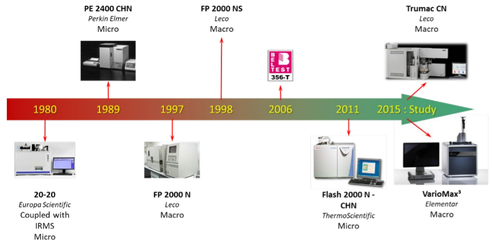
MACRO, MICRO? COMPARISON OF DUMAS ANALYSERS IN REAL CONDITIONS

Jean-Michel Romnee | CRAW, Walloon Agricultural Research Centre - Valorization of agricultural products, biomass and wood Unit | Gembloux, Belgium
Charles Ojeimi | ELEMENTAR France | Lyon, France
Alexis Sorokovsky | LECO France | Villepinte, France

Introduction

The comparison of two pieces of equipment allowing the determination of nitrogen and carbon using the Dumas method was carried out as part of an investment designed to supplement the means available in the laboratory. The micro-dumas installed (**Flash 2000®**, *ThermoScientific*) do not allow tests above 50 mg, which may limit the technique, particularly for samples with insufficient particle size (heterogeneous sample).



1. History of the Dumas method within the CRAW

The Walloon Agricultural Research Centre is required to determine the organic or total nitrogen content in numerous matrices related to agronomy (soils, plants, agricultural products, human and animal food, fertilisers, fermentation products, etc.). His experience with the Dumas method goes back more than forty years, with the dosage of nitrogen 15, used for tracing fertilisers (Figure 1).

Goal of the study

This study aims to objectify the quality of the two equipment (**TruMac CN®**, *LECO* – **VarioMax³®**, *ELEMENTAR*) likely to meet the specifications and thus choose the equipment that best meets the needs of the laboratory. *Skalar Analytique* preferred to decline the offer to participate in this comparison with the **PrimacsSNC-100®**. To evaluate the improvement of the laboratory analysis scheme, the results of the **TruMac CN®** and of the **VarioMax³®** were compared with the results obtained using **Flash 2000®** (*ThermoScientific*), accredited according to the ISO17025 standard for the determination of the nitrogen content in cereals, cereal products and in food and feed.

Techniques implemented

The determination of the nitrogen content in gaseous effluents resulting from the combustion of samples is based on three principles.

The simplest principle corresponds to determination by gas chromatography (**Flash 2000®** – *ThermoScientific*) in which all of the gas effluent is analysed through a catharometer, after chromatographic separation of the various gases to be analysed. In this analysis, no special precautions are taken: the sample is burnt and all of the nitrogen in gaseous form (N_2) is determined. It is therefore possible to relate this quantity to the test sample and to determine a concentration in the sample. However, this implies limiting the number of tests in order to maintain good chromatography.

The second approach is based on the principle of "purge and trap" (**VarioMax³®** – *ELEMENTAR*). In this case, the combustion gases are selectively trapped on molecular sieves and released successively to the catharometer. In this approach too, all the gases emitted pass through the detector.

In the third detection mode, only an aliquot of the gases produced during combustion is analysed (**TruMac CN®** – *LECO*). Analysing only an aliquot of gas increases the size of the test portion and reduces the amount of reducing agent per sample. However, the very principle of aliquoting requires collecting a sufficient quantity of gas to be homogenized in the ballast so that the aliquot is representative of all the gases produced.

Experimental comparison protocol

In order to overcome the lack of control of equipment by laboratory staff, the responsibility for analyses has been delegated to the application laboratories (*ELEMENTAR* and *LECO*). The samples were conditioned for direct analysis (grinding and homogenization carried out at Gembloux before sending the samples).

About twenty samples were selected from the stock available to the laboratory. The selected solid samples (18) (cereals, animal feed, protein crops, fodder, soils) were ground on a mill complying with the particle sizes required in the standards used for the determination of the nitrogen content in cereals and in animal feed (ISO 16634-1 and ISO/TS 16634-2). The last two samples are liquid samples (milk and beer).

