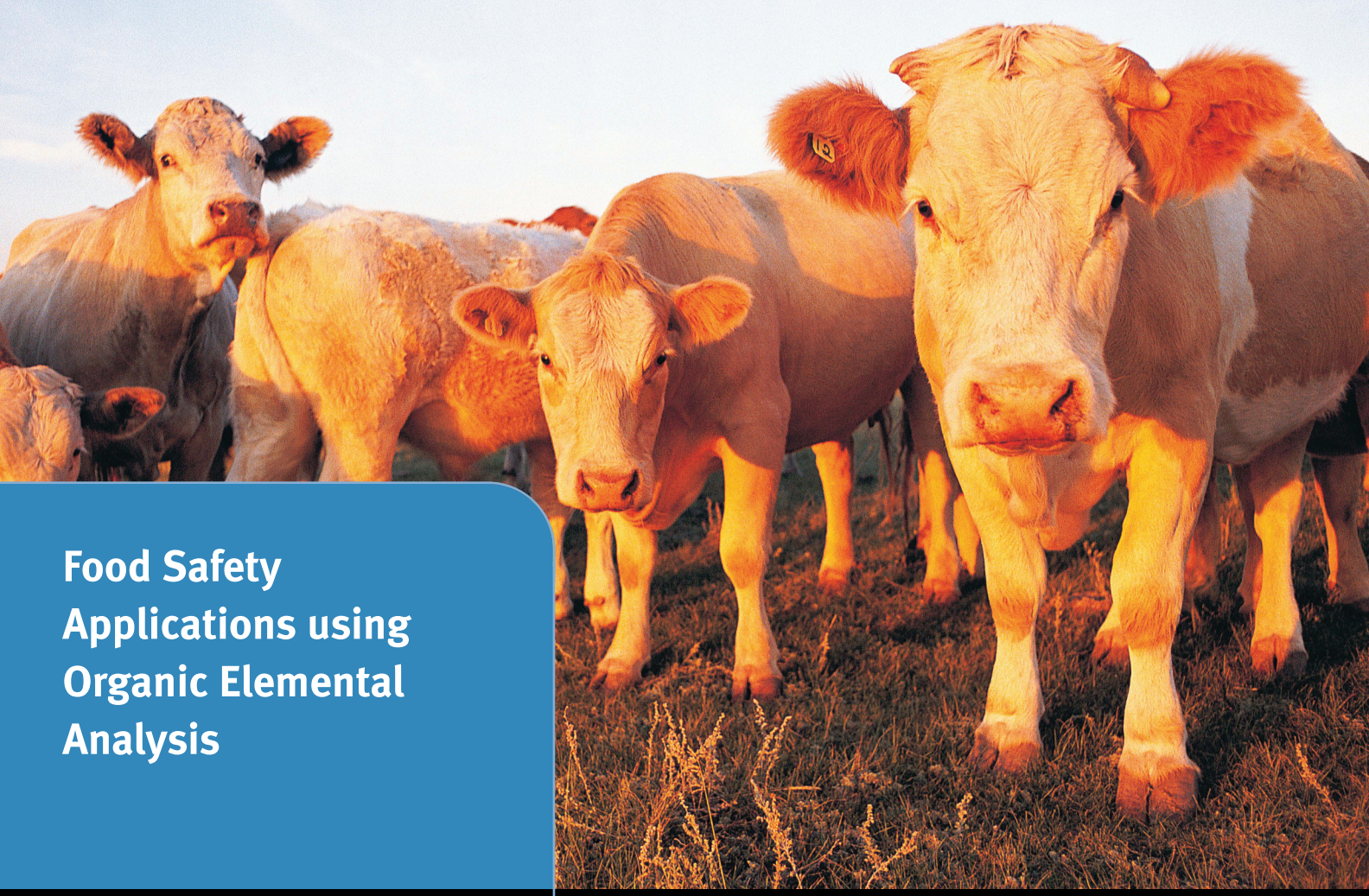

Food Safety Applications using Organic Elemental Analysis

Thermo Scientific FLASH CHNSO Analyzers

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Welcome to the food safety application pack for Organic Elemental Analysis (OEA)

Over the past few years, food safety has become an increasingly important topic in the world of consumer safety. As scientific understanding and consumer awareness increases, more and more stringent testing is required for foodstuffs, particularly relating to toxic contamination (for example from over fertilization), quality and nutritional value. Determination of nitrogen and protein content is an accurate and precise measure of these components across a wide range of sample types including soils, animal feed, and final product testing for human consumption.

This information pack has been compiled to present a range of food safety applications, demonstrating how organic elemental analysis using the dynamic flash combustion technique offers a faster, safer and more reliable alternative to the traditional Kjeldahl method for the determination of nitrogen and protein content in food. The pack also includes information on our Thermo Scientific FLASH range of OEA products to facilitate automated, accurate and high throughput analysis.

Macro or Micro Organic Elemental Analysis?

Thermo Scientific organic elemental analyzers are available for both macro and micro analytical methodologies.

The **Thermo Scientific FLASH 4000** Analyzer has been designed to determine Nitrogen in larger sample weights, up to 2 g (2000mg). This is the same sample weight used by the traditional Kjeldahl method.

The **Thermo Scientific FLASH 2000** Analyzer offers micro analysis for CHNS, CHN, Oxygen or Sulfur determination when introducing a low sample size (just a few mg). With an optional upgrade, it can also be used for N/Protein and Nitrogen-only determinations, with a sample amount between 100 – 500 mg.



CASE STUDY

Gruppo CSA SpA Research Institute Selects Thermo Scientific FLASH 4000 Nitrogen/Protein Analyzer to Expand its Services and Ensure High Sample Throughput

Gruppo CSA, a leading Italian environmental research institute required an instrument with the capacity to efficiently and accurately analyze nitrogen/protein in animal feed and in human food samples.

The Thermo Scientific FLASH 4000 organic elemental analyzer (OEA) was selected by the organization due to its unique ability to conduct nitrogen/protein analysis while also providing optimal accuracy, increased sample capacity and high sample throughput.

Introduction

The Research Institute - Gruppo CSA S.p.A. was founded in 1985 by a group of self-professed 'young and enthusiastic' researchers, with the intent of creating an institute that nurtured the innovative development of environmental analyses in a multidisciplinary way. Located in Rimini, Italy, the laboratory processes 70,000 samples and determines over 800,000 parameters every year. Divisions within Gruppo CSA cover a broad range of areas including environmental sampling, physical analyses, industrial hygiene, food hygiene, water analyses, research and development and agriculture. The institute is accredited according to the standard UNI CEI EN ISO/IEC 9001:2008 and UNI CEI EN ISO/IEC 14001: 2004 for chemical analysis and environmental services, certifying the quality of both the methods used and the data obtained within the institute laboratories.

Working in collaboration with multiple university research institutes and private and public laboratories, CSA offers an innovative and professional service to a diverse customer base.

The Challenge

The institute's food laboratory division performs a broad range of analyses including nutritional evaluation, multi-residual pesticide analysis and the analysis of protein within animal feed and human food samples. Protein is one of the most important nutrients when determining the nutritional quality of a product and is typically calculated through the determination of nitrogen. When determining the quantity of protein in a product, Gruppo CSA must adhere to the AOAC method 992.23 which indicates that the suitable fineness of grind must be determined to achieve precision that gives RDS of ≤2% for 10 successive determinations of nitrogen.

On an ongoing basis the company is required to conduct nitrogen/protein analysis on alfalfa, a common hay crop primarily used as live stock feed due to its high protein content. A recent initiative established by The Commission of the European Communities provides a financial incentive for farmers producing dehydrated alfalfa with levels of protein above 15%. As a result, Gruppo CSA receives a particularly high demand for the rapid analysis of nitrogen/protein in high quantities of dehydrated alfalfa.Since the company's inception, the laboratory has utilized the traditional Kjeldahl method for protein analysis. However, this method suffers from a number of challenges. One of the key drawbacks for Gruppo CSA is that the Kjeldahl method is extremely time consuming, requiring over four hours to complete a single analytical cycle while also being limited to a maximum of just 20 samples per cycle. This is due to the various time consuming stages involved in the process, including sample digestion in boiling sulfuric acid, neutralization with sodium hydroxide solution, distillation of the resulting ammonia gas into a trapping solution, titration with an acid solution and determination of nitrogen/protein content by calculation.

Dr. Pierpaolo Tentoni, chairman of Gruppo CSA comments: "Although the Kjeldahl method has been modified and improved since its initial development, the method still requires the use of acids at extremely high temperatures. This can have a corrosive effect on our instrumentation alongside posing a serious health and safety threat to our technicians." Additionally, the Kjeldahl method suffers from issues of toxic waste generation due to the deployment of mercury or selenium as catalysts for digestion. As a result, a chimney is required within the laboratory to remove the waste, which incurs a high expense and raises the problem of pollution excreted into the environment. Due to these factors, the Kjeldahl method cannot be operated continuously which reduces sample throughput, and as a consequence drives up the cost of these services for the institute.

To overcome these challenges the laboratory required a solution that would increase overall productivity and cost-efficiency, while also improving health and safety conditions and remaining compliant with existing industry regulations. In order to achieve its goals, Gruppo CSA began to explore alternative protein analysis techniques and instrumentation.

Implementation

Gruppo CSA has recently implemented the Thermo Scientific FLASH 4000 nitrogen/protein analyzer for the determination and quantification of protein in dehydrated alfalfa and other forms of animal feed. The analyzer has been designed to work with the Dumas combustion method for protein analysis which is recognized by the Association of Official Analytical Chemists (AOAC) and the American Oil Chemist Society (AOCS) as a valid alternative to the Kjeldahl method.

Prior to the purchase of the FLASH 4000 nitrogen/protein analyzer, Gruppo CSA had extensive experience with Thermo Scientific instruments, which instilled confidence in their new selection. Dr. Tentoni comments: "Before selecting the Thermo Scientific FLASH 4000 nitrogen/protein analyzer, we spent time comparing competitive solutions. After conducting a comprehensive evaluation of the FLASH 4000 OEA it became clear that the analyzer is unmatched in terms of capacity for accuracy, sample throughput and sheer efficiency when facilitating the Dumas technique. Our decision was also informed by the institute's positive experiences with a range of other Thermo Scientific instrumentation, including an OEA unit which was purchased eight years ago and continues to be an efficient and valuable tool in the laboratory."

Key Benefits

Since the implementation of the new analyzer, Gruppo CSA has experienced significant benefits for its protein analyses. Previously when using the Kjeldahl method, the laboratory was limited to a batch of 20 samples per cycle. The FLASH 4000 OEA now enables the team to process up to 50 samples in a single cycle. In addition, the four hour cycle time has been dramatically reduced to just 10 minutes, increasing throughput by over 50%. It is estimated that the laboratory will now be able to process over 7,000 samples each year.

Dr. Tentoni comments: "In contrast to the Kjeldahl method which requires constant monitoring by our technicians, the FLASH 4000 analyzer requires no human presence. As a result, analytical cycles can be left to run overnight and unattended, saving technician time and increasing overall throughput. Due to the limited sample capacity and the high cost associated with the Kjedlahl method, we were previously unable to analyze animal feed samples for farmers and producers, who required large quantities of samples to be analyzed at low cost. With the high sample capacity and cost-efficiency of the FLASH 4000 nitrogen/protein analyzer, for the first time ever we have been able to provide animal feed analysis as a key service."

An additional benefit of the FLASH 4000 OEA is the elimination of aggressive mixtures such as base acid and fumes. Dr. Tentoni says: "The new instrument does not require the use of corrosive acids, making it much safer for our laboratory technicians and more environmentally friendly. Furthermore, the removal of the acids and toxic reagents has meant that the instrument is much easier to clean and maintain, particularly with the inclusion of the self-cleaning filter, saving both time and money."

Conclusion

To meet its objectives Gruppo CSA needed an alternative to the Kjeldahl method for protein analysis that would improve overall productivity within the laboratory while also ensuring the safety of the institute's technicians and reducing overall costs. The institute also required a method that would enable the team to provide cost-effective animal feed analysis as a service, particularly with complex matrices such as dehydrated alfalfa which is currently in high demand within the agricultural community.

Prior to the purchase of the FLASH 4000 nitrogen/protein analyzer, Gruppo CSA was unable to provide nitrogen/protein analysis of animal feed as a service due to sample throughput being limited to just 20 samples per cycle. Since implementing the new analyzer, the laboratory's throughput has doubled enabling the institute to expand its services and customer base significantly. In addition, with the removal of dangerous and corrosive acids used for the Kjeldahl method, the health and safety conditions within the laboratory have improved.

Dr. Tentoni comments: "We selected the Thermo Scientific FLASH 4000 nitrogen/protein analyzer based on its capacity to work with the Dumas method. Since its deployment we have been able to expand our services and significantly increase the speed and quantity of samples being processed, while also improving the precision and accuracy of our results. Our decision to implement the FLASH 4000 analyzer was not only based on the unique merits of the instrument and the impressive reputation of the company, but on our experience of the excellent customer service provided by Thermo Fisher Scientific."

Product Specifications

Thermo Scientific FLASH 4000 Nitrogen/Protein Analyzer - Technical Specifications

FLASH 4000 BASE UNIT

Dimensions	667 x 635 x 695 mm (w x d x h) including MAS 4000 autosampler
Power supply	230 V ; 9,3 A max ; 2140 watt ; 50 / 60 Hz
Weight	93 kg (net value)

MULTI AUTOSAMPLER MAS 4000

The MAS 4000 can accommodate the samples in a single tray, of solid or liquids (supported on solid inert material) placed in tin containers

First tray capacity	31 samples
Max capacity (4 drums of 31 positions)	124 samples

UTILITIES

Gases	Helium GC grade (99.995 % minimum purity) Oxygen GC grade (99.995 % minimum purity)
He flow	300 ml/min (measurement channel) / 20 – 500 ml/min flow range 300 ml/min (reference channel) / 20 – 500 ml/min flow range 250 - 300 ml/min (Regenerative CO_2 filters)
Helium gas pressure	100 – 150 kPa
Oxygen gas pressure	450 kPa
Gas connectors	1/8" Swagelok
Important: in Stand-By condition the Helium flow is brought to 20 ml/min , the Oxygen flow is cut off while the temperatures of the Left and Right furnaces are reduced by 50 % versus operating temperatures	
Furnace voltage	48 V
Furnace temperature sensor	Pt / Pt-Rh thermocouple
Furnace temperature	max 1050 °C (2008 °F)

ENVIRONMENTAL CONDITIONS:

Operating temperatures	15 – 35 °C (54 – 95 °F)
Maximum relative humidity between 5 % and 95 %	
Voltage variations not exceeding +/- 10 % of the nominal value	

TECHNICAL FEATURES

Detector	Thermal Conductivity Detector (TCD)
External interface	RS 232 serial line, Local Area Network (LAN)
Instrumental control	Eager Xperience for Windows / Vista

OPTIONS

AI 3000 or AS 3000 liquid autosampler
Manual liquid injector

Analytical specifications

Sample matrix	Solid and Liquid
Sample size	0.100 – 2 g or more (depending on the sample nature)
Working range	100 ppm – 100 %
Calibration	K factors or Linear Regression
Analysis time	5 - 7 min (depending on the sample nature)

ACCURACY FOR N DETERMINATION

Theoretical Value	Experimental Value
0.01 % (100 ppm)	100 ppm ± 10
0.10 %	0.1 % ± 0.01
1.00 %	1.00 % ± 0.02
10.00 %	10.00 % ± 0.1
50.00 %	50.00 % ± 0.3
90.00 %	90.00 % ± 0.3

Conformance tested by pure organic elemental analysis standard.

