

DYNAMIC PREGL-DUMAS TECHNIQUE APPLIED IN NITROGEN DETERMINATION FROM INPUTS USED IN ORGANIC AGRICULTURE

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Abstract

Nitrogen is one of the most important component of fertilizers. Quantifying nitrogen, along with other elements, is an essential operation for calculating the efficiency of these inputs. Because Romanian regulations doesn't provide any methodology for determining nitrogen from inputs used in organic agriculture, each laboratory uses its own method for these determinations. This study presents a fast and efficient dry combustion method of determining the total nitrogen from organic inputs, using a CHNS elemental analyzer (Eurovector EA 3100). This Automated Pregl-Dumas technique is a very good alternative to the classic Kjeldahl method, which has several drawbacks: it does not quantify nitrogen from nitrates (although fertilizers contain a large amount of nitrogen in this form), needs a longer time for the sample to be analysed, it is not environmental friendly, and has lower accuracy of the results. To ensure the quality of the results, the necessary parameters for the validation of this method were calculated according to international guidelines, and the acceptance criteria of results were verified based on certified reference materials.

Key words: dry combustion, method validation, nitrogen, organic input.

INTRODUCTION

Ordinary laboratory determinations tend to become more and more automated, computer-coordinated, environmentally friendly, and with shorter execution times. Although old-fashioned methods of analysis are still widely used, technology is gaining ground and new devices are being developed which greatly facilitate the work of the operators.

Nitrogen determination is a routine analysis and a quick and accurate method is required (Beljkas et al., 2010). There are many ways to determine nitrogen, depending on the nature of the sample. Some of these methods are standardized and can be applied directly, with the certainty of suitable results. Most methods have been developed for the determination of protein in food and feed. Methods for determining soil nitrogen and fertilizers have also been developed for agriculture, but on a much smaller scale.

Two of the most widely used methods for determining nitrogen are based on principles discovered more than 100 years ago: Kjeldahl method (published in 1883) and Dumas method (published in 1831).

The limitations of both methods have been studied intensively and a lot of improvements

have been made to each of them over the past century (Sapan & Lundblad, 2015; Tomé et al., 2019; Sáez-Plaza et al., 2013). High precision and good reproducibility, along with the simplicity of use, have made the Kjeldahl method the standard method for comparison against all other methods. Starting with the 1990's, due to the development of technology (high purity gases, high precision detectors, high precision microbalances), the Dumas method gained recognition versus the traditional Kjeldahl method that was the dominating method for nitrogen and protein analysis for more than 100 years (Müller, 2017).

Some differences between Kjeldahl and Dumas methods are presented in the Table 1 (Mihaljev et al., 2015; Walter, 2018; Moore et al., 2010).

Due to the fact that Kjeldahl method does not quantify nitrogen from nitrates, the use of Dumas method is a suitable alternative for the analysis of fertilizers, which contain large amounts of nitrates. There are also some modified Kjeldahl method that can be used. There are a few official methods for total N determination in fertilizers recommended by International Fertilizer Industry Association (IFA), presented on Table 2.

Table 1. Comparison of Kjeldahl and Dumas methods

Kjeldahl	Dumas
Manual	Automatic
Time consuming (hours)	Fast (minutes)
Very dependent on the operator	Slightly dependent on the operator
Partial N determination (NOT NO ₃ ⁻ /NO ₂ ⁻ , azo)	All N components determination
Use toxic reagents	No toxic reagents
Low cost/sample	Low cost/sample
Low price equipment	Expensive equipment
Medium sample quantity required (1-5 grams)	Small sample quantity required (1-20 mg)
Precision influenced by operator and preparation	High precision provided by standards
Suitable for protein determination	Suitable for protein/total N in soil/fertilizers

Table 2. Official methods for total N determination in fertilizers

Standard	Method type
ISO 5315:1984	Kjeldahl
AOAC 978.02	Kjeldahl
AOAC 955.04	Kjeldahl
AOAC 993.13	Dumas

As the number of certified producers in organic agriculture is growing, the need of fertilizers testing is increasing as well. In order to keep their certification, they need to use fertilizers that respect regulation EC 2008/889 (Ion et al., 2021). As the above methods refer to fertilizers in general, the development of specific methods for inputs used in organic farming is a requirement.