The samples were then divided into two series of vials of +/- 10 g (per sample) and were randomly identified (CRAW-01 to CRAW-40). The CRAW-21 and CRAW-28 (dehydrated alfalfa) samples were limited to approximately 5 g per vial, due to quantity available. The CRAW-06 and CRAW-16 samples (extruded soy-beans) were repackaged under vacuum in an opaque aluminium bag to protect them from any changes (oxidation, in particular).

EIL BIPEA	Nature	Nitrogen content (%) mean ± 2·σ	EIL BIPEA	Nature	Nitrogen content (%) mean ± 2·σ
2014-06	Straw	0,566 ± 0,080	2014-05	Dehydrated alfalfa	2,904 ± 0,146
2014-02	Dactyl hay	1,040 ± 0,080	2014-04	Colza	3,024 ± 0,096
2014-10	Corn	1,184 ± 0,064	2014-06	Pea	3,072 ± 0,096
2014-04	Alveograph flour	1,468 ± 0,040	2014-02	Feed for turkey	3,856 ± 0,112
2014-09	Alveograph flour	1,613 ± 0,046	2014-05	Dog feed	4,736 ± 0,144
2014-05	Alveograph flour	1,797 ± 0,051	2014-12	Extruded soya beans	5,856 ± 0,176
2014-01	Alveograph flour	2,069 ± 0,058	2014-04	N corrector with urea	6,976 ± 0,208
2014-11	Feed for laying hens	2,640 ± 0,080	2014-11	Fish meal	11,024 ± 0,336
	Milk	0.550		Soil 01	0.060
	Beer	1.120		Soil 02	1.180

Table 1: List of samples studied in the context of the comparison study

The BIPEA results for alveograph flours are expressed at 100% dry matter. To avoid determining the dry matter in the Application labs (the determination of the dry matter on flour requires a special oven that is only found in the laboratories concerned with the analyses of cereals), the nitrogen contents have been reduced to an expression on fresh matter, like the other samples.

These samples cover a particularly wide range of nitrogen contents and have different natures (cereals, animal feed, protein crops, food supplement, soil, fodder, etc.). For BIPEA samples, the nitrogen content, the result of an inter-laboratory test (together with its standard deviation) is considered as the target value to be reached. For the four samples from CRAW (milk, beer and soils), the reference value was calculated by averaging the 8 measurements for the sample (double blind * two days * two laboratories). The series of forty samples was analysed twice (two different days) under reproducibility conditions.

Results

As part of the study carried out, not all the parameters used for the validation of analytical methods will be studied. Only the following parameters were analysed: trueness, precision (repeatability, short-term repeatability and reproducibility) and robustness, with reference to the standards relating to the different matrices analysed: **ISO 16634-1:2009** (Food products – Oilseeds and animal feeding stuffs) – **ISO 16634-2:2016** (Food products – Cereals, pulses and milled cereal products) – **ISO 14891:2002** (Milk and milk products) – **NBN EN 16168 (2012)** (Sludge, treated bio-waste and soil) – **EBC 9.9.2. (1999)** (Total Nitrogen in Beer: Dumas Combustion Method).

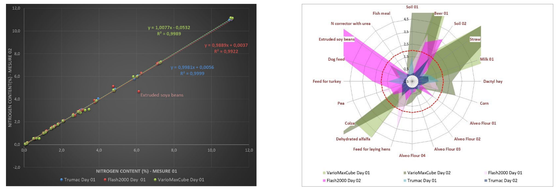
Repeatability

The repeatability was evaluated from the doubles (results obtained in double blind) on days 01 and 02. The difference between double was compared for each sample, with the corresponding standard (when it was available) and the relation between both measurements was characterized for both days and for all data.

		Flash 2000®	TruMac CN®	VarioMax³®
Day 01	Respect for limits	16 / 17	17 / 17	14 / 17
	Regression (M1;M2)	M2 = 1,005 M1 – 0,0257	M2 = 1,0047 M1 – 0,0067	M2 = 1,0034 M1 – 0,0555
	R^2	0,9997	0,9999	0,9991
Day 02	Respect for limits	16 / 17	17 / 17	15 / 17
	Regression (M1;M2)	M2 = 0,9728 M1 + 0,0332	M2 = 1,0003 M1 – 4E-5	M2 = 1,012 M1 – 0,051
	R^2	0,9848	1,0000	0,9988
Global	Respect for limits	32 / 34	34 / 34	29 / 34
	Regression (M1;M2)	M2 = 0,9889 M1 + 0,0037	M2 = 0,9981 M1 + 0,0056	M2 = 1,0077 M1 – 0,0532
	R^2	0,9922	0,9999	0,9989