Official methods include:

AACC (American Association of Cereal Chemist) 46 -30;

AOAC (Association of Official Analytical Chemists) 990.03;

AOAC 992.15,

AOAC 992.23,

AOAC 993.13;

AOCS (American Oil Chemists Society) Ba 4e;

ASBC (American Society of Brewing Chemists)

ISO 13878, Soil Quality - Det. Of Total Nitrogen.

Gazzetta Ufficiale, New regulation for fertilizer's control

Office International de la Vigne et du Vin, OENO 13 Quantification of Total Nitrogen

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Application Note: 43029

The Thermo Scientific FLASH 4000 N/Protein Analyzer: Performance Through Food Reference Materials Analysis

Dr. Liliana Krotz, Product Specialist, OEA and Dr. Guido Giazzi, Product Manager, OEA, Thermo Fisher Scientific, Milan, Italy

Key Words

- Flash Combustion
- N / Protein
- Accuracy

Introduction

The globalization of the food market has increased the demand for reliable controls in order to determine the safety and quality of produce. It is for this reason that the accurate and precise determination of protein content has become very important to ensure the commercial quality and the nutritional value of the produce. In addition, analyzing for protein will assist in determining the condition of denaturation and degradation. Therefore the accurate and precise determination of protein content has become critical to ensure commercial quality and nutritional value. The use of an accurate instrumental analytical technique is required in order to avoid the use of toxic chemicals.

An alternative to the classical Kjeldahl method, based on Dumas (combustion) method has been developed and approved by a number of governing associations.

With the increased demand for improved sample throughput, a reduction operational costs and the minimization of human error, it is very important to have a simple and automatic technique which allows fast analysis combined with excellent reproducibility.

The Thermo Scientific FLASH 4000 Elemental Analyzer is based on the dynamic combustion of the sample and therefore requires no sample digestion or toxic chemicals. In addition, the FLASH 4000 enables significant advantages, including time-saving automation as well as the quantitative determination of Nitrogen in a large concentration range. The correct amount of Oxygen, correlated to the weight and type of sample, is determined automatically by OxyTune® assuring complete combustion of the sample.

Analytical Configuration

The sample is weighed in a Tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 autosampler together with the correct amount of Oxygen by OxyTune® function (ensuring the complete combustion of the sample).

After combustion, the produced gases are carried by a Helium flow to a second reactor filled with Copper. The water is trapped through a Water Condensation Drainage Device, while the CO_2 is adsorbed by the NoStop Twin Traps. Then the Nitrogen passes through a GC column and is detected by a thermal conductivity detection device (see Figure 2).

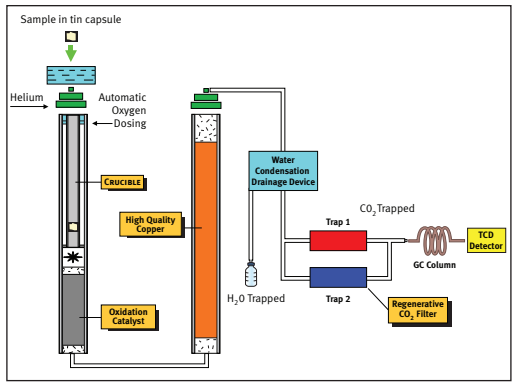

Figure.2: Flash 4000 Configuration Layout

Analytical conditions

T left tube: 950 °C
T right tube: 840 °C
T oven: 50 °C

Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min

Standard: 500 mg EDTA (9.59 %N)
EDTA: EthyleneDiamineTetraAcetic acid

Note: *The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.*

The Thermo Scientific FLASH 4000 Performance Evaluation by Reference Materials

The accuracy and precision of the FLASH 4000 was evaluated through the analysis of Reference Materials. The results obtained were compared with the average and range indicated in the relative Reference Materials Certificates, which include the Kjeldahl and Combustion data.

Results

The samples analyzed were selected on the basis of their differing nature and Nitrogen content. This ensures that the amount of Oxygen and combustion required is completely different. The calibration of the system was performed with EDTA (9.59 %N) using K factor as calibration method and the samples were analyzed exactly as they were received from the Reference Materials suppliers.

Table 1 and 2 show the sample information and the reproducibility of 10 consecutive analysis of Bipea Reference Materials (Bureau InterProfessionnel d'Etudes Analytiques, France) using a sample weight of about 1000 mg. The materials were characterized through a laboratory intercomparison using Kjeldahl and Combustion methods. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.

Table 1 – Bipea sample information available

Sample	Moisture %	Fat %	Carbohydrate %	Kjeldahl Protein Av. %	Kjeldahl Protein Tolerance	Combustion Protein Av. %	Combustion Protein Tolerance
Bipea - Feed for Sow 3/2009	9.8	2.8	48.7	16.0	0.6	16.2	0.6
Bipea - Dehydrated Alfalfa 3/2009	7.7		29.3	14.8	0.6	15.1	0.6
Bipea - Hyperproteic Powder 1/2008		0.8		85.4	3.4	86.4	3.5

Table 2 – Reproducibility of Nitrogen / Protein determination in Bipea Reference Materials

Sample %	Bipea – Feed for Sow N %	Bipea – Feed for Sow Protein %	Bipea – Dehydrated Alfalfa N %	Bipea – Dehydrated Alfalfa Protein %	Bipea - Hyperproteic Powder N %	Bipea - Hyperproteic Powder Protein %
	2.65	16.54	2.42	15.14	13.49	84.33
	2.63	16.47	2.46	15.36	13.46	84.12
	2.64	16.50	2.44	15.22	13.47	84.18
	2.61	16.32	2.43	15.20	13.48	84.26
	2.67	16.66	2.48	15.52	13.55	84.69
	2.69	16.78	2.47	15.41	13.51	84.41
	2.66	16.63	2.48	15.53	13.48	84.28
	2.65	16.56	2.47	15.44	13.45	84.08
	2.64	16.51	2.47	15.43	13.45	84.08
	2.67	16.68	2.48	15.50	13.46	84.15
Average %	2.65	16.56	2.46	15.38	13.48	84.25
RSD %	0.86	0.78	0.90	0.92	0.23	0.23

Table 3 and 4 show the sample information and the reproducibility of Nitrogen and Protein determination in Meat Reference Materials (SMRD 2000) using a sample weight of about **1000 mg**. This Material consists of a mixture of lean pork, water, potato flour and nitrate salt properly homogeneized. It was characterized through a laboratory intercomparison ("*certification tria*") coordinated by the National Food Administration, Uppsala (Sweden) in which 22 labs participate for Nitrogen determination using Kjeldahl and Combustion methods. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.

Table 3 – SMRD 2000 sample information available

Sample	Moisture %	Fat %	Nitrogen % Average	Nitrogen % Uncertainty
Meat SMRD 2000	68.8	14.3	1.63	1.57 – 1.69

Table 4 – Reproducibility of Nitrogen / Protein determination in Meat Reference Materials

Weight (mg)	N %	Average N %	Protein %	Average Protein %	RSD %
996.7	1.679	1.672	10.491	10.452	1.112
1010.2	1.679		10.494		
1000.3	1.686		10.536		
1000.5	1.680		10.500		
1020.8	1.697		10.607		
999.2	1.688		10.550		
997.6	1.673		10.456		
1012.9	1.642		10.265		
1003.5	1.654		10.339		
1030.8	1.646		10.286		

Conclusion

The data obtained demonstrated excellent reproducibility. No memory effect was observed when changing the sample type which indicated the complete detection of Nitrogen present in the sample. The Thermo Scientific FLASH 4000 Analyzer was able to analyze Nitrogen in a wide range (from a low to high content) without a matrix effect. The resulting Nitrogen data was inside the tolerance that is indicated in the Reference Material Certificates for both Kjeldahl and the Combustion method which clearly demonstrates the high performance of the instrument.

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Application Note: 43030

Nitrogen Determination in Soils and Plants by the Thermo Scientific FLASH 4000 Elemental Analyzer

Dr. Liliana Krotz, Product Specialist, OEA and Dr. Guido Giazzi, Product Manager, OEA, Thermo Fisher Scientific, Milan, Italy

Key Words

- Flash Combustion
- Nitrogen determination
- Soils
- Plants

Introduction

Figure1: Flash 4000 elemental analyzer.

The analysis of nitrogen content in soils is important for both the evaluation of organic matter and the calculation of the ideal fertilizer quantities required to maximize plant growth. This is enabled by providing information regarding the deficiency or excess of nutritional elements important to growth. Nitrogen content analysis is required when determining the quality of various types of crops for feeding and processing, as well as for N-cycle and N-fixation monitoring in agricultural and environmental research.

The determination of nitrogen in soils requires the use of accurate instrumental analytical techniques and the avoidance of toxic chemicals. To achieve this, an alternative to the classical Kjeldahl method - based on Dumas (combustion) method - has been developed and approved by a number of different associations.

As the demand increases for improved sample throughput, reduction operational costs and a reduction in human error, it has become very important to have a simple and automatic technique which enables fast analysis combined with excellent reproducibility.

The Thermo Scientific FLASH 4000 elemental analyzer (Fig 1.) is based on the dynamic combustion of the sample. The FLASH 4000 does not require sample digestion of toxic chemicals, in addition, this analyzer provides significant benefits which include: Time saving, automation and the quantitative determination of nitrogen in a large range of concentrations. The correct quantity of oxygen, (correlated to the weight and type of sample), is determined automatically by OxyTune® which facilitates the complete combustion of the sample.

Analytical Configuration

The sample is weighed in a Tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 autosampler together the correct amount of oxygen which has been automatically calculated by OxyTune function, to ensure the complete combustion of the sample. After combustion, the produced gases are passed by a helium flow to a second reactor which is filled with copper. The water is trapped through a Water Condensation Drainage Device, while the CO_2 is adsorbed by the No-Stop Twin Traps. The nitrogen is then passed through a GC column and finally detected by a thermal conductivity detector (see Figure 2).

Analytical conditions

T left tube: 950°C
T right tube: 840°C
T oven: 50°C

Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min

Standard weight: 500 mg EDTA (9.59 % N)
EDTA: EthyleneDiamineTetraAcetic acid

Note: *The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.*

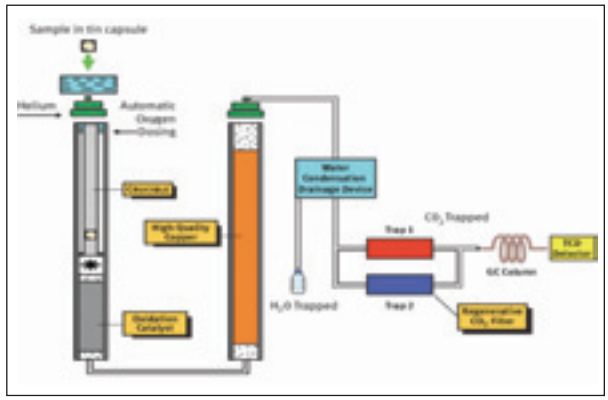

Figure 2: Flash 4000 configuration layout

The Thermo Scientific FLASH 4000 performance evaluation by WEPAL Round Robin Tests

The accuracy and precision of the FLASH 4000 was evaluated through the participation in International Round Robin Tests WEPAL (Wageningen Evaluating Programs for Analytical Laboratories, Wageningen University, Netherlands). For soil samples, the data was compared with the range accepted by WEPAL statistic studies, including all methods for nitrogen determination. While for plant samples, the results were compared with the range accepted for both Kjeldahl and Total Nitrogen methods – which include the combustion method.

Results

The samples selected for analysis where chosen on the basis of their differing nature and nitrogen content. These differences mean that the combustion and the amount of oxygen required is completely different. The data obtained demonstrated the no-matrix effect in the determination of nitrogen, indicating complete combustion for all the sample types. The calibration of the system was performed with EDTA (9.59 %N) using K factor as the calibration method. The samples were analyzed as they were received by WEPAL, without treatment.

Table 1 – Reproducibility of Nitrogen Determination in Soil WEPAL Samples

Cat Clay (code WEPAL 2007.2, no. 887)				Sandy Soil (code WEPAL 2008.2, no. 918)			
FLASH 4000			WEPAL - N %	FLASH 4000			WEPAL - N %
Weight (mg)	N %	RSD %		Weight (mg)	N %	RSD %	
1043.5	0.1053	0.334		1011.1	0.204	0.997	
1021.1	0.1042			1030.0	0.204		
1089.9	0.1065			1015.6	0.202		
1000.9	0.1069		Average: 0.105	1008.7	0.199		Average: 0.200
1087.7	0.1043			1018.9	0.203		
1054.2	0.1054		Range: 0.086 – 0.116	1025.5	0.201		Range: 0.178 – 0.221
1034.7	0.1085			1009.2	0.202		
1023.9	0.1062			1022.8	0.199		
1056.6	0.1059			1002.1	0.203		
1055.9	0.1048			1006.8	0.199		

Table 1 shows the reproducibility of 10 consecutive runs of Soil WEPAL samples using a sample weight of about 1000 mg. The nitrogen data obtained was inside in the range of nitrogen concentration approved by the WEPAL statistic studies.

Table 2 – Reproducibility of Nitrogen Determination in Plant WEPAL Samples

Lucerne/Medicago Sativum (code WEPAL 2008.3 , no. 939)				Oil Palm Leaves (code WEPAL 2008.1 , no. 165)			
FLASH 4000			WEPAL - N %	FLASH 4000			WEPAL - N %
Weight (mg)	N %	RSD %		Weight (mg)	N %	RSD %	
1001.8	2.867	0.682		1056.5	2.725	0.773	
1010.0	2.889		Kjeldahl methods:	1067.6	2.761		Kjeldahl methods:
1005.3	2.891		Average: 2.830	1003.4	2.742		Average: 2.670
1000.9	2.868		Range: 2.660 – 2.990	1020.0	2.747		Range: 2.500 – 2.830
1008.3	2.898			1002.5	2.739		
1015.3	2.878			1080.9	2.762		
1004.6	2.889		Total Nitrogen Methods	1077.8	2.749		Total Nitrogen Methods
1012.2	2.835		Average: 2.970	1025.3	2.735		Average: 2.790
1020.1	2.864		Range: 2.820 – 3.110	1003.8	2.728		Range: 2.700 – 2.960
1009.8	2.898			1098.4	2.755		

Table 2 shows the reproducibility of 10 consecutive runs of Plant WEPAL samples using a sample weight of about 1000 mg. The nitrogen data obtained are inside in the range of nitrogen concentration approved by WEPAL statistic studies for both Kjeldahl and Total Nitrogen methods.

