quality, the development and validation of analysis meth-



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). ods are necessary, including for micro(oligo)elements. The advantage of simultaneous determination of various elements in fertilizers by ICP-AES/ICP-OES has already been emphasized [9].

The complex matrix of organic fertilizers represents the main challenge in developing analytic methods for fertilizers. Therefore, two extraction methods were used to optimize the sample preparation methods, and the performance parameters for the subsequent determination of Fe, Mn, Zn, Cu, and Mo by ICP-OES were evaluated. The method we developed has been validated, meeting the performance parameters reported here. In this study, the concentrations of micronutrients from an organic fertilizing product were determined by the ICP-OES validated method.

## 2. Materials and Methods

## 2.1. Reagents and Samples

The reagents used for micronutrients (Fe, Zn, Mn, Cu, Mo) determination were analytical grade. Three multielement standard solutions were used: CertiPur XVI 100 mg L<sup>-1</sup>, Supelco CertiPur VIII 100 mg L<sup>-1</sup>, and Supelco CertiPur IV 1000 mg L<sup>-1</sup> concentration, from Merck Group (Darmstadt, Germany), HNO<sub>3</sub>  $\geq$  69.0% Trace SELECT<sup>TM</sup>, Sigma-Aldrich, HCl 37% from Scharlau (Barcelona, Spain) and H<sub>2</sub>O<sub>2</sub> 30% from Merck Group (Darmstad). For the preparation of working solutions and samples, ultrapure water was used by Milli-Q Plus (Merck-Millipore, Bedford, MA, USA). For ICP-OES, argon 5.0 was used, >99.999% purity (Siad Gas, Călărași, Romania).

Two fertilizer samples were used in this study, as follows: a sample of liquid organic fertilizer with micronutrients chelated in complexing agent allowed in organic agriculture (lignosulphonate), totally water-soluble, and a sample liquid fertilizer, reference material (M.R.) from an interlaboratory comparison scheme.

## 2.2. Extraction of Micronutrients from Samples

The extraction of water-soluble micronutrients from liquid organic fertilizer was performed by shaking the fertilizer sample in ultrapure water at  $(20 \pm 2)$  °C for 60 min, using an incubated shaker (Binder, Tuttlingen, Germany). The extracts are acidified after extraction to avoid hydrolysis. The organic matter is removed by boiling with the addition of hydrogen peroxide. In parallel, a blank sample of reagents is prepared.

## 2.3. Micronutrient Analysis by ICP-OES

For the determination of micronutrient (Fe, Zn, Mn, Cu, Mo) content, the Optima 2100 DV ICP-OES System (Perkin Elmer, Waltham, MA, USA) was used, with the characteristics described in the previous work [10]. ICP-OES experimental operating conditions and method parameters are presented in other work of our group [11]. The plasma viewing mode was axial or radial, and the selected wavelengths ( $\lambda$ ), nm, are the following: (Fe = 238.204, Mn = 257.610, Cu = 327.393, Zn = 213.857, Mo = 202.031).

Preparation of standard calibration solutions and standard concentration verification solutions in the domains:  $10 \div 50 \ \mu g \ L^{-1}$ ,  $0.1 \div 1 \ mg \ L^{-1}$ , and  $1 \div 10 \ mg \ L^{-1}$  were obtained by diluting the standard ICP, Certipur solutions of 1000 mg  $L^{-1}$  and 100 mg  $L^{-1}$ . The analytical procedure was: the blank solution, the standard solution for tracing the calibration curve, the standard solution for independent verification of the calibration curve, and the sample solution.

## 2.4. Validation of the Method for Micronutrient Determination from Liquid Organic Fertilizer

The performance parameters of the developed method were linearity, limits of detection (LOD) and quantification (LOQ), precision (repeatability), accuracy (degree of recovery), and measurement uncertainty. The validation parameters, acceptance criteria, and acceptance criteria, as well as the reference works, are presented in Table 1.

No. Crt.	Parameters	Acceptance Criteria	Reference Documents
1.	Linearity	$\begin{array}{l} \mbox{Linear regression coefficient} \\ \mbox{value (correlation),} \\ \mbox{R} \geq 0.997 \end{array}$	[12]
2.	Detection limit	$\rm LOD \leq 10 \ mg \ kg^{-1}$	[12]
3.	Quantification limit	$LOQ \le 35 \text{ mg kg}^{-1}$	[12]
		$s_{r\text{-}Fe} \leq 0.130\% \text{ (w/w)}$	
	Repeatability - (Precision) -	$s_{r-Mn} \le 0.075\%$ (w/w)	
4.		$s_{r\text{-}Zn} \leq 0.023\% \text{ (w/w)}$	[13]
		$s_{r-Cu} \le 0.007\%$ (w/w)	
		$s_{r\text{-}Mo} \leq 0.006\% \text{ (w/w)}$	
5.	Accuracy (Recovery degree)	90 < R < 110	[14]
6.		$U_{max\_Fe} \leq 0.26\%  (w/w)$	
	Measurement uncertainty, (k = 2, P = 95%) -	$U_{max\_Mn} \leq 0.15\% \text{ (w/w)}$	
		$U_{max\_Zn} \leq 0.045\% \ (w/w)$	-
		$U_{max\_Cu} \leq 0.014\% \text{ (w/w)}$	
		$U_{max\_Mo} \leq 0.012\% \text{ (w/w)}$	