Table 2: Summary of the "Repeatability" comparison between the three devices

Table 2 presents a summary of the comparison for the parameter "Repeatability". Of the three devices compared, only the **TruMac CN®** gave daily results that met all repeatability standards. The R^2 obtained for the **TruMac CN®** relations are higher, without being different from the others. The **VarioMax³®** encountered problems when determining the nitrogen content in three different samples (Milk, Dehydrated alfalfa and Colza). For the **Flash 2000®**, the problems were at the level of feed for poultry (hens and turkeys).



2. Repeatability: example of Day 01 and coefficient of variation measured for each pair of samples

The coefficient of variation, calculated for each pair of results over the two days, completes the information on repeatability. Thus, only the **TruMac CN®**, for all of its results (Figure 2), respects an arbitrary limit of 2% for the coefficient of variation.

The results presented in Figure 1 clearly show that the three equipment are particularly well suited to determining the protein content in cereals since the variation coefficients are much less than one percent for this type of matrix. They deteriorate once the matrix becomes more complex and becomes more difficult to grind (fatty food, for example). This matrix-test socket combination can lead to very high coefficients of variation for **Flash 2000®**.

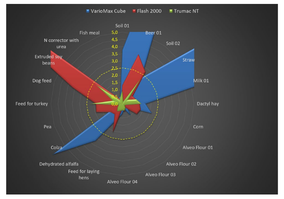
Intermediate precision

Intermediate precision is evaluated by comparing the results obtained for the same sample on the two days of analysis. The first approach lies in the relation between these two values (linear regression) and the second in the comparison between the difference measured for a double and the reproducibility limits defined in the standards (function of the matrix) (Table 3).

	Flash 2000®	TruMac CN®	VarioMax³®
Respect limits	34 / 34	33 / 34	33 / 34
Equation $D_2 = f(D_1)$	D_2 = 0,98 D_1 + 0,018	D_2 = 0,9993 D_1 + 0,0011	D_2 = 1,0005 D_1 – 0,0065
R^2	0,9905	0,9994	0,9992

Table 3: Summary of the parameter "Intermediate precision"

A complementary approach to reproducibility is based on the calculation of the coefficients of variation calculated not on the duplicates but on the samples, or by integrating the four measurements (doubles from day 01 and doubles from day 02). Figure 3 shows that the **TruMac CN®** exhibits coefficients of variation of less than 2.5% for all samples. The results obtained for straw and rapeseed lead to CVs (%) greater than 5% for the **VarioMax³®**.



*3. Intermediate precision: Coefficients of variation calculated per sample (2 days * 2 measurements)*

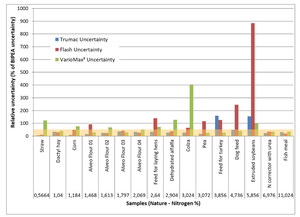
Accuracy

For each of the samples from the BIPEA, the laboratory has a reference value with an uncertainty. These two elements were incorporated into the trueness assessment. Table 4 provides the regression equations calculated on the results obtained for each of the techniques.

Flash 2000®	TruMac CN®	VarioMax³®
Lab = 1.0101 BIPEA – 0.103	Lab = 1.0082 BIPEA + 0.003	Lab = 1.0199 BIPEA – 0.0414
R^2 = 0,9993	R^2 = 0,9991	R^2 = 0,9989

Table 4: Summary of the parameter "Intermediate precision"

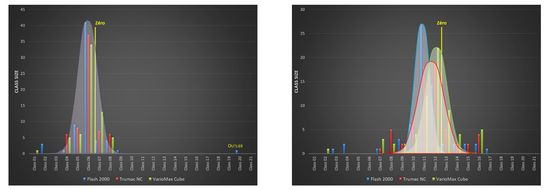
The three devices monitored show an excellent relationship between the values obtained in the laboratory and the BIPEA reference values. These relationships can be completed by observing the relative uncertainties (Lab uncertainty related to the BIPEA uncertainty, in %, Figure 4).