Table 3 – Nitrogen determination in soil samples

Soil Sample	N %	RSD %
Moist Clay	0.1372	0.621
	0.1355	
	0.1363	
Braunerde	0.2758	0.255
	0.2744	
	0.2750	
Calcareous soil	0.1978	0.838
	0.1945	
	0.1961	
Braunerde pseudoclay	0.1719	0.613
	0.1739	
	0.1723	
Sandy Clay	0.0979	0.845
	0.0995	
	0.0983	
Riverclay	0.2835	1.048
	0.2859	
	0.2800	

Table 3 shows the reproducibility of nitrogen determination in soils in a wide level of concentration. Samples were analysed in triplicate and the weight of sample used was 500 – 1500 mg depending of the matrix.

Table 4 – Nitrogen determination in plant samples

Plant Sample	N %	RSD %
Straw	0.636	0.877
	0.629	
	0.640	
Grass	2.305	0.153
	2.298	
	2.302	
Fig Leaves	2.657	0.260
	2.657	
	2.669	
Grape	0.519	0.111
	0.520	
	0.518	
Alfalfa	2.830	0.462
	2.804	
	2.818	
Potato (plant)	0.251	0.345
	0.253	
	0.253	

Table 4 shows the reproducibility of nitrogen determination in plants in a wide level of concentration. Samples were analysed in triplicate and the weight of sample used was 500 – 1500 mg depending of the matrix.

Conclusion

The resulting data obtained demonstrated excellent reproducibility, and no memory effect was observed when changing the type of sample, indicating the complete detection of the nitrogen present in the sample. The Thermo Scientific FLASH 4000 Analyzer is able to analyze nitrogen in a wide range from low to high content without matrix effect.

The nitrogen data obtained by FLASH 4000 are inside in the tolerance accepted by WEPAL International Round Robin Tests demonstrating the high performance of the instrument.

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Application Note: 42145

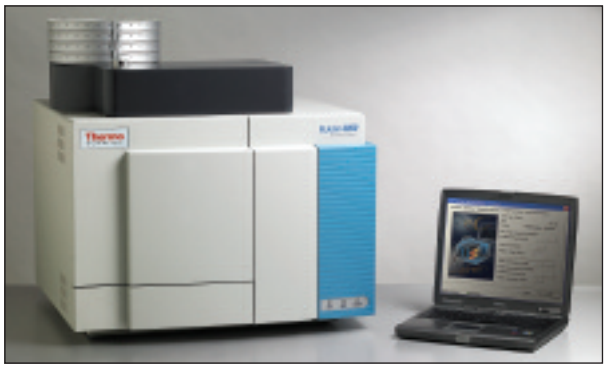
Nitrogen/Protein determination in Animal Feed by the Thermo Scientific FLASH 4000 Series

Liliana Krotz and Guido Giazzi, Thermo Fisher Scientific, Milan, Italy

Key Words

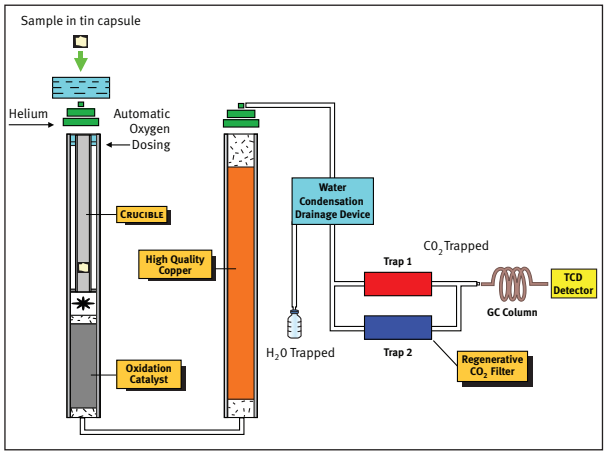
- Food Safety
- DDG
- Flash combustion
- Nitrogen/Protein

Introduction



One of the most important nutrients in animal food is protein. Protein intake provides the building blocks needed by the animal to produce its own proteins in order to grow, maintain or produce muscles, enzymes, hormones, milk, wool, etc. The quantity of Protein required by animals is very precise as excess amounts of lead to amino acid deficiency and generates unnecessary amounts of energy. The precise and accurate determination of its amount, through the determination of Nitrogen, is fundamental to achieving high nutritional quality of finished animal products.

Analytical Configuration



The capabilities of the combustion method for the determination of Nitrogen have been greatly improved to make analyses faster, safer and more reliable than the traditional Kjeldahl method. For this reason, the Dumas Combustion method has been approved and adopted by the Association of Official Analytical Chemists for this application (AOAC Official Method 990.03. Protein crude in Animal Feed 4.2.08).

The FLASH 4000 Nitrogen/Protein Analyzer, based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility and high sample throughput.

Analytical conditions

T left tube: 950°C
T right tube: 840°C
T oven: 50°C

Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min

Standard: 500 mg EDTA 9.59 %N
EDTA: EthyleneDiamineTetraAcetic acid

Sample weight: 1 gram

Note: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.

Official method requirements for Protein determination by Combustion

AOAC (Method 990.03) indicates that the suitable fineness of grind must be determined (for each different material analyzed) to achieve precision shown by an RSD of ≤ 2 % for 10 successive determinations of Nitrogen. The fineness of 0.5 mm required to achieve this precision must be used for all mixed feeds and other non-homogenous materials.

Results

Different animal feed products were chosen to demonstrate the suitability of the method for all ranges of Protein content without matrix effect and with reduced sample preparation. The Protein content is calculated automatically by the dedicated Thermo Scientific Eager Xperience software using a Protein factor of 6.25.

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Table 1 shows the Nitrogen/Protein determination in **maize flour, wheat flour** and **wheat midd** samples. Samples were homogenized to particle size 2 mm.

Table 1 – N/Protein determination in flours samples

Maize flour			Wheat flour			Wheat midd		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
1006.1	1.274	7.963	1020.3	1.836	11.475	1001.3	2.737	17.109
1005.0	1.257	7.858	1016.3	1.856	11.600	1008.3	2.763	17.271
1005.8	1.288	8.053	1017.5	1.832	11.450	1002.1	2.759	17.242
1007.2	1.259	7.867	1008.4	1.841	11.506	999.3	2.776	17.347
1002.9	1.268	7.928	1009.8	1.844	11.525	1006.7	2.789	17.434
1004.1	1.287	8.043	1004.4	1.848	11.550	1004.5	2.769	17.309
996.4	1.285	8.032	1002.6	1.859	11.619	1015.3	2.776	17.349
994.8	1.267	7.918	1012.4	1.849	11.556	1003.5	2.722	17.015
1003.1	1.257	7.856	1000.6	1.857	11.606	1000.7	2.729	17.054
996.1	1.275	7.967	1007.3	1.848	11.550	1015.3	2.730	17.063
Average	**1.272**	**7.949**	**Average**	**1.844**	**11.525**	**Average**	**2.757**	**17.232**
RSD %	**0.959**	**0.959**	**RSD %**	**0.459**	**0.459**	**RSD %**	**0.858**	**0.858**

Table 2 shows the analysis of 10 consecutive determinations of **soya** and **sunflower** samples homogenized to 2 mm particle size.

Table 2 – N/Protein determination in soya and sunflower

Soya			Sunflower		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
995.8	7.452	46.574	998.1	3.088	19.302
1000.4	7.464	46.647	694.9	3.092	19.328
999.5	7.451	46.569	991.5	3.041	19.008
1010.3	7.454	46.587	795.1	3.029	18.9300
1007.2	7.453	46.582	692.2	3.074	19.216
999.6	7.448	46.550	994.9	3.052	19.077
1005.7	7.461	46.631	802.4	3.066	19.161
108.8	7.443	46.518	996.5	3.077	19.233
1008.2	7.447	46.544	897.6	3.061	19.129
1002.1	7.469	46.681	1002.1	3.058	19.115
Average	**7.454**	**46.55**	**Average**	**3.064**	**19.150**
RSD %	**0.161**	**0.161**	**RSD %**	**0.655**	**0.655**

Table 3 shows the N/Protein data obtained from **DDG, gluten** and **fish meal** samples. DDG is the dried distilled grain fraction after removing ethyl alcohol from the yeast fermentation.

Table 3 – N/Protein determination in gluten, DDG and fish meal

DDG			Gluten			Fish meal		
Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %	Weight (mg)	N %	Protein %
1010.5	5.298	33.112	1001.5	9.538	59.614	999.8	11.404	71.275
1002.4	5.324	33.277	1001.7	9.542	59.635	1007.3	11.396	71.226
1008.5	5.367	33.545	1009.7	9.621	60.134	1003.6	11.369	71.055
1009.2	5.345	33.407	1010.2	9.623	60.146	1010.2	11.416	71.350
1012.3	5.377	33.604	1012.3	9.544	59.651	998.9	11.357	70.978
1008.6	5.350	33.438	1011.8	9.549	59.683	1004.6	11.418	71.365
1001.4	5.326	33.290	1029.8	9.600	59.998	1000.3	11.418	71.362
1008.3	5.363	33.519	1015.7	9.560	59.748	1008.4	11.339	70.868
1012.2	5.336	33.353	1006.4	9.559	59.745	1008.2	11.277	70.483
1015.7	5.309	33.185	1008.8	9.587	59.916	997.5	11.321	70.755
Average	**5.340**	**33.373**	**Average**	**9.572**	**59.827**	**Average**	**11.372**	**71.072**
RSD %	**0.479**	**0.479**	**RSD %**	**0.343**	**0.343**	**RSD %**	**0.421**	**0.422**

Conclusion

The data obtained of 10 consecutive determinations show an excellent reproducibility. In all cases the relative standard deviation was less than 2 %, according to the official method indicating that it is not necessary a fine homogenization and no memory effect was observed when changing the type of sample, indicating the complete detection of the Nitrogen present in the sample. This demonstrates that the Thermo Scientific FLASH 4000 Analyzer is able to analyze Nitrogen in a wide range from low to high content without matrix effect.

Note: Thanks to Cargill Animal Feed Division, Spessa(Pv), Italy, for the collaborative studies. Part of this application data was presented at PittCon 2009 as an oral lecture.

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Application Note: 42170

Determination of Nitrogen/Protein in Brewing Industry Products with the Thermo Scientific FLASH 4000 Elemental Analyzer

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Key Words

- Combustion
- Malt and barley
- Nitrogen
- Wort

Introduction

The brewing industry needs to perform routine analytical testing to ensure the quality of products and raw materials. Nitrogen/protein determination is a key process and enables manufacturers to monitor the content and stability of a product.

Control and measurement of protein throughout the brewing process is important in order to ensure the survival, growth and productivity of the yeast utilized to convert sugars to ethanol and carbon dioxide. The yeast organisms depend on a variety of conditions, including the availability of amino groups derived from enzymatic hydrolysis of protein during the brewing process. While in the past methods have traditionally focused on investigating foam and haze-forming properties of protein in beer, there is now an increasing demand for techniques to accurately measure and quantify protein concentration.

The traditional method for determining protein content is the Kjeldahl method through the determination of nitrogen (N x 6.25 = protein concentration). An advanced analytical technique based on the Dumas combustion method has been developed to offer an alternative to the classical Kjeldahl method. The method is approved by a broad range of recognized associations (AOAC®, AACC®, ISO® and IFFO®), including the American Society of Brewery Chemists (ASBC®).

The Thermo Scientific FLASH 4000 Nitrogen/Protein Analyzer (Figure 1) utilizes the Dumas combustion method and requires no sample digestion or toxic chemicals. Due to the automated and accessible nature of the instrument, it provides significant advantages for the quantitative determination of nitrogen/protein, including reproducibility, high accuracy, minimization of human error and high sample throughput.

Experimental data compared to results obtained by spectrophotometric, near infrared spectroscopy (NIR) and Kjeldahl methods as well as correlation with Round Robin Tests values, demonstrate the validity of the new instrument as an alternative to traditional wet chemistry procedures.

Figure 1: FLASH 4000 Nitrogen/Protein analyzer

Methods

FLASH 4000 Method (in accordance with the European Brewery Convention Method, EBC 4.9.3 Soluble Nitrogen of Dumas Combustion Method)

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 Autosampler together with a controlled amount of oxygen using the Thermo Scientific OxyTune function, ensuring a complete combustion of the sample. Malt and barley samples are homogenized and weighed directly in the tin capsule in the range of 700 – 1000 mg. A 2 ml sample of wort is pipetted onto filter paper in the tin capsule, then dried in an oven at 105 °C for 90 minutes. The sample is not dried completely as the filter still has to be moist to be able to close the capsule for analysis (Figure 2).

After combustion, the resulting gases are carried by a helium flow to a second reactor filled with copper. The water is trapped through a Peltier system while the CO_2 is adsorbed by the NoStop Twin Traps. The nitrogen is then passed through a GC column and finally detected by a thermal conductivity detector (Figure 3). A complete report is automatically generated by the dedicated Thermo Scientific Eager Xperience data handling software.

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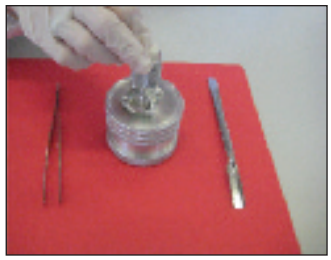
Tools: tin disk,capsulator, filter paper, wort sample.

The tin disk is rested on the capsulator.



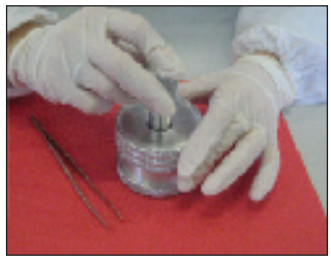
The tin disk is pressed through a cylindrical tool.

Filter paper is placed in the tin capsule.

The wort sample is adsorbed by the filter paper.

The sample is dried in an oven at 105°C for 90 min.

The tin disk is closed by hand using the capsulator.



Using the cylindrical tool, the disk is pressed into the cavity.

The top of the device is pressed downwards to release the capsule.

Figure 2: Wort sample weighing technique

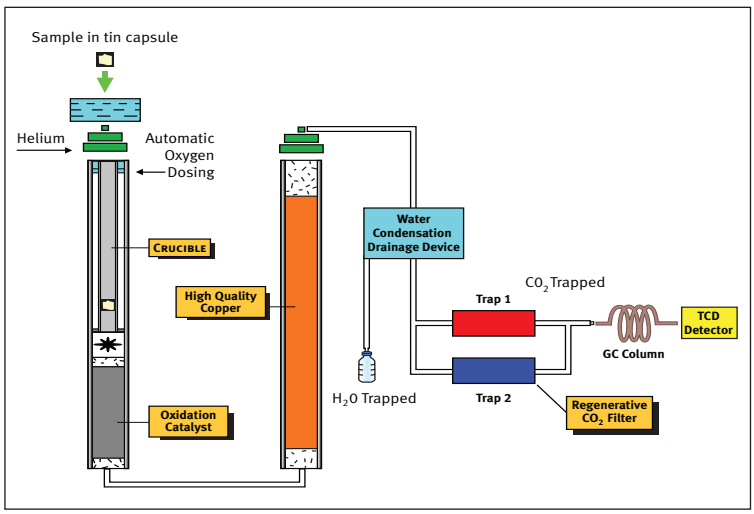


Figure 3: FLASH 4000 Nitrogen/Protein configuration

Analytical conditions:
Oxidation reactor (Left furnace): 950 °C
Reduction reactor (Right furnace): 840 °C
Oven Temperature: 50 °C
Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min

Note: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Eager Xperience software.