**Table 1.** Validation parameters and general acceptance criteria of the method.

The Evaluation Criteria

• Linearity;

The statistical verification of the linear calibration function is performed by evaluating the calibration linearity given by the equation y = a + bx from which the coefficients are obtained: b—slope of the calibration line b (represents a measure of the sensitivity of the method), a—ordered at origin (calculated control sample of reagents) and the value of the linear regression coefficient (correlation),  $r \ge 0.997$ .

Limit of detection and limit of quantification;

Given the level of fertilizer concentrations on the market and the level of performance of analytical methods (Horwitz [13]), for the detection and quantification limits, we propose that LOD be  $\leq$  with the concentration corresponding to the lower working range limit for liquid organic fertilizer:

 $LOD \le 10 \text{ mg kg}^{-1}$ 

 $LOQ \le 35 \text{ mg kg}^{-1}$ 

The limits of detection (LOD) and quantification (LOQ) were calculated using the formula (Equations (1) and (2)) [13].

$$LOD = 3 \times s_r \tag{1}$$

$$LOQ = 3 \times LOD$$
 (2)

where s<sub>r</sub> is the standard deviation.

Repeatability and reproducibility;

The evaluation criteria for repeatability by different concentration ranges are established based on Horwitz's report as follows [13]:

$$RSD < 0.6 \times 2^{(1-0.5\lg C)} \tag{3}$$

The accepted values of the standard deviation depend on the analyte concentration expressed as % (w/w) mass fraction (C).

• The acceptance criterion for measurement uncertainty; U is defined according to [10]:

$$U \le 2 \times s_R \tag{4}$$

where  $s_R$  is standard deviation and is calculated with Formula [13]:

$$s_R = \frac{RSD \times C}{100} \tag{5}$$

where RSD is calculated using Horwitz Equation (3).

For concentration at the limit of detection, the acceptance criterion is 20% of the concentration.

# 3. Results and Discussions

3.1. Linear Calibration Function

For the statistical evaluation of the linear calibration function, the working areas were chosen according to the concentration level present in the analyzed samples. The data regarding the equations of the calibration curves and the coefficients of the linear calibration function are presented in Table 2.

Table 2. Summary of method characteristics and linear calibration function.

Analyte/ Sample Type	Calibration Line Equation (y = bx + a)	Correlation Coefficient, r	Working Range, (mg L <sup>-1</sup> )
Cu/liquid organic fertilizer	y = 6344.6x + 476.73	0.9999	1–10
Fe/liquid organic fertilizer	y = 7841.8x - 35.1	1	1–10
Mn/liquid organic fertilizer	y = 6891583.4x - 29969.5	0.9999	1–10
Zn/liquid organic fertilizer	y = 7139.1x + 4.67	0.9999	1–10
Mo/liquid organic fertilizer	y = 15.36x + 10.54	0.9999	0.01–0.3

The calibration was performed from six standard concentrations distributed equidistantly throughout the work area. Also, it can be observed that the value of the linear regression coefficient (correlation) criterion ( $r \ge 0.997$ ) is fulfilling for all tested parameters, according to the requirements for the method validation.

## 3.2. Detection and Quantification Limits

To establish the limit of detection and quantification, from eight to ten determinations of the samples from the analysed liquid fertilizer with plant micronutrients were performed. The samples were diluted to arrive at the lowest point of the calibration curve. The obtained results were used for the calculation of the limit of detection (LOD) and limit of quantification (LOQ) using the already presented formula (Equations (1) and (2)). The results are presented in Table 3.

Analyte	Average Samples, mg kg <sup>-1</sup>	Standard - Deviation, s <sub>r</sub>	LOD, mg kg $^{-1}$		LOQ, mg kg $^{-1}$	
			Determined	Acceptance Criteria	Determined	Acceptance Criteria
Fe	14.1	0.695	2.09	10	6.95	35
Zn	14.8	0.084	4.14	10	13.8	35
Mn	11.9	0.995	3.10	10	10.3	35
Cu	11.7	1.25	4.06	10	13.5	35
Мо	14.8	0.825	2.48	10	8.25	35

Table 3. LOD and LOQ of liquid organic fertilizer sample elements.

The criteria for detection and quantification limits of the used method were met for all micronutrients tested.