The N determination using combustion method was successfully tested for the determination of proteins in food (VELP Scientifica, 2018; Cortes-Herrera et al., 2021) and soils (Matejovic, 1993). Regarding fertilizers, studies on the advantages and disadvantages of the combustion method began more than 50 years ago when Morris et al. (1969) made a comparison between the Kjeldahl method and the dry combustion method for conventional fertilizers. In 2016 Incorporated Administrative Agency, Food and Agricultural Materials Inspection Center (FAMIC) Japan, published a guide “Testing Methods for Fertilizers 2016” where an impressive number of different types of fertilizer samples were analyzed by several laboratories both by the Kjeldahl method and by the dry combustion method. The results showed

that both methods can be successfully used for the fertilizers analysis.

In order to be sure that a newly developed method can be used successfully, a series of steps are necessary to validate the method, to demonstrate that its performance characteristics are adequate for use for a particular purpose. According to EURACHEM Guide, these steps usually refer to precision (repeatability, reproducibility, precision limits) and accuracy/bias estimates (Magnusson & Örnemark, 2014). The aim of this paper is to develop and to validate a method based on automated Pregl-Dumas technique for the quantification of total nitrogen in organic fertilizers. This method can be also optimised for other type of organic fertilizers based on biomass waste material, soils and food.

MATERIALS AND METHODS

Chemicals and reagents

The combustion tube used by the elemental analyzer Eurovector EA 3100 at high temperature (950°C) oxidizes the samples in the presence of pure O₂ gas (Messer Oxigen 5.0, 99.999% purity). Helium (Messer He 6.00, 99.999% purity) gas is used as carrier and flushing gas.

Tin capsules (8 x 5 mm) were used for weighing and packing the samples and also to enhance the combustion. The samples were weighted in the tarred capsules and tweezers were used to close them to exclude atmospheric air. High purity acetanilide (Certif. no. B2061-1) from Elemental Microanalysis Ltd was used as calibration standard (N content % = 10.34%).

Samples

Three organic fertilizers and a standard reference material (SRM) were used as test matrices:

- Commercial liquid water-soluble fertilizer which contains trace elements chelated with citric acid and lignosulfonic acids - F1;
- Commercial semi-solid organic fertilizer obtained from soy and seaweed protein hydrolysis - F2;
- Commercial semi-solid organic fertilizer obtained from soy and seaweed protein hydrolysis fortified with micronutrients - F3;

- NIST® SRM® 695 (National Institute of Standards and Technology).

The samples were used without any treatments and were stored at room temperature during the analysis.

Basic principles

In the combustion process (furnace at 950-1100°C), C is converted to CO₂; H to H₂O; N to N gas/oxides of N and S to SO₂. If other elements such as Cl are present, they will also be converted to combustion products, such as HCl gas. To remove these additional combustion products, a variety of traps and absorbents are used, as well as some of the main elements, like water or sulphur, if the determination of these elements is not required.

The combustion products are carried through the combustion tube by a carrier gas (usually helium) and passed over heated high purity copper, situated at the base of the combustion tube. The function of this copper is to convert all forms of N to N₂, C to CO₂ and S to SO₂ gases and to remove any oxygen not consumed in the initial combustion. This copper can be situated at the base of the combustion chamber or in a separate furnace.

The gases are then passed through the absorbent traps in order to leave only carbon dioxide, water, nitrogen, and sulphur dioxide.

Detection of the gases are carried out by a GC separation followed by quantification using a thermal conductivity detector (TCD). Quantification of these elements need calibration for each element by using high purity standards such as cystine, acetanilide, benzoic acid, ethylenediaminetetraacetic acid (ETDA), etc. (AMC TB, 2008).