Spectrophotometric Method (in accordance with the European Brewery Convention Method, EBC 4.9.2 Soluble Nitrogen of Malt)

The soluble nitrogen in wort is determined using a spectrophotometric method, from absorbance measurements at 215 nm and 225 nm. The sample is diluted 1/100 with 5 g/l sodium chloride solution and the absorbance of the diluted wort is measured at both 215 and 225 nm with the 5 g/l sodium chloride solution set at zero. The soluble protein content is calculated based on the difference in absorbance between 215 and 225 nm. A wort with a known content of soluble protein is used for calibration of the linear equation. A Skalar continuous flow analyzer was used for the analysis.

Results

The samples analyzed were chosen on the basis of their varying nature and different content of nitrogen. These differences meant that the combustion and the amount of oxygen required were completely different. The data obtained demonstrated no-matrix effects in the determination of nitrogen, indicating complete combustion for all types of samples.

For malt and barley samples, calibration was performed using Thermo Scientific Pasta Reference Material on the FLASH 4000 Analyzer. A K factor calibration method was used.

For wort samples, a Boortmalt Wort Reference Material (0.075 N %) and a glycine solution were used for calibration. The protein factor used to calculate the protein content via the conversion of nitrogen value was 6.25.

The data obtained were compared with the spectrophotometric and NIR methods. In addition, the quality of the measurements obtained by the FLASH 4000 Analyzer were evaluated through participation in International Round Robin Tests.

Table 1 shows the reproducibility and comparison of total nitrogen/protein data obtained from the malt and barley samples with the FLASH 4000 Analyzer.

Sample	FLASH 4000 Analyzer		
	Nitrogen %	Protein %	RSD %
Malt 1	1.748 - 1.756	10.923 - 10.975	0.336
Malt 2	1.723 - 1.716	10.768 - 10.727	0.269
Barley 1	1.622 - 1.638	10.140 - 10.237	0.673
Barley 2	1.646 - 1.635	10.285 - 10.222	0.434

Table 1: Reproducibility of Nitrogen/Protein data of malt and barley samples

Table 2 and Table 3 present the Total Protein and Soluble Protein data obtained during a Circuit of Interlaboratories Malt Test and iFBM Ring Test (Institute Francais des Boissons, de la Brasserie et de la Malterie). In each round, two malt A & B samples were analyzed using the FLASH 4000 Analyzer. The protein data obtained were within the range of nitrogen concentration approved by the iFBM statistic studies (minimum and maximum values accepted).

Month /	Sample	FLASH 4000	iFBM Ring Test		
Year	Code	Total Protein %	Total Protein %	Minimum %	Maximum %
			Assigned value		
8/2010	A	9.2	9.36	9.00	9.75
	B	9.1	9.32	9.05	9.65
9/2010	A	9.9	9.74	9.40	10.10
	B	9.8	9.69	9.30	10.00
10/2010	A	9	9.27	8.85	9.67
	B	8.9	9.27	8.90	9.64
11/2010	A	9.00	9.73	9.00	10.37
	B	9.80	9.74	9.20	10.54

Table 2: iFBM Total Protein data 4.3.1 / 4.3.2 EBC Method (percentage dry matter)

Month /	Sample	Spectrophometric method	FLASH 4000	iFBM Ring Test		
Year	Code	Soluble Protein %	Soluble Protein %	Soluble Protein%	Minimum %	Maximum %
				Assigned value		
8/2010	A	4.11	3.98	3.89	3.67	4.11
	B	4.02	4.10	3.86	3.56	4.16
9/2010	A	4.21	3.90	3.86	3.60	4.10
	B	4.15	4.17	3.90	3.64	4.15
10/2010	A	4.22	4.22	4.10	3.84	4.25
	B	4.30	4.33	4.13	4.00	4.40
11/2010	A	4.20	3.93	3.86	3.57	4.20
	B	4.20	3.88	3.86	3.50	4.26

Table 3: iFBM Soluble Protein data 4.9.1 / 4.9.2 EBC Protein (percentage dry matter).

Tables 4, 5 and 6 show Total Nitrogen and Soluble Nitrogen data of malt, barley and wort obtained during a MAPS Ring Test (Malt Analytes Proficiency Testing Scheme, LGC Standards Proficiency Testing, UK). In each cycle, a malt or barley sample was analyzed with the FLASH 4000 Analyzer. The nitrogen values obtained were within the range of nitrogen concentration approved by the MAPS statistic studies. The data were comparable with the Kjeldahl, combustion, NIR and spectrophotometric methods.

Round	FLASH 4000	MAPS Ring Test					
No.	Analyzer	Kjeldahl		Combustion		NIR	
	Total N %	Total N %	Range %	Total N %	Range %	Total N %	Range %
163	1.78	1.76	1.70-1.82	1.79	1.73-1.85	1.79	1.71-1.87
164	1.52	1.54	1.48-1.60	1.55	1.49-1.61	1.55	1.47-1.63
165	1.38	1.39	1.31-1.49	1.40	1.35-1.44	1.40	1.32-1.48
166	1.63	1.65	1.59-1.71	1.67	1.61-1.73	1.67	1.59-1.75
167	1.39	1.36	1.27-1.41	1.35	1.25-1.48	1.41	1.33-1.49
169	1.89	1.83	1.77-1.89	1.86	1.86-1.92	1.86	1.78-1.94

Table 4: MAPS Total Nitrogen data of malt samples.

Round	FLASH 4000	NIR	MAPS Ring Test					
No.	Analyzer	Analyzer	Kjeldahl		Combustion		NIR	
	Total N %	Total N%	Total N %	Range %	Total N %	Range %	Total N %	Range %
163	1.57	1.63	1.55	1.48-1.60	1.55	1.49-1.61	1.55	1.49-1.61
166	1.71	1.74	1.74	1.68-1.80	1.77	1.71-1.83	1.77	1.71-1.83
167	1.47	1.49	1.46	1.40-1.52	1.49	1.43-1.55	1.49	1.43-1.55
168	1.76	1.73	1.71	1.65-1.77	1.77	1.71-1.83	1.74	1.68-1.80
169	1.55	1.60	1.55	1.49-1.61	1.57	1.51-1.63	1.57	1.51-1.63

Table 5: MAPS Total Nitrogen data of barley samples.

Round	FLASH 4000	Spectrophotometric	MAPS Ring Test			
No.	Analyzer	method	Kjeldahl		Combustion	
	Soluble N %	Soluble N %	Soluble N %	Range %	Soluble N %	Range %
164	0.59	0.60	0.57	0.51-0.63	0.58	0.54-0.62
165	0.66	0.68	0.66	0.60-0.72	0.66	0.60-0.72
166	0.85	0.85	0.82	0.76-0.88	0.84	0.78-0.90
167	0.62	0.62	0.60	0.54-0.66	0.60	0.54-0.66
168	0.69	0.67	0.66	0.60-0.72	0.66	0.60-0.72
169	0.82	0.81	0.81	0.75-0.87	0.82	0.76-0.88

Table 6: MAPS Soluble Nitrogen data of wort samples.

Conclusion

The results demonstrate that the FLASH 4000 Analyzer offers excellent reproducibility, with no memory effect observed when using large sample weights. The system's ability to accurately analyze nitrogen in a wide range from low to high content without matrix effects and without the use of sample digestion or toxic chemicals is also demonstrated. The data obtained through the use of the instrument were within the range accepted for the Kjeldahl, Combustion and NIR methods included in the iFBM and MAPS Ring Tests, indicating the high performance of the system. In addition, the FLASH 4000 results are comparable to those obtained using the spectrophotometric and NIR methods, demonstrating the validity of the instrument as an alternative to traditional wet chemistry procedures.

Thanks to: Geert van D'Huynslager, Boortmalt (Antwerpen, Belgium) FLASH 4000 user, and Roel Wuyts and Jean-Louis Brix, Interscience (Louvain-la-Neuve, Belgium).

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Application Note: 42171

Determination of Nitrogen/Protein in Fishmeal using Thermo Scientific FLASH 4000 Elemental Analyzer

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Key Words

- Animal feed
- Combustion
- Fishmeal
- Nitrogen/Protein

Introduction

High quality fishmeal is recognized by animal nutritionists as an excellent source of protein, energy, minerals and vitamins. With millions of tons produced annually worldwide, the majority of fishmeal is used across the aquaculture, livestock and poultry industries. Fishmeal increases productivity and improves the efficiency with which feed is converted to animal produce (feed conversion), alongside improving food palatability and enhancing nutrient uptake, digestion and absorption. High quality fishmeal provides a balanced amount of all essential amino acids, phospholipids, and fatty acids, optimizing development, growth and reproduction. The nutrients in fishmeal also help to protect animals from disease by boosting and helping to maintain a healthy functional immune system.

Fishmeal is produced by the process of cooking, drying and grinding raw fish. As fish is a highly perishable raw material, process control in the factory is vital to ensure freshness and maintain the high quality of the protein in the final product. All fishmeal is traded on its protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum quantity of protein content. As a result, producers must be able to accurately evaluate and determine the levels of protein in their product by quantifying the nitrogen concentration.

To facilitate the efficient production and sale of fishmeal, an accurate, rapid and reproducible analytical technique for the determination of nitrogen/protein is required that avoids the use of toxic chemicals. The analytical technique based on the Dumas combustion method has been developed to offer an alternative to the classical Kjeldahl method. The method is approved by a broad range of recognized associations,(AOAC®, AACC®, AOCS®, ASBC® and ISO®) including the International Fishmeal and Fish Oil Organisation (IFFO). The IFFO has recommended that its members adopt the Dumas method as an official method for nitrogen and crude protein determination.

As the demand for increased sample throughput and reduced operational costs increases, there is a growing need for a simple and automated technique which enables rapid, reproducible analysis using the Dumas combustion method. The Thermo Scientific FLASH 4000 Elemental Analyzer (Figure 1) is based on the dynamic combustion of the sample and requires no sample digestion or toxic chemicals. Due to the automated and accessible nature of the method, it provides significant advantages for the quantitative determination of nitrogen, including the reduction of operational costs, minimization of human error and improved sample throughput.

Analytical Configuration

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 Autosampler together with a specified amount of oxygen using the Thermo Scientific OxyTune® function. This ensures a complete combustion of the sample. After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper. The water is trapped through a water condensation drainage device while the CO_2 is absorbed by the NoStop Twin Traps. The nitrogen is then passed through a GC column and is finally detected by a thermal conductivity detector (see Figure 2).

Figure 1: FLASH 4000 Nitrogen/Protein analyzer

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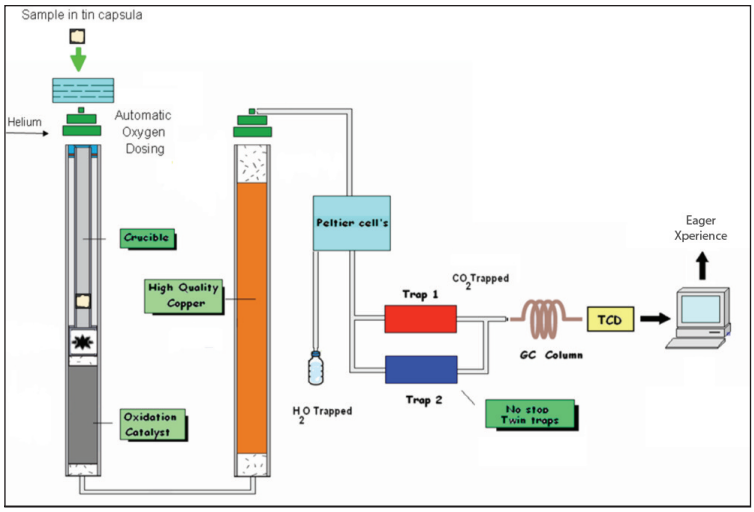

Figure 2: FLASH 4000 Nitrogen/Protein configuration

Analytical conditions:

Oxidation reactor temperature: 950 °C
Reduction reactor temperature: 840 °C
Oven Temperature: 50 °C
Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min
Standard: 400-700 mg EDTA (9.59 %N)
EDTA: (EthyleneDiamineTetraacetic acid)
Sample weight: 400-700 mg

Note: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.

Results

A broad range of fishmeal samples were selected for analysis, originating from different countries and composed of varying nutritional content (fat, salt, moisture and ashes). The data obtained demonstrate the no-matrix effect in the determination of nitrogen, indicating complete combustion for all sample types. The calibration of the system was performed with EDTA (9.59 N%) using K factor as calibration method. The protein factor used to calculate the protein content via the conversion of nitrogen value was 6.25.

The performance of the FLASH 4000 Analyzer was evaluated in the following terms:

- Evaluation of the reproducibility through the Relative Standard Deviation obtained (RSD %) analyzing the samples in triplicate.
- Evaluation of results using different weight of sample
- Comparison with Kjeldahl method data

Table 1 shows the nitrogen/protein reproducibility analyzing several fishmeal samples in triplicate. In all cases the RSD % obtained was excellent.

Sample	N %	Protein %	RSD %
A	11.06	69.12	0.17
	11.10	69.35	
	11.08	69.23	
B	9.59	59.93	0.33
	9.63	60.18	
	9.65	60.32	
C	9.56	59.78	0.32
	9.53	59.54	
	9.59	59.92	
D	9.75	60.92	0.43
	9.77	61.07	
	9.69	60.56	
E	9.93	62.06	0.14
	9.90	61.90	
	9.93	62.03	
F	9.77	61.04	0.31
	9.80	61.27	
	9.74	60.89	
G	10.60	66.24	0.27
	10.57	66.09	
	10.54	65.88	
H	11.01	68.84	0.42
	10.95	68.41	
	11.03	68.96	
I	10.35	64.67	0.25
	10.38	64.90	
	10.40	64.99	
J	10.93	68.34	0.49
	10.99	68.68	
	10.88	68.01	
K	10.77	67.28	0.53
	10.73	67.08	
	10.65	66.58	
L	10.64	66.47	0.60
	10.54	65.85	
	10.52	65.73	

Table 1: Nitrogen/Protein reproducibility of fishmeal

Table 2 shows two fishmeal samples analyzed at different weights to evaluate the influence on the results. No memory effect was observed when changing the amount of sample, indicating a complete combustion of the sample. No significant difference in nitrogen and protein content was observed.

Sample	Weight (mg)	N %	Average N%	Protein %	Average Protein %	RSD %
B	400	9.70 9.61 9.62	9.64	60.60 60.04 60.15	60.26	0.49
	500	9.59 9.63 9.65	9.62	59.93 60.18 60.32	60.14	0.39
I	500	10.42 10.37 10.40	10.40	65.16 64.81 64.99	64.99	0.27
	700	10.35 10.38 10.40	10.38	64.67 64.90 64.99	64.85	0.25

Table 2: Nitrogen/Protein determination of fishmeal at different weight

Table 3 shows a comparison between FLASH 4000 Analyzer results and Kjeldahl method data. The values demonstrate a perfect correlation between both methods.