## 3.3. Repeatability

Eight and ten determinations were performed on the following samples at different concentrations to determine the repeatability. The samples were liquid organic fertilizer extracted in pure water according to the described method. The average of the results obtained, analyzed in triplicate, are presented in Table 4.

Table 4. Repeatability data for the liquid organic fertilizer sample.

Analyte	Average Liquid	Standard Deviation, sr		RSDr, %	
	Organic Fertilizer, % (w/w)	Determined	Horwitz Criteria	Determined	Horwitz Criteria
Fe	4.02	0.044	0.13	1.10	1.95
Mn	2.10	0.045	0.075	2.14	2.15
Zn	0.497	0.006	0.022	1.11	2.67
Cu	0.125	0.002	0.007	1.68	3.28
Мо	0.105	0.001	0.006	1.34	3.38

The method acceptance criterion for repeatability was met for all metals tested.

## 3.4. Accuracy (Recovery)

The obtained results are presented in Table 5.

Table 5. Evaluation of external reproducibility data for the liquid fertilizer sample.

Analyte	MR Concentration,	Measured	Recovery, % (w/w)		
	% (w/w)	Value, % (w/w)	Value	<b>Evaluation</b> Criteria	
Fe	0.0444	0.0435	97.9	$90 \le R \le 107$	
Mn	0.0423	0.0432	97.9	$90 \le R \le 107$	
Zn	0.0349	0.0367	95.2	$90 \le R \le 107$	
Cu	0.0103	0.0109	94.9	$90 \le R \le 107$	
Мо	0.00399	0.00412	96.8	$90 \le R \le 110$	

The experimental data for the recovery method were obtained by performing six to ten determinations on the following reference material: liquid mineral fertilizer with micronutrients. The acceptance criterion for recovery of the used method was met for all metals tested.

#### 3.5. Measurement Uncertainty

The significant sources of uncertainty that affect the measured concentration are the calibration curve, repeatability, accuracy of the method, and purity of the standards.

The acceptance criterion for uncertainty is met for all metals tested, and the obtained results are presented in Table 6.

Analyte	Sample Concentration, % (w/w)	Composed Uncertainty, u <sub>c</sub> , % (w/w)	The Extended Uncertainty, $U \le 2 \times u_c$ (k = 2, P = 95%), % (w/w)	Evaluation Criteria (Horwitz) $U \le 2 \times s_R$ % (w/w)
Fe	4.02	0.06	0.12	0.26
Mn	2.10	0.057	0.11	0.15
Zn	0.497	0.009	0.018	0.044
Cu	0.125	0.004	0.008	0.014
Мо	0.105	0.002	0.004	0.012

Table 6. Measurement uncertainties for the liquid organic fertilizer sample.

The optimized analytical method for the determination of Fe, Mn, Zn, Cu, and Mo by ICP-OES was applied to two fertilizer samples. The concentrations of micronutrients are shown in Tables 4 and 5. The concentration for the liquid organic fertilizer sample found for the micronutrients ranged from  $0.105 \pm 0.004\%$  (w/w) for Mo,  $0.125 \pm 0.008\%$  (w/w) for Cu,  $0.497 \pm 0.018\%$  (w/w) for Zn,  $2.10 \pm 0.11\%$  (w/w) for Mn to  $4.02 \pm 0.12\%$  (w/w) for Fe.

The repeatability data calculated as RSD (%) was less than 4%, so the liquid organic fertilizer sample ranged from 1.10% to 2.14%, which meets the acceptance criterion established based on Horvitz's equation [13].

The detection and quantification limits are given in Table 3. The sensitivity of the method proposed in this study is comparable to that of the methods reported in the literature [15]. According to the results obtained, the performance criteria imposed in this *"in-house"* validation study were met.

# 4. Conclusions

In this article, an analytical method for the micro(oligo)nutrient determination from inputs used in organic farming was developed. The correlation coefficients of the calibration curves obtained for the determined elements demonstrated a good linearity (r > 0.999). The precision, expressed as RSD, was lower than 4.0% for micronutrients (Fe, Mn, Zn, Cu, and Mo).

LOQ for liquid fertilizer ranged from 6.95 mg Kg<sup>-1</sup> for Fe to 13.8 mg Kg<sup>-1</sup> for Zn. Reference materials were used to determine the accuracy of the method developed in this study. Recoveries liquid fertilizer samples were in the range of 94.9–97.9%. Extended uncertainties with a probability of 95% (k = 2) for the liquid organic fertilizer sample were between 0.004% for Mo and 0.12% for Fe. The results obtained from this study have shown good accuracy and reproducibility.

The presented method meets the validation criteria for micro/oligoelements (Fe, Mn, Zn, Cu, and Mo) in liquid organic fertilizers and is suitable for their determination from inputs used in organic farming—(bi)fertilizers and biostimulants.

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