Elemental analysis instrument and parameters

The development and validation of the analytical method was performed using an automated CHNS elemental analyzer (Eurovector EA 3100) with the following accessories:

- Eurovector CHN combustion tube for 250 sample analysis of C, N and H;
- double autosampler (carousel) with 40+40 positions;
- Mettler Toledo microbalance (precision 0,000001 g);
- H₂O trap for water elimination;

- PTFE GC Column, outer diameter 8 mm, length 2 m;
- Weaver.NET 1.8.0.0 software.

The parameters used for method development are described in the Table 3.

Table 3. Method parameters for N determination using elemental analyzer Eurovector EA3100

Parameter	Unit	Value
Carrier Pressure	kPa	90
Reference Pressure	kPa	10
Furnace #1	°C	950
GC Oven	°C	90
Transfer Line	°C	100
Run Time	s	400
Sample Delay	s	6
O ₂ Volume	mL	20
O ₂ Injection Rate		slow

This analysis lasts for 400 seconds per sample and is fully automated.

Method validation

The following method-performance parameters were determined: linearity, accuracy, intra-day and inter-day precision.

Linearity

Linearity is recommended to be determined using a minimum of 6 standards whose concentration spans from 80% to 120% of expected concentration levels. Linearity report should include the slope, linear range and correlation coefficient data. Correlation coefficient must be greater or equal to 0.999 in the working range.

Calibration curve, made with acetanilide as a calibration standard, was constructed from six points and covered a wide range of nitrogen masses, corresponding from 0.5 to 2.5 mg of standard material.

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

Precision expresses closeness of a series of measurements of the same sample under identical conditions. A high precision does not necessarily mean high accuracy. Precision can be expressed as variance, standard deviation or as coefficient of variation of a series of measurements. Minimum of five replicate

sample determinations should be carried out for accurate results.

The precision of the method was evaluated based on the relative standard deviation (RSD) of the intra-day precision (RSD_I %) and the inter-day precision (RSD_R %).

For determination of repeatability precision, acetanilide (Microelemental Analysis), SRM 695 and three organic fertilizers was successively analyzed 10 times as 10 different sample weights in the same day, by one analyst. Intra-laboratory reproducibility precision was determined by analyzing the same sample in 10 replicates during 5 days by two different analysts.

Accuracy and BIAS

Accuracy indicates the closeness of the measured value to the true value. BIAS is determined as the difference between the mean obtained from a large number of replicate measurements with a sample having a reference value.

For determination of accuracy two standard materials were used. 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.

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

where:

\bar{x} = the mean of MRS samples

μ = "real" value from MRS certificate

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

Measurement Uncertainty

In order to assess the measurement uncertainty for each matrix, several sources were taken into consideration. From these sources, the following have been considered to contribute the most when combined uncertainty is calculated: uncertainty from weighing, uncertainty from method calibration data, uncertainty derived from inter-day repeatability on the matrix, and uncertainty from accuracy and bias tests.

RESULTS AND DISCUSSIONS

The software enables a real-time view of the analytical process and it can be used for running

the equipment, storing the data, and for post-run 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 nitrogen retention time is at 54 s.

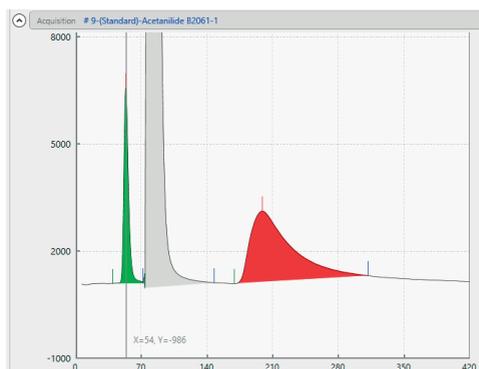


Figure 1. A typical chromatogram for CHNS analysis generated by Weaver.NET software (green peak - N; grey peak - Carbon; red peak - Hydrogen)

Linearity

Calibration curve, made with acetanilide as a calibration standard, was constructed from six points and a correlation coefficient $R^2 = 0.9999$ was obtained (Figure 2).