Sample	FLASH 4000 Analyzer		Kjeldahl Method		Difference
	N %	Protein %	N %	Protein %	in Protein %
A	11.08	69.23	11.09	69.30	-0.07
B	9.62	60.14	9.60	60.00	+0.14
C	9.56	59.75	9.54	59.60	+0.15
D	9.74	60.85	9.73	60.80	+0.05
E	9.92	62.00	9.92	62.00	+0.00
F	9.77	61.07	9.78	61.10	-0.03
G	10.57	66.07	10.66	66.60	-0.53
H	11.00	68.74	10.94	68.40	+0.34
I	10.38	64.85	10.35	64.70	+0.15
J	10.93	68.34	10.92	68.20	+0.14
K	10.72	66.97	10.64	66.50	+0.47
L	10.57	66.02	10.48	65.50	+0.52

Table 3: FLASH 4000 Analyzer data vs Kjeldahl values

Conclusion

The results obtained through this experiment demonstrate excellent reproducibility and RSD %, with no memory effect observed when changing the type of sample or when using large sample weight. This indicates the complete and accurate detection of the nitrogen present in the sample. As a complete automatic system, the FLASH 4000 Analyzer is approved by multiple official organizations, including IFFO, and is able to accurately analyze nitrogen in a wide range from low to high content without matrix effect. Providing an ideal solution for nitrogen determination, the system offers consistently accurate data that is comparable with data produced by the Kjeldahl method, while also removing the requirement for sample digestion or toxic chemicals.

Thanks to: Intertek (Bremen, Germany) FLASH 4000 user.

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Application Note: 42172

Determination of Nitrogen/Protein in Canned Meat Reference Materials with the Thermo Scientific FLASH 4000 Elemental Analyzer

Dr. Liliana Krotz, OEA Product Specialist, Walter Galotta, OEA Product Support Engineer, Dr. Guido Giazzi, OEA Product Manager, Thermo Fisher Scientific, Milan, Italy

Key Words

- Canned meat
- Combustion
- Nitrogen
- Protein
- Reference Materials

Introduction

In order to safeguard consumer health and protect the commercial value of a product and the reputation of manufacturers, there is a growing need within the canned meat industry to ensure nutritional levels of products are controlled and accurately represented. Current regulations in Europe, Canada and the US stipulate that product descriptions should correctly reflect product composition and all ingredients should be accurately declared. These regulations recognize the importance of enabling consumers to make an informed decision, particularly with regard to quality comparisons based on the ratio of meat protein content.

To ensure compliance with the varying regulations across the globe, manufacturers require an analytical technique that enables accurate, rapid and reproducible determination of nitrogen/protein levels in canned meat products. The analytical technique based on the Dumas combustion method has been developed to offer an alternative to the classical Kjeldahl method that additionally avoids the use of toxic chemicals. The Dumas method has been approved by a broad range of recognized associations, including the Association of Official Analytical Chemists (AOAC®).

As the demand for increased sample throughput and reduced operational costs increases, there is a growing need for a simple and automated technique which enables rapid, reproducible analysis. The Thermo Scientific FLASH 4000 Elemental Analyzer (Figure 1) is based on the dynamic combustion of the sample and requires no sample digestion or toxic chemicals. Due to the automated and accessible nature of the method, it provides significant advantages for the quantitative determination of nitrogen, including the reduction of operational costs, minimization of human error and improved sample throughput.

Figure 1: FLASH 4000 Nitrogen/Protein analyzer

Analytical Configuration

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 Autosampler together with a controlled amount of oxygen using the Thermo Scientific OxyTune function. This ensures complete combustion of the sample. After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper. The water is trapped through a water condensation drainage device, while the CO_2 is adsorbed by the NoStop Twin Traps. The nitrogen then passes through a GC column and is finally detected by a thermal conductivity detector (Figure 2).

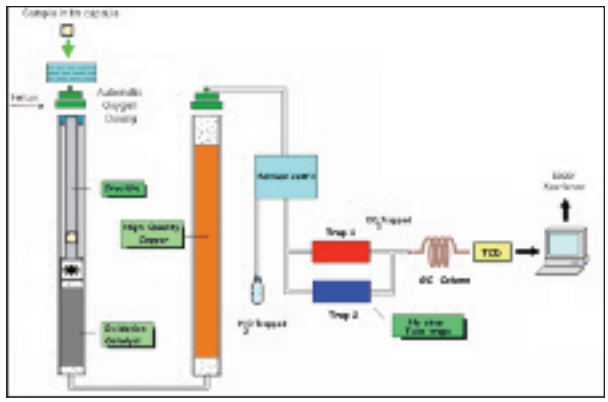

Figure 2: FLASH 4000 Nitrogen/Protein configuration

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Analytical conditions:

Oxidation reactor (Left furnace): 950 °C
Reduction reactor (Right furnace): 840 °C
Oven Temperature: 50 °C
Carrier Flow: 300 ml/min
Reference Flow: 300 ml/min
Standard: 500 mg EDTA (9.59 % N)
EDTA: (EthyleneDiamineTetraaceticAcid)
Sample weight: 400-800 mg

Note: The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Eager Xperience software.

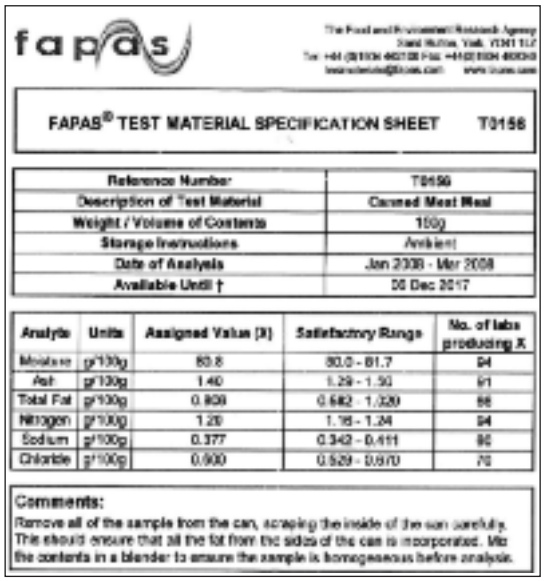
FLASH 4000 performance evaluation by Reference Materials

The performance of the FLASH 4000 Analyzer was evaluated through the analysis of canned meat reference materials by FAPAS® (Food Analysis Performance Assessment Scheme, part of the Food and Environment Research Agency, an executive agency of the UK). The results obtained were compared with the average and satisfactory ranges indicated in the relative reference materials certificates.

Results

Four canned meat samples were selected representing a range of varying levels of nitrogen and fat content. The data obtained demonstrates the no-matrix effect in the determination of nitrogen, indicating complete combustion for all types of samples. The calibration of the system was performed with EDTA acid (9.59 N %) using the K factor calibration method. The samples were analyzed as received by the reference materials supplier. The protein factor used to calculate the protein content via the conversion of Nitrogen value was 6.25.

The certificates of the four samples FAPAS T0156, T0158, T0160 and T0166 can be seen below:



fapas

FAPAS® TEST MATERIAL SPECIFICATION SHEET T0156

Reference Number	T0156
Description of Test Material	Canned Meat Meal
Weight / Volume of Contents	150g
Storage Instructions	Ambient
Date of Analysis	Jan 2008 - Mar 2008
Available Until †	06 Dec 2017

Analyte	Units	Assigned Value (X)	Satisfactory Range	No. of labs producing X
Moisture	g/100g	80.8	80.0 - 81.7	94
Ash	g/100g	1.40	1.29 - 1.50	91
Total Fat	g/100g	0.809	0.682 - 1.020	88
Nitrogen	g/100g	1.20	1.16 - 1.24	94
Sodium	g/100g	0.377	0.342 - 0.411	86
Chloride	g/100g	0.900	0.529 - 0.970	70

Comments:
Remove all of the sample from the can, scraping the inside of the can carefully. This should ensure that all the fat from the sides of the can is incorporated. Mix the contents in a blender to ensure the sample is homogeneous before analysis.

fapas

FAPAS® TEST MATERIAL SPECIFICATION SHEET T0158

Reference Number	T0158
Description of Test Material	Canned Meat
Weight / Volume of Contents	150g
Storage Instructions	+4°C
Date of Analysis	Aug 2008 - Sep 2008
Available Until †	30 Jun 2018

Analyte	Units	Assigned Value (X)	Satisfactory Range	No. of labs producing X
Moisture	g/100g	65.4	64.5 - 66.3	89
Ash	g/100g	3.14	2.93 - 3.35	86
Total Fat	g/100g	9.07	8.13 - 10.01	87
Nitrogen	g/100g	3.65	3.52 - 3.78	87
Hydroxyproline	g/100g	0.823	0.716 - 0.930	83

Comments:
You may use any method of analysis you wish. Remove all of the sample from the can, scraping the inside of the can carefully. This should ensure that all the fat from the sides of the can is incorporated. Mix the contents in a blender to ensure the sample is homogeneous before analysis.

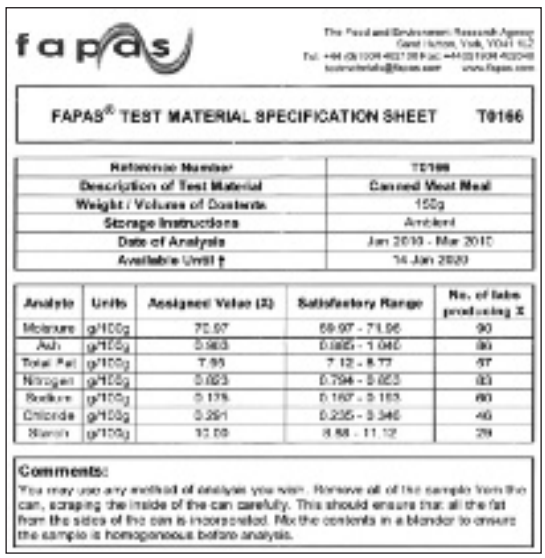
fapas

FAPAS® TEST MATERIAL SPECIFICATION SHEET T0160

Reference Number	T0160
Description of Test Material	Canned Meat
Weight / Volume of Contents	150g
Storage Instructions	Ambient
Date of Analysis	Nov 2008 - Jan 2009
Available Until †	18 Nov 2018

Analyte	Units	Assigned Value (X)	Satisfactory Range	No. of labs producing X
Moisture	g/100g	66.07	59.99 - 61.15	107
Ash	g/100g	2.07	1.92 - 2.21	98
Total Fat	g/100g	17.24	14.83 - 19.65	65
Nitrogen	g/100g	2.26	2.16 - 2.35	97
Hydroxyproline	g/100g	0.288	0.248 - 0.328	70

Comments:
Remove all of the sample from the can, scraping the inside of the can carefully. This should ensure that all the fat from the sides of the can is incorporated. Mix the contents in a blender to ensure the sample is homogeneous before analysis.



fapas

FAPAS® TEST MATERIAL SPECIFICATION SHEET T0166

Reference Number	T0166
Description of Test Material	Canned Meat Meal
Weight / Volume of Contents	150g
Storage Instructions	Ambient
Date of Analysis	Jan 2010 - Mar 2010
Available Until †	14 Jun 2020

Analyte	Units	Assigned Value (X)	Satisfactory Range	No. of labs producing X
Moisture	g/100g	73.97	69.97 - 73.96	90
Ash	g/100g	0.963	0.885 - 1.040	89
Total Fat	g/100g	7.55	7.12 - 8.77	87
Nitrogen	g/100g	0.923	0.794 - 0.923	83
Sodium	g/100g	0.176	0.167 - 0.185	60
Chloride	g/100g	0.291	0.235 - 0.346	46
Starch	g/100g	10.09	8.68 - 11.12	29

Comments:
You may use any method of analysis you wish. Remove all of the sample from the can, scraping the inside of the can carefully. This should ensure that all the fat from the sides of the can is incorporated. Mix the contents in a blender to ensure the sample is homogeneous before analysis.

Table 1 shows the nitrogen/protein reproducibility of canned meat sample T0156. The sample was analyzed over five days at different weights. All data obtained were inside the range specified in the FAPAS certificate.

Day	1		2		3		4		5	
Weight (mg)	500 - 600		500 - 600		600 - 700		700 – 800		400 - 500	
Analysis	N %	Prot %	N %	Prot %	N %	Prot %	N %	Prot %	N %	Prot %
Run	1.194	7.463	1.190	7.422	1.188	7.425	1.163	7.271	1.214	7.589
	1.219	7.618	1.188	7.425	1.175	7.342	1.176	7.349	1.209	7.556
	1.211	7.569	1.196	7.476	1.189	7.428	1.180	7.375	1.232	7.701
	1.221	7.634	1.169	7.303	1.202	7.510	1.211	7.570	1.183	7.395
	1.221	7.628	1.181	7.378	1.210	7.560	1.214	7.587	1.209	7.555
	1.196	7.475	1.154	7.210	1.208	7.551	1.209	7.557	1.210	7.562
	1.214	7.588	1.170	7.311	1.198	7.490	1.189	7.430	1.218	7.615
	1.195	7.466								
	1.167	7.295								
	1.176	7.350								
	1.193	7.456								
	1.179	7.368								
	1.777	7.356								
	1.210	7.565								
	1.210	7.564								
Average %	**1.199**	**7.496**	**1.178**	**7.361**	**1.196**	**7.472**	**1.192**	**7.448**	**1.211**	**7.568**
RSD %	**1.496**	**1.497**	**1.248**	**1.240**	**1.043**	**1.048**	**1.672**	**1.670**	**1.212**	**1.214**

Table 1: Nitrogen/Protein reproducibility of Canned Meat sample T0156

Table 2 shows the nitrogen/protein reproducibility of canned meat sample T0158. The sample was analyzed at different weights. All data obtained were inside the range specified in the FAPAS certificate.

Weight (mg)	400 - 500		500 - 600		600 - 700	
Analysis	N %	Prot %	N %	Prot %	N %	Prot %
Run	3.68	23.03	3.75	23.44	3.73	23.30
	3.76	23.47	3.75	23.46	3.77	23.55
	3.75	23.44	3.69	23.03	3.70	23.12
	3.69	23.03	3.73	23.29	3.78	23.62
	3.76	23.52	3.72	23.27	3.75	23.44
	3.75	23.42	3.75	23.45	3.71	23.21
	3.70	23.13	3.71	23.21	3.79	23.67
	3.73	23.31	3.75	23.47	3.77	23.57
	3.70	23.12	3.71	23.18	3.78	23.64
	3.76	23.48	3.68	22.99	3.76	23.48
Average %	**3.73**	**23.30**	**3.72**	**23.28**	**3.75**	**23.46**
RSD %	**0.86**	**0.85**	**0.71**	**0.76**	**0.82**	**0.81**

Table 2: Nitrogen/Protein reproducibility of canned meat sample T0158

Table 3 shows the nitrogen/protein reproducibility of canned meat samples T0160 and T0166. The samples were analyzed at two different weights. All data obtained were inside the range specified in the FAPAS certificate. The canned meat T0160 contained the highest concentration of fat and was the least homogenous.