4. Relative uncertainty (Lab uncertainty referred to the BIPEA uncertainty, expressed in %)

Figure 4 shows that, although the regressions are excellent for the three series of samples, the relative uncertainties are greater than 100% for 7 samples for the **Flash 2000®** and for the **VarioMax³®**, while the overshoot is only found for 2 samples for the **TruMac CN®**.

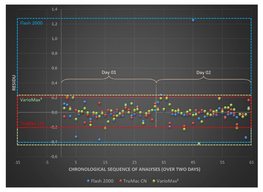
The accuracy of the results obtained for each piece of equipment was supplemented by an evaluation of the distribution of residuals. These are defined as the difference between the Lab value, estimated from the BIPEA, and the measured Lab value. The relationship defined between the lab values and the BIPEA values makes it possible to calculate the estimated Lab value. The distribution of the residuals must have the appearance of a Gaussian curve, centred at zero, which is found in Figure 5, for the three equipment studied.



5. Distribution of residuals (left : 21 classes with amplitude of 0.09, from -0.5 – right : 21 classes with amplitude of 0.045, from -0.5, with elimination of the "Outlier" value)

All three distributions appear centred on the same maximum when the extreme value (**Flash 2000®**) is taken into account. The average of the residuals is equal to 0.0001 (**TruMac CN®** and **VarioMax³®**) and -0.0001 (**Flash 2000®**). Dividing the class amplitude by two reveals some differences for the class with the maximum number of students and for the spread of the distribution (width at the base).

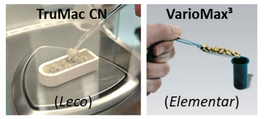
The chronological distribution of the residuals over the two days that the analyses were performed (Figure 6) effectively shows that the residuals range of the **TruMac CN®** is well centred on zero and has the lowest amplitude. Both the **Flash 2000®** and the **VarioMax³®** would have the same distribution if there were only a few samples that were largely out of range. The central area of the samples clearly shows the accuracy of the flour analysis, with very low residues. The amplitude of the residues increases with the type of sample (fat, heterogeneous grinding).



6: Chronological distribution of residuals

Implementation of the analysis

The practicality of the analysis was also assessed during the study. Weighing and cup filling, which is simple for one or two samples, proved to be less cumbersome for the **TruMac CN®** than for the **VarioMax³®**. This is because the filling of the basket is done in a more natural motion for the **TruMac CN®** (Figure 7). The preparation of the nickel foils for the **Flash 2000®** was not taken into account, even though this step is particularly "time-consuming".



7: Ease of filling the buckets

Conclusion

The objective of this study was to compare two macro-dumas equipment (**TruMac CN®**, *LECO* – **VarioMax³®**, *ELEMENTAR*) allowing the determination of the nitrogen content in different agricultural and agro-food matrices according to the Dumas method, using test samples of the order of 500 mg, in order to complete the micro-dumas equipment available in the laboratory.

The study involved the analysis of 40 samples (20 in double blind) repeated two days in a row. Sixteen samples came from the BIPEA sample stock (for which the laboratory has a target value and uncertainty), to which were added two soils, one milk and one beer. The application laboratories of the two firms concerned carried out the analyses and sent the results to CRAW, which analysed the data.

The study showed that, despite being ISO17025 accredited, the **Flash 2000®** (*Interscience*, micro-dumas) showed greater variability in the results than the two pieces of equipment studied, which can easily be explained by the reduced size of the test sample (maximum 50 mg compared to 500 mg).

The differences between **TruMac CN®** and **VarioMax³®** were particularly marked for the more specific samples (high fat content, difficult to grind, heterogeneous matrix). The performance of TruMac CN was better for these samples, whereas for easy to prepare samples (e. g. flour) both machines gave equivalent results.

While the regressions obtained between Lab and BIPEA values are excellent, the relative uncertainties are better for the **TruMac CN®** for which only two samples have a relative uncertainty greater than 100% (BIPEA Uncertainty) while seven samples exceed this threshold for the **Flash 2000®** and **VarioMax³®**.