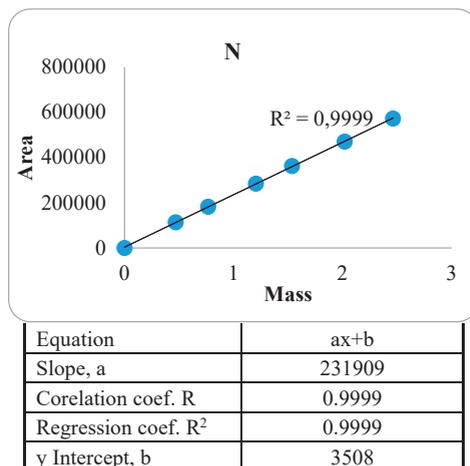


Figure 2. Calibration curve for N determination

Repeatability and reproducibility precision

The intra-day and inter-day precision was evaluated using calibration standard (acetanilide - Microelemental Analysis), SRM 695 and the above-mentioned three organic fertilizers (F1, F2, F3).

The results obtained for both standard materials and fertilizer samples are showed in Table 4.

Table 4. Intra-day and inter-day precision

Sample	Quantity used (mg)	Mean Conc (%)	RSD _r (%)	RSD _R (%)
Acetanilide	0.5-2.5	10.376	0.486	0.667
SRM 695	0.5-2.5	13.139	0.218	0.349
F1	2.5-7	0.213	3.744	11.641
F2	1-3.5	3.712	1.505	3.409
F3	0.5-3.5	3.439	2.316	4.879

It was observed that the method has good results for the sample quantities used above. 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 1%, from 0.218% for SRM 695 to 0.486% for Acetanilide, which indicated that the equipment method is highly repeatable. The RSD_R% (reproducibility precision) values for all three organic fertilizers were less than 20% which means a good precision of reproducibility according to EURACHEM Guide.

Except F1 which still falls within the limits imposed by the validation guidelines, the values are comparable with published values of relative repeatability and reproducibility standard deviations (RSD_r range 0.3-2.8% and RSD_R range 0.8-4.3%) for 12 different types of analyzed fertilizers (FAMIC, 2016).

Accuracy and Bias

For determination of accuracy, 10 samples of SRM (NIST 695) and 10 samples of acetanilide were successively analyzed. The obtained results were compared with the certificate value (Table 5).

Both standard materials obtained a good accuracy, in the interval of 94-101%, in

accordance with EURACHEM Guide (Magnusson & Örnemark, 2014), which recommends that the accuracy should fall within the range 70-110%. BIAS (trueness) also obtained good values, 0.35% for Acetanilide and -5.47% for SRM 695 (Table 5).

Table 5. Accuracy and BIAS of standard materials

Material	Acetanilide	SRM 695
Used quantity (mg)	0.5-3.0	0.5-3.5
Mean value	10.376	13.139
SD	0.087	0.029
Certificate value (%N)	10.34	13.9
Accuracy (%)	100.35	94.53
BIAS (%)	0.35	-5.47

Measurement Uncertainty

Good results were obtained for measurement performances when using Dumas method to determine the total nitrogen. As expected, the expanded uncertainty U_{ext} (%) is higher at lower concentrations, as seen in Table 6 (U_{ext} - 23.43% for a concentration of 0.213% N₂). The smallest uncertainty was obtained for the reference material (U_{ext} - 2.68%), which had the largest concentration and also the best sample homogeneity.

Table 6. Uncertainty results for matrix and reference materials

Sample	Mean Conc (%)	Ustd %	Uext %
SRM 695	13.139	1.34	2.68
F1	0.213	11.71	23.43
F2	3.712	3.65	7.29
F3	3.439	5.05	10.10

CONCLUSIONS

The method development highlighted good results for:

- linearity,
- accuracy,
- precision of repeatability,
- precision of reproducibility.

The method was successfully applied to organic fertilizers, obtaining fast results, in accordance with the certificates of used materials.

The method can be optimized for other type of samples, as soils, plants or food, after a proper preparation of them.

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