Sample	FAPAS T0160				FAPAS T0166			
Weight (mg)	500 - 600		600 - 700		500 - 600		600 - 700	
Analysis	N %	Prot %	N %	Prot %	N %	Prot %	N %	Prot %
Run	2.33	14.58	2.31	14.42	0.833	5.205	0.829	5.180
	2.35	14.69	2.30	14.36	0.814	5.084	0.840	5.251
	2.29	14.32	2.35	14.69	0.814	5.088	0.850	5.314
	2.32	14.50	2.35	14.67	0.824	5.150	0.828	5.174
	2.29	14.32	2.34	14.65	0.837	5.232	0.847	5.293
	2.31	14.56	2.34	14.60	0.832	5.202	0.852	5.324
	2.32	14.48	2.29	14.33	0.839	5.243	0.829	5.180
	2.28	14.26	2.28	14.27	0.823	5.144	0.850	5.314
	2.27	14.19	2.30	14.36	0.834	5.215	0.850	5.311
	2.31	14.43	2.30	14.38	0.822	5.139	0.852	5.322
Average %	**2.31**	**14.42**	**2.32**	**14.47**	**0.827**	**5.170**	**0.843**	**5.266**
RSD %	**1.06**	**1.06**	**1.14**	**1.11**	**1.097**	**1.109**	**1.219**	**1.223**

Table 3: Nitrogen/Protein reproducibility of canned meat samples T0160 and T0166

Table 4 shows the comparison between the data obtained by FLASH 4000 and the values included in the relative FAPAS certificates. All data obtained were inside the range specified in the FAPAS certificate.

FAPAS	FAPAS Specification		FLASH 4000
Code	Assigned N%	Satisfactory Range N%	N %
T0156	1.20	1.16 – 1.24	1-18 - 121
T0158	3.65	3.52 – 3.78	3.72 – 3.75
T0160	2.26	2.18 – 2.35	2.31 – 2.32
T0166	0.823	0.794 – 0.853	0.827 – 0.843

Table 4 : Summarized results

Conclusion

The nitrogen/protein data obtained through this experiment are inside the tolerance range specified by the reference materials certificates, demonstrating the high performance of the FLASH 4000 Analyzer. The results also demonstrate excellent reproducibility, with no memory effect observed when changing the type of sample. This indicates the complete and accurate detection of the nitrogen present. As a complete automatic system, the FLASH 4000 Analyzer is able to accurately determine nitrogen content in a wide range from low to high concentrations without matrix effect and without the use of sample digestion or toxic chemicals.

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Technical Note: 42177

Analytical Comparison of the Thermo Scientific FLASH 4000 Nitrogen/Protein Analyzer with the traditional Kjeldahl Method

Dr. Liliana Krotz, OEA Product Specialist, and Dr. Guido Giazzi, OEA Product Manager, Thermo Fisher Scientific, Milan, Italy

Key Words

- Food analysis
- Food quality
- Food reference materials (Kjeldahl)
- Protein content
- TDF

Introduction

One of the most important nutrients of food and animal feed is protein. The quantity of protein required by animals is very precise as excess amounts lead to amino acid deficiency and generate unnecessary amounts of energy. As a consequence, the exact determination of protein amount in animal feed is fundamental in achieving high nutritional quality of animal feed and securing the safety of final food products intended for human consumption.

In the case of fish meal, for example, the analysis of nitrogen is critical for daily quality control of production and for specification in contracts. All fish meal is traded on its protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum quantity of protein content. If the amount of nitrogen is multiplied by a factor depending on the kinds of protein expected to be present in the food, then the total protein content can be determined.

Determination of nitrogen is also very important in dietary fibre analysis as part of the enzymatic-gravimetric method to correct the fibre residue values for protein. The precise and accurate determination of nitrogen is fundamental to assessing the nutritional quality of foods, such as ready-to-eat meals, processed foods, grain and cereal products, fruits and vegetables, chocolate, fish, meat and meat products, etc. Dietary fibre and proteins are both important values to label the nutrition facts and are used for the calculation of the total carbohydrates.

The globalisation of the food market requires accurate and reliable control of the characteristic of products for the protection of commercial value, but mainly to safeguard the consumer's health and manufacturer's reputation. Official regulations establish the protein content and labeling requirements which enable consumers to make price and quality comparisons based on % protein declarations.

For this reason the use of an accurate analytical technique is required. Furthermore, as the demand for improved sample throughput, reduction of operational costs and minimization of human errors is becoming every day more notable, it is very important to have a simple and automatic technique which allows fast analysis with an excellent reproducibility, and can avoid the risk of handling toxic chemicals.

An alternative to the classical Kjeldahl method, based on the Dumas (combustion) method, has been developed and approved by different associations (AOAC, AACC, AOCS, ASBC, ISO and IFFO). The Thermo Scientific FLASH 4000 Elemental Analyzer (Figure 1), based on the dynamic combustion of the material, requires no sample digestion or toxic chemicals, while providing important advantages in terms of time, automation and quantitative determination of nitrogen in a large range of concentration. This document presents the FLASH 4000 analyzer as a powerful analytical alternative to the Kjedahl method.

Figure 1: FLASH 4000 Nitrogen/Protein analyzer

Analytical Configuration

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 Autosampler together with a proper amount of oxygen, calculated by the Thermo Scientific OxyTune® function, which ensures a complete combustion of the sample.

After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper. The water is trapped through a water condensation drainage device while the CO_2 is adsorbed by the NoStop Twin CO_2 Traps. Then the nitrogen is swept through a GC column and is finally detected by a thermal conductivity detector (see Figure 2).

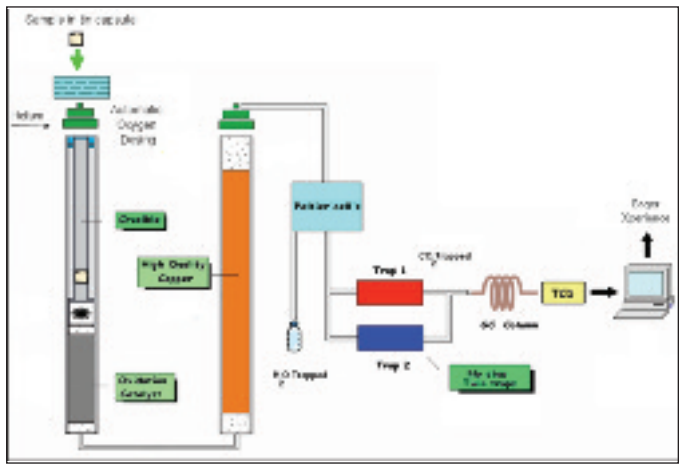


Figure 2: FLASH 4000 Nitrogen/Protein configuration

Analytical conditions:

Left reactor temperature: 950 °C
Right reactor temperature: 840 °C
Oven temperature: 50 °C
Standard: EDTA (9.59 % N)
EDTA: EthyleneDiamineTetraAcetic acid

Note: The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.

FLASH 4000 performance evaluation

The performance of the FLASH 4000 Analyzer was evaluated through the analysis of **FAPAS** (Food Analysis Performance Assessment Scheme, part of The Food and Environment Research Agency, an executive agency of the UK) and **BIPEA** (**B**ureau **I**nter**P**rofessionnel d'**E**tudes **A**nalytiques, France) Reference Materials. The results obtained were compared with the average and range indicated in the relative Reference Materials Certificates.

The validity of the results obtained by the FLASH 4000 Analyzer was evaluated through the participation in International Round Robin Tests such as **MAPS** (Malt Analytes Proficiency Testing Scheme, LGC Standards Proficiency Testing, UK). The data were compared with the range accepted by statistic studies with Kjeldahl, NIR and Combustion methods.

Results

Tables 1 and 2 show the sample information and the reproducibility of 10 consecutive analyses of **BIPEA Reference Materials**, using a sample weight of about **1000 mg**. The materials were characterized through a laboratory inter-comparison using Kjeldahl and Combustion methods. The protein factor used to calculate the protein content was the default value 6.25 present in the Thermo Scientific Eager Xperience software.

Four **FAPAS Canned Meat**, and one **FAPAS Animal Feed Reference Materials** were tested in basis of different content of nitrogen, content of fat and homogeneity. The data obtained (see table 3) demonstrate the no-matrix effect in the determination of nitrogen, indicating complete combustion for all type of samples. The samples were analyzed as received by the reference materials supplier.

Sample	Moisture	Fat	Carbohydrate	Kjeldahl Protein		Combustion Protein	
	%	%	%	Av. %	Tolerance	Av. %	Tolerance
BIPEA – Feed for Sow	9.8	2.8	48.7	16.0	0.6	16.2	0.6
BIPEA – Dehydrated Alfalfa	7.7		29.3	14.8	0.6	15.1	0.6
BIPEA – Hyperproteic Powder		0.8		85.4	3.4	86.4	3.5

Table 1: BIPEA sample information available

Sample	BIPEA – Feed for Sow		BIPEA – Dehydrated Alfalfa		BIPEA – Hyperproteic Powder	
%	N %	Protein %	N %	Protein %	N %	Protein %
	2.65	16.54	2.42	15.14	13.49	84.33
	2.63	16.47	2.46	15.36	13.46	84.12
	2.64	16.50	2.44	15.22	13.47	84.18
	2.61	16.32	2.43	15.20	13.48	84.26
	2.67	16.66	2.48	15.52	13.55	84.69
	2.69	16.78	2.47	15.41	13.51	84.41
	2.66	16.63	2.48	15.53	13.48	84.28
	2.65	16.56	2.47	15.44	13.45	84.08
	2.64	16.51	2.47	15.43	13.45	84.08
	2.67	16.68	2.48	15.50	13.46	84.15
Average %	2.65	16.56	2.46	15.38	13.48	84.25
RSD %	0.86	0.78	0.90	0.92	0.23	0.23

Table 2: Reproducibility of Nitrogen / Protein determination in BIPEA Reference Materials

FAPAS Matrix	FAPAS Code	FAPAS Specification		FLASH 4000
		Assigned N%	Satisfactory Range N%	N %
Canned Meat	T 0156	1.20	1.16 – 1.24	1-18 - 121
Canned Meat	T 0158	3.65	3.52 – 3.78	3.72 – 3.75
Canned Meat	T 0160	2.26	2.18 – 2.35	2.31 – 2.32
Canned Meat	T 0166	0.823	0.794 – 0.853	0.827 – 0.843
Animal Feed	PT 1089	2.32	2.19 – 2.45	2.39 – 2.43

Table 3: Nitrogen determination of FAPAS Canned Meat Reference Materials

		MAPS Ring Test					
Round No.	FLASH 4000 Analyzer	Kjeldahl		Combustion		NIR	
	Total N %	Total N %	Range %	Total N %	Range %	Total N %	Range %
163	1.78	1.76	1.70-1.82	1.79	1.73-1.85	1.79	1.71-1.87
164	1.52	1.54	1.48-1.60	1.55	1.49-1.61	1.55	1.47-1.63
165	1.38	1.39	1.31-1.49	1.40	1.35-1.44	1.40	1.32-1.48
166	1.63	1.65	1.59-1.71	1.67	1.61-1.73	1.67	1.59-1.75
167	1.39	1.36	1.27-1.41	1.35	1.25-1.48	1.41	1.33-1.49
169	1.89	1.83	1.77-1.89	1.86	1.86-1.92	1.86	1.78-1.94

Table 4: MAPS Total Nitrogen data of malt samples.

			MAPS Ring Test					
Round No.	FLASH 4000 Analyzer	NIR Analyzer	Kjeldahl		Combustion		NIR	
	Total N %	Total N%	Total N %	Range %	Total N %	Range %	Total N %	Range %
163	1.57	1.63	1.55	1.48-1.60	1.55	1.49-1.61	1.55	1.49-1.61
166	1.71	1.74	1.74	1.68-1.80	1.77	1.71-1.83	1.77	1.71-1.83
167	1.47	1.49	1.46	1.40-1.52	1.49	1.43-1.55	1.49	1.43-1.55
168	1.76	1.73	1.71	1.65-1.77	1.77	1.71-1.83	1.74	1.68-1.80
169	1.55	1.60	1.55	1.49-1.61	1.57	1.51-1.63	1.57	1.51-1.63

Table 5: MAPS Total Nitrogen data of barley samples.

			MAPS Ring Test			
Round No.	FLASH 4000 Analyzer	Spectrophotometric Method	Kjeldahl		Combustion	
	Soluble N %	Soluble N %	Soluble N %	Range %	Soluble N %	Range %
164	0.59	0.60	0.57	0.51-0.63	0.58	0.54-0.62
165	0.66	0.68	0.66	0.60-0.72	0.66	0.60-0.72
166	0.85	0.85	0.82	0.76-0.88	0.84	0.78-0.90
167	0.62	0.62	0.60	0.54-0.66	0.60	0.54-0.66
168	0.69	0.67	0.66	0.60-0.72	0.66	0.60-0.72
169	0.82	0.81	0.81	0.75-0.87	0.82	0.76-0.88

Table 6: MAPS Soluble Nitrogen data of wort samples

Tables 4, 5 and 6 show some Total Nitrogen and Soluble Nitrogen data of **malt, barley and wort** obtained during **MAPS Ring Test**. In each period (Round No.), a malt or barley sample was analyzed with the FLASH 4000 Analyzer. The nitrogen values obtained are inside in the range of nitrogen concentration approved by the MAPS statistic studies. The data are comparable with Kjeldahl, Combustion, NIR and Spectrophometric methods.

Table 7 shows a comparison between FLASH 4000 results and Kjeldahl method data of nitrogen/protein determination in fish meal samples. The values demonstrate a perfect correlation between both methods. The several fish meal samples were chosen in basis of the different country of origin and different nutritional composition (content of fat, salt, moisture and ashes). The data obtained demonstrate the no-matrix effect in the determination of nitrogen, indicating complete combustion for all type of samples.

Sample	FLASH 4000 Analyzer		Kjeldahl Method		Difference
	N %	Protein %	N %	Protein %	in Protein %
A	11.08	69.23	11.09	69.30	-0.07
B	9.62	60.14	9.60	60.00	+0.14
C	9.56	59.75	9.54	59.60	+0.15
D	9.74	60.85	9.73	60.80	+0.05
E	9.92	62.00	9.92	62.00	+0.00
F	9.77	61.07	9.78	61.10	-0.03
G	10.57	66.07	10.66	66.60	-0.53
H	11.00	68.74	10.94	68.40	+0.34
I	10.38	64.85	10.35	64.70	+0.15
J	10.93	68.34	10.92	68.20	+0.14
K	10.72	66.97	10.64	66.50	+0.47
L	10.57	66.02	10.48	65.50	+0.52

Table 7: FLASH 4000 vs Kjeldahl values of fish meal samples

Table 8 shows a nitrogen/protein comparison between the FLASH 4000 and Kjeldahl methods. The **food samples** were chosen due to the different content of nitrogen, fat, carbohydrate and moisture.

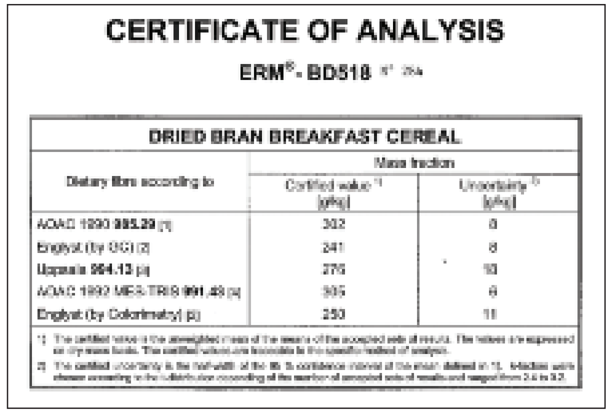
Sample	FLASH 4000 Analyzer		Kjeldahl Method	
	N %	Protein %	N %	Protein %
Biscuits with chocolate	1.18	7.37	1.15	7.19
Animal feed	2.64	16.50	2.62	16.37
Fish feed	9.59	59.94	9.63	60.19
Grapes	0.52	3.25	0.51	3.19
Cattle food	8.14	50.87	8.12	50.75
Ham 1	2.53	15.81	2.55	15.94
Ham 2	2.95	18.44	2.89	18.06
Meat loaf	2.02	12.62	1.97	12.31
Wheat 1	1.62	10.15	1.65	10.30
Wheat 2	1.65	10.33	1.64	10.31
Flour 1	1.77	11.07	1.78	11.10
Flour 2	1.34	8.40	1.32	8.24
Bread	1.91	11.92	1.92	12.00
Biscuit 1	0.88	5.51	0.87	5.45
Biscuit 2	1.41	8.80	1.39	8.72
Biscuit 3	1.36	8.51	1.34	8.37
Coffee granules A	3.07	19.18	3.06	19.15
Coffee granules B	3.81	23.83	3.82	23.85
Coffee granules C	2.57	16.09	2.54	15.85
Raw Egg	1.97	12.31	1.96	12.25
Essence of chicken	0.80	5.03	0.82	5.15
Milk powder	1.93	12.33	1.92	12.24

Table 8: Comparison FLASH 4000 vs Kjeldahl values of food samples

Regarding the trueness of the FLASH 4000 Analyzer in comparison to the Kjeldahl method for the determination of nitrogen in dietary fibre analysis (celite), a certified Reference Material was tested. Table 9 shows the reproducibility and comparison of TDF (Total Dietary Fibre) data obtained in comparison with Kjeldahl and the values indicated in the certificate (Figure 3), while Table 10 shows the reproducibility and comparison of TDF data in peas, crackers and lasagna samples obtained with both methods.

Kjeldahl method % TDF	FLASH 4000 Analyzer % TDF
29.9	30.2
29.9	30.3
29.9	30.4
30.3	30.9
30.9	31.0

Table 9: Reproducibility & comparison of TDF data



CERTIFICATE OF ANALYSIS

ERM®- BD518

DRIED BRAN BREAKFAST CEREAL		
Dietary fibre according to	Mass fraction	
	Certified value [1] [g/kg]	Uncertainty [2] [g/kg]
AOAC 1990 985.29 [1]	302	8
Englyst (by GC) [2]	241	8
Uppsala 994.13 [3]	276	10
AOAC 1992 MES-TRIS 991.43 [4]	305	8
Englyst (by Colorimetry) [5]	250	11

Figure 3: TDF Reference Material Certificate

Peas		Crackers		Lasagna	
Kjeldahl % TDF	FLASH 4000 % TDF	Kjeldahl % TDF	FLASH 4000 % TDF	Kjeldahl % TDF	FLASH 4000 % TDF
5.2	5.6	4.4	4.4	1.3	1.2
5.5	5.9	4.6	4.4	1.4	1.2
5.6	6.0	4.7	4.5	1.4	1.3
5.8	6.0	4.7	4.6	1.5	1.3
5.9	6.3	4.8	4.8	1.6	1.7
6.5	6.7				

Table 10: Determination of TDF in peas, crackers and lasagne samples

Conclusion

The results showed demonstrate that the Thermo Scientific FLASH 4000 Analyzer is the best solution for nitrogen determination offering excellent reproducibility, with no memory effect observed when using large sample weight. The data obtained through the use of the instrument were within the tolerance in the Reference Materials Certificates and in the range accepted for Kjeldahl, NIR and Combustion methods included in the Round Robin Tests, indicating the high performance of the system. Providing an ideal solution for nitrogen determination, the system offers consistent results comparable to the data obtained to the Kjeldahl method, while also removing the requirement for sample digestion or toxic chemicals. At last, as a complete automatic system based on the combustion of the sample, the FLASH 4000 is approved by multiple official organizations and is able to accurately analyze nitrogen in a wide range from low to high content without matrix effect.

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Technical Note: 42178

Technical Comparison of the Thermo Scientific FLASH 4000 Nitrogen/Protein Analyzer with the traditional Kjeldahl Method

Dr. Liliana Krotz, OEA Product Specialist, and Dr. Guido Giazzi, OEA Product Manager, Thermo Fisher Scientific, Milan, Italy

Key Words

- Combustion method
- Food and Animal Feed
- Kjeldahl method
- Official methods
- Protein analysis

Introduction

One of the most important nutrients in human food and animal feed is protein. The precise determination of protein amount, through the determination of nitrogen, is fundamental to achieving high nutritional quality of raw and finished products. For many years, the Kjeldahl method has been the internationally-recognized method for estimating protein concentration. However, due to its several disadvantages, the capabilities of the combustion method have been greatly improved to make it faster, safer and more reliable than the traditional method. In this way, the Thermo Scientific FLASH 4000 N/Protein Analyzer, based on the dynamic flash combustion of the sample, offers many features and benefits to make it an excellent alternative to the classical procedure.

This article shows a comparison between the FLASH 4000 Analyzer (Figure 1) and the conventional Kjeldahl methodology from a technical point of view and highlights the industry associations who have approved the capabilities of the dynamic flash combustion method.

Figure 1: FLASH 4000 Nitrogen/Protein analyzer

Description of the Methods

Kjeldahl Method

The Kjeldahl method is a multi-stepped process, including sample digestion in boiling sulfuric acid, neutralization with sodium hydroxide solution, distillation of the resulting ammonia gas into a trapping solution, titration with an acid solution and determination of the amount of nitrogen and protein by calculation. The entire process may require many hours to be completed while all steps, excluding digestion, require continuous technician contribution. In addition, handling boiling sulfuric acid, especially with the addition of concentrated caustic solution, is a particularly hazardous task. Kjeldahl analyses also generate toxic waste since they involve the use of mercury or selenium catalysts during the digestion step.

FLASH 4000 Nitrogen/Protein Method

The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 4000 Autosampler together with a proper amount of oxygen using the Thermo Scientific OxyTune® function, insuring a complete combustion of the sample.

After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper. The water is trapped through a water condensation drainage device while the CO2 is adsorbed by the NoStop Twin CO2 Traps. The nitrogen is then swept through a GC column and is finally detected by a thermal conductivity detector (see Figure 2).

Analytical conditions:

Left Furnace Temperature : 950°C
Right Furnace Temperature : 840°C
Oven Temperature: 50°C
Standard: EDTA (9.59 %N)
EDTA: EthyleneDiamineTetraAcetic acid

Note: The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Thermo Scientific Eager Xperience software.

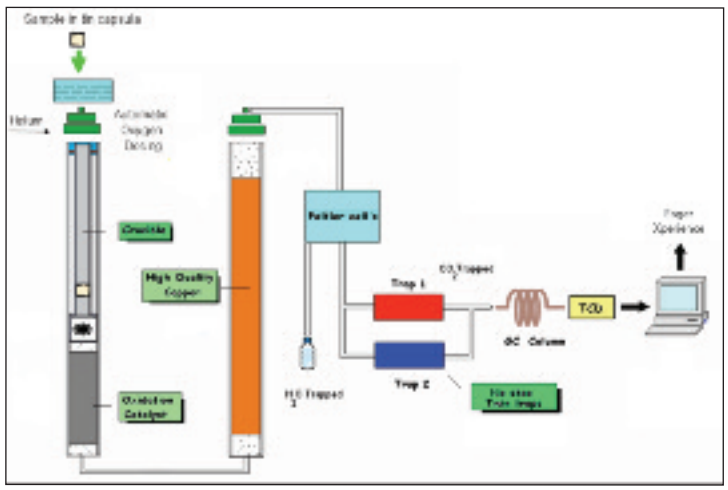

Figure 2: FLASH 4000 Nitrogen/Protein configuration

Technical Comparison

Information	Kjeldahl Method	FLASH 4000 Analyzer
Range of sample weight	500 – 1000 mg (2000 mg)	500 -2000 mg (or more)
Procedure steps	Sample preparation-weighing-digestion-distillation-titration-calculations-results.	Sample preparation-weighing-analysis-results
Timing		
Warming up	10 - 45 minutes (once a day)	30 minutes (from St-by condition). Warming up is not needed using Wake-up automatic function.
Preparation of the sample	5 – 20 minutes	5 - 20 minutes This can be done when the system is analyzing samples (not possible with Kjeldhal)
Preparation of reagents	Manual preparation: 10 - 20 min,	Filling of two reactors: 20 min
Digestion	2 - 6 hours	No
Cool Down	20 minutes - 2 hours	No
Nitrogen analysis time	10 minutes	7 - 10 minutes.
Other steps (specify)	5 - 10 minutes (distillation/tritation, washing of distillation system)	7 - 10 minutes
Total time for one N determination (excluding the warming up)	3 - 10 hours	7 - 10 minutes/sample (samples can be weighing during a sequence of analyses)
Safety (specify chemicals used)	High cost Concentrated acids at boiling temperature Toxic catalyst and chemicals Glass tubes Consumables: H_2SO_4 96-98%, NaOH 40 %, H_3BO_3 3%, HCl 0.1N, $CuSO_4.5H_2O$, $ZnSO_4$ KSO4, H_2SO_4 0.1N, NaOH 0.1N, Acetanilide 10 N NaOH in a 25 litre vessel	Low cost No fumes, acids and toxic reagents No atmospheric pollution Special stainless steel tubes Consumables: EDTA, Glucose, CuO-Pt on alumina, copper, quartz wool, ceramic disks The separation column is **not** a consumable
Waste disposal	Alkali waste High cost Large amount ***Note: destruction of 1 batch of 20 tubes takes almost 3 hours***	Ashes in the crucible (the amount of ashes depends of the sample nature). Exhausted catalyst and copper
Equipment		
Lifetime of instrument	Moderate lifetime due to acidic environment Frequent servicing every 3-4 months	Long lifetime of instrument
Leakage problems	Often rubber tubing breaks, leading to leaking	None. Automatic Leak Test (Eager Xperience software function) is performed after maintenance.
Damage to safety cabinet	Slight damage due to accident spillage of corrosive chemicals	No
Laboratory requirements	Workplace often looks like a mess	Clean workplace
Anti acid table	Yes	No
Need of chimney	Yes	No
Gases	No	Yes, helium and oxygen
User knowledge	High Basic laboratory and chemistry knowledge. Knowledge of tritrimetric methods Lab technician, analytical training needed Labor intensive	Low Basic laboratory and chemistry knowledge Basic knowledge in gas chromatography Lab technician, analytical training needed, instrument operation and maintenance training needed.

Information	Kjeldahl Method	FLASH 4000 Analyzer
Maintenance	High Regular maintenance of sealings, reagent pumps, glass parts of the system. Distillated system, washing of tubes and filters every 3 months. Frequent replacement of rubber tubings and modules (hot block digestion). Manual cleaning of glass tubes Daily care of instrument	Low Catalyst and copper lifetime: about 1000 runs Cleaning the ashes into the crucible: 80 - 120 runs. Maintenance scheduled in the Eager Xperience software, automatic signal when needed Large number of runs with same reactors Easy to maintain: special stainless reactors and crucible for faster ash removal
Capacity (number of analysis in continuous cycle)	6 -20 by day The capacity is limited by the digestion time	1 drum for 30 samples With 3 extra drums, the FLASH 4000 can reach 124 samples.
Automation and unattended analysis (number of analysis)	Not automated. There is no automation in digestion/distillation No automatic sample loading	Automatic autosampler MAS 4000 Truly unattended operation Ability to add extra samples during the analyses
Quantitative recovery of N	Matrix problems Incomplete N recovery from some samples even after hours of digestion	Total conversion of organic and inorganic material to elemental gases. Results unbiased by sample matrix due to the flash combustion method.
Manual and/or automatic protein calculation	Manual (Excel file) In house spreadsheet for keying in data and final protein calculation	Dedicated Software Eager Xperience, automatically calculates the protein content. It is possible to use different protein factors according to sample nature.
Software	No dedicated software	Eager Xperience dedicated software with the following features: Stand-By, Wake-Up and Auto-Start automatic functions Maintenance program OxyTune® option to calculated in automatic the amount of oxygen necessary for a complete combustion of the sample Automatic transfer of the weight from the balance Standard and personal reports Automatic calculation of the Protein % using different protein factors Automatic protein corrections according to the humidity value Automatic Leak Test

Official Methods

The combustion method, principle of the FLASH 4000 Analyzer, has been approved and adopted by several International Associations.

Official methods	Performance Requirements
AACC (American Association of Cereal Chemists). Crude Protein in Cereal, 46-30, 1999.	The system must be capable of measuring nitrogen in ***materials containing 0.2-20 %N.*** The accuracy is demonstrated by making 10 successive determinations of nitrogen in nicotinic acid and lysisne-HCl. Samples must be ground to ***Suitable granularity*** (which is different for each material analyzed) 1. to achieve precision 2. which gives ***RSD of*** ≤ ***2%*** for 10 successive determinations of nitrogen.
AOAC (Association of Official Analytical Chemists). Official Method 990.03. Protein (crude) in Animal Feed 4.2.08	The system must be capable of measuring nitrogen in ***feed materials containing 0.2-20 %N***. The accuracy is demonstrated by making 10 successive determinations of nitrogen in nicotinic acid and lysisne-HCl. ***Suitable granularity*** is that which gives ***RSD*** ≤ ***2%*** for 10 successive determinations of nitrogen in mixture of corn grain and soybeans (2:1) that has been ground for analysis. ***Granularity (0.5 mm)*** required to achieve this precision must be used for all mixed feeds and other nonhomogeneous materials.
AOAC (Association of Official Analytical Chemists). Official Method 992.15. Crude Protein in Meat & Meat Products including Pet Foods 39.1.16	Applicable to meat and meat products (including pet foods) with 10-20% crude protein. Pass samples through ***grinder 2x*** in succession for emulsified meat products; mix thoroughly after each grinding. ***Pass 3x*** in succession for nonemulsified (coarse or whole muscle) products.
AOAC (Association of Official Analytical Chemists). Official Method 992.23. Crude Protein in Cereal Grains & Oilseeds 32.2.02	The system must be capable of measuring nitrogen in ***materials containing 0.2-20 %N***. The accuracy is demonstrated by making 10 successive determinations of nitrogen in nicotinic acid and lysisne-HCl or tryptophan. Grind samples to ***suitable granularity*** (determined for each different material analysed) to obtain ≤ ***2% RSD*** for 10 successive nitrogen determinations for that material type. Nitrogen is converted to protein using the conventional factor of 6.25 (5.70 in case of wheat).
ASBC (American Society of Brewing Chemists). Official method 1996. Nitrogen determination in Barley.	***All samples must be milled*** before analysis Refs 1-3. Total Nitrogen is converted to protein using the conventional factor of 6.25.
ASBC (American Society of Brewing Chemists). Total Nitrogen in wort and beer by combustion method.	***No sample preparation*** is needed.
AOAC (Association of Official Analytical Chemists). Official Method 993.13. Nitrogen (Total) in Fertilizers 2.4.02	The system must be capable of measuring nitrogen in ***fertilizer materials containing 1-67 %N***. Grind samples to ***suitable granularity*** to give ***RSD*** ≤ ***1%*** for 10 successive nitrogen determinations. Moisture content of solid fertilizer test sample must be same before and after grinding to ensure correct analytical result.
AOCS (American Oil Chemists Society). Official Method Ba 4e-93 (revised 1995). Combustion method for determination of Crude Protein.	The system must be capable of measuring nitrogen in ***materials containing 0.2-20 %N***. The accuracy is demonstrated by making 10 successive determinations of nitrogen in nicotinic acid and lysisne-HCl which gives relative standard deviation (***RSD)*** ≤ ***2%*** for 10 successive determinations of nitrogen in mixture of corn grain and soybeans (2:1) that has been ground for analysis. ***Fineness (0.5 mm)*** required to achieve this precision must be used for all mixed feeds and other nonhomogeneous materials.
IFFO (International Fishmeal & Fish Oil Organization Ltd)	IFFO has recommended that its members adopt the Dumas method as an official method for nitrogen and crude protein determination.

Conclusion

The Thermo Scientific FLASH 4000 Analyzer demonstrates the best solution for nitrogen determination as alternative to the traditional Kjeldahl method because is a complete automatic system which is very easy to use and easy to maintain. The system's ability to accurately analyze nitrogen in every food and animal feed without matrix effects, and without the use of sample digestion or toxic chemicals, is also demonstrated. As a combustion system, the FLASH 4000 is approved by multiple official organizations allowing also the determination of nitrogen not only for food and feed sample but also in: soil, leaves, plants, compost, feaces, urine, polymers, oils, etc.

The protein content is automatically calculated in using different protein factors through the dedicated software Eager Xperience.

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Thermo Scientific FLASH 4000 N / Protein analysis Bursting with productivity

Beyond the Kjeldahl Method

Food

Beverages

Animal Feed

Thermo SCIENTIFIC

FLASH 4000 N / Protein analysis
Bursting with productivity

FLASH 4000

N/Protein Analyzer

Expand your laboratory's capabilities with an automated, accurate and reliable method to determine Nitrogen/Protein in any type of food and beverages using large sample amounts - up to 2g!

The Thermo Scientific FLASH 4000 is a step forward for determination of N/Protein concentration in any food matrix.

Based on the Dynamic FLASH Combustion technique – well known as a modified Dumas method – the FLASH 4000 is the optimum choice for Quality Control and R&D environments in the food industry.

With proven accuracy and unbeatable cost-per-analysis, the FLASH 4000 gives lab managers an automated 24/7 solution for solid and liquid samples that they can be confident in.

Based on more than 40 years experience in the OEA field, incorporating the Carlo Erba and Fisons Instruments, the FLASH 4000 model is the new generation of high quality and productive Thermo Scientific Organic Elemental Analyzers.

Bursting with Productivity

now with a large sample volume capacity

Beyond Kjeldahl

The classical Kjeldahl Nitrogen method (involving sample oxidation by digestion in sulphuric acid followed by distillation and off-line titration measurements), is extremely time-consuming, expensive, and requires laboratory safety measures.

As Kjeldahl becomes inadequate in meeting international safety rules, many official methods (e.g. AOAC, AOCS, AACC), support the Dynamic Flash Combustion/ modified Dumas method as an alternative to the well known Kjeldahl method. Furthermore, the Kjeldahl method suffers from high waste cost, the inability to operate continuously, and is dependent on the user's experience and capabilities.

The FLASH 4000 N/Protein analyzer overcomes all concerns of the Kjeldahl method, by reducing sample preparation. This in turn eliminates safety concerns, operator experience requirements, costs of preparation materials and most significantly, the time involved.

Reduces Kjeldahl analysis time from *hours* to *minutes!*

Automation

for today's busy laboratories

Simplicity in design ensures accurate results for any material requiring Nitrogen / Protein determination. The simple configuration of the FLASH 4000 includes a sample loader, oxidation and reduction furnace, a Peltier system for water elimination, a regenerative CO_2 trap (patent pending) for eliminating the CO_2 produced during the combustion, a GC separation column and a thermal conductivity detector.

The large sample capacity of the FLASH 4000 model combined with the unique Flash combustion principle ensures optimum conversion of Nitrogen bounded compounds into elemental gases without dilution or splitting of the gases. This reduces handling time and increases accuracy.

Automated running

The **Thermo Scientific MAS 4000 Autosampler** provides fully automatic running of 31 solids samples (loaded in Tin capsules to eliminate contamination). The multiple tray concept of the MAS 4000 sampler can enhance productivity to a capacity of 124 sample positions.

Automated maintenance

Patent pending **Regenerative CO_2 Traps** improve the autonomy of the FLASH 4000. Consisting of two CO_2 traps which are automatically activated, the traps are always ready to adsorb any CO_2 generated during the flash combustion and so increase the up-time of the analyzer.

Cost savings

for the conscientious lab manager

Water condensation drainage device

To remove water out of the combustion gases, the FLASH 4000 is fitted with a Peltier device. As this eliminates the need to use and purchase solid adsorbers, operating costs are reduced.

Electronic Flow Controller

Reproducibility, performance and accuracy are increased with the FLASH 4000 by the presence of an **Electronic Flow Controller (EFC)** which regulates gas flow. The EFC system is also connected to the dedicated Eager Xperience software, allowing users to perform an Automatic Leak Check Test.

FLASH 4000 N / Protein analysis
Bursting with productivity

Xperience simplicity

Thermo Scientific Eager Xperience Software

Straightforward Operation

Simply place the sample container in the MAS4000 autosampler and press the start key. The analytical conditions (Flows, Temperature, Timing, Oxygen injection etc.) are controlled and evaluated by the Eager Xperience according to the sample nature and the sample weight.

New Levels of Automation – and Cost Savings!

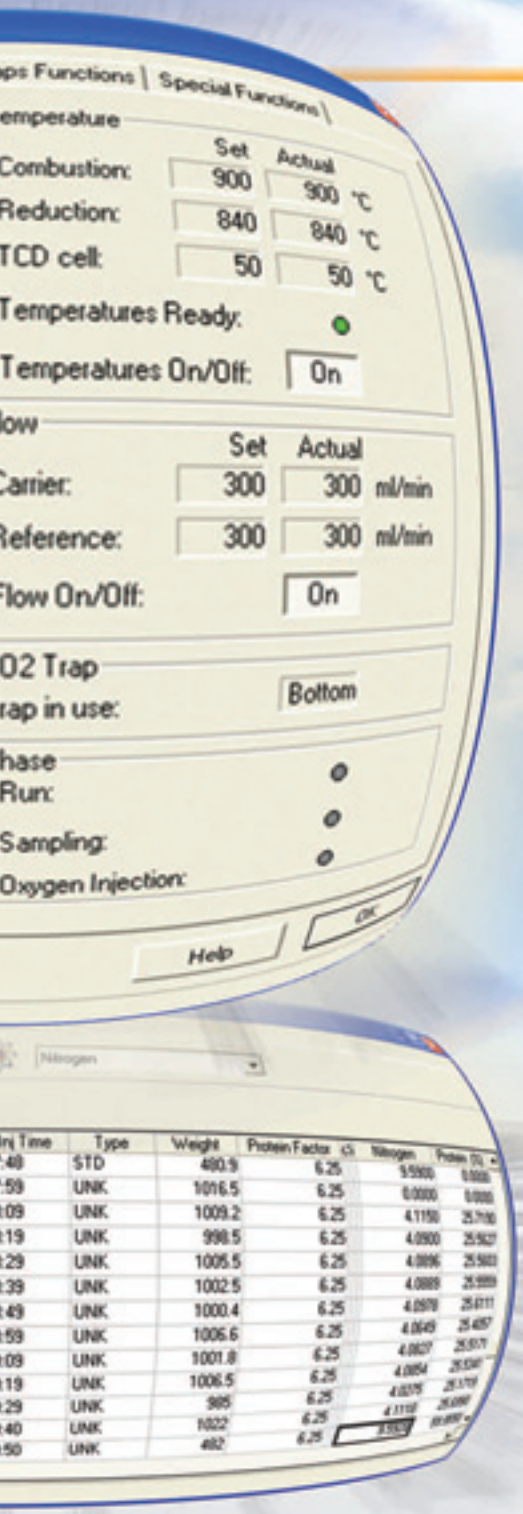
According to the sample nature and sample weight, the ***Thermo Scientific OxyTune®*** function automatically computes the quantity of Oxygen required to achieve complete combustion of the material without any user input. This drastically increases the catalyst lifetime: reducing the instrument downtime for maintenance and saving significant costs per analysis.

Other cost saving functions are **Auto-Ready, Auto-Off, Auto-Start and Auto-Standby**, which allow un-attended

24/7 operation.

Simple and Precise Results

At the end of the analytical cycle, the dedicated Eager Xperience software reports the results as **Nitrogen percent and/or Protein percent** using an operator selected protein conversion factor. Protein conversion factors can be changed according to different types of food applications.



Calibration Flexibility

Results can be evaluated using a standard calibration system, as prescribed in official methods (such as AOAC, AOCS, ASBC, AACC*) or a multiple standard linear regression calibration.

Easy to Control

Users often prefer visual aids when performing Quality Control of the results.
Average data visualization provides control and variation analysis at-a-glance. This visual graphic can be used for preparing a complete bespoke analytical report.

QC Functionality

Evaluating if the measured Nitrogen concentration is within the acceptable control limit range could not be easier with our **Red/Green Light Indicators**. The red/green light function provides an on-sight YES/NO result, which is ideal for QC analysts who need answers fast. The Control Limits can be pre-set by the user according to the characteristic of the compound, the sample nature and the lab precision required.

Maintaining Productivity

Pre-program the maintenance of the instrument and monitor the status of the crucible, oxidation and reduction catalysts at any time. A colour change from green to yellow indicates the catalyst usage, while the red suggests to the user that the maintenance needs to be performed.

Typical results

FLASH 4000 N / Protein determination - Applications

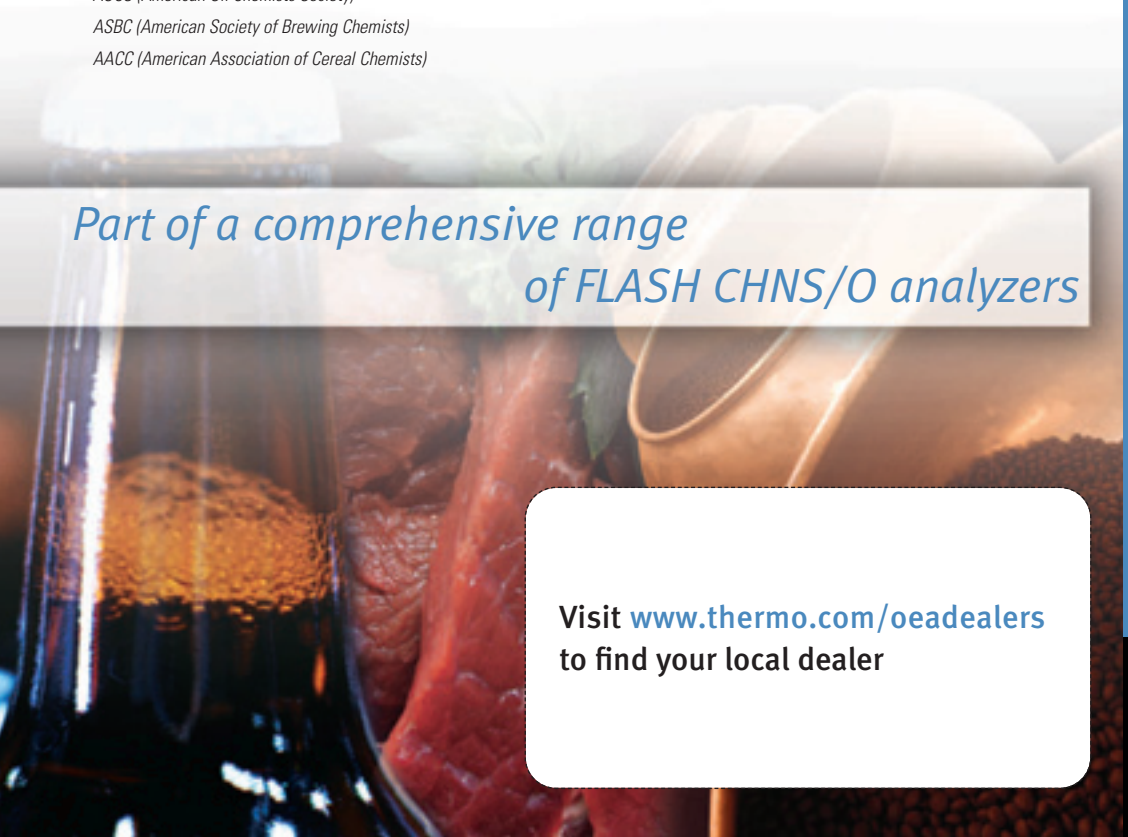
SAMPLE	RANGE WEIGHT (MG)	N %	PROTEIN %	RSD %
Pasta	500 - 1800	1.9330	12.0812	0.1551
Starch	800 - 2000	0.2527	1.5794	1.0520
Cocoa	300 - 1200	3.7662	23.5387	0.1883
Wheat flour	500 - 1800	1.8165	11.3529	0.5967
Corn flour	500 - 1800	1.2630	7.8936	0.6431
Rice flour	500 - 1800	1.5408	9.6301	0.4682
Soy flour	500 - 1800	5.4548	34.0925	0.4039
Rice	600 - 1600	1.2017	6.8500	0.5362
Barley	600 - 1600	1.9467	12.1669	0.8790
Red beans	600 - 1600	4.0262	25.1641	0.4432
Green Peas	600 - 1600	4.0241	25.1507	0.4060
Chickpeas	600 - 1600	3.5462	22.1637	0.5213
Lentils	600 - 1600	4.0196	25.1227	0.6970
Sunflower	500 - 1200	3.0640	19.1500	0.6546
Ham	400 - 2500	2.8204	17.6278	1.0746
Salame	500 - 1800	4.4888	28.0552	1.3629

Other market applications which are covered by the FLASH 4000 are:
- Baby and Diet Food applications
- Breweries and Beverages
- Agronomy
- Material Characterization

FLASH 4000 Validation

A comprehensive FLASH Validation kit ensures quick and efficient validation to meet stringent prerequisites required for the different Food Industry areas.

** : AOAC (Association of Official Analytical Chemists)*
AOCS (American Oil Chemists Society)
ASBC (American Society of Brewing Chemists)
AACC (American Association of Cereal Chemists)



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Application Note: 42156

Thermo Scientific FLASH 2000 Sulfur Analyzer for Agronomy

Luigi Ragaglia, Dr. Liliana Krotz and Dr. Guido Giazzi, Thermo Fisher Scientific, Milan, Italy

Key Words

- Easier sample handling
- Automated, unattended analysis
- Fast analysis: less than 4 minutes
- Low cost per analysis
- Reliable results

Introduction

Sulfur is an essential component of living matter. A deficiency of sulfur has an influence in the growth of vegetables, in the quality of proteins through the synthesis of amino acids such as cysteine, cystine and methionine, synthesis of vitamins and also has an effect on the formation of chlorophyll. Additionally sulfur in leaves can be considered an indicator of pollution, such as acid rain. Sulfur content in pine needles gives an accurate evaluation of this phenomenon.

The importance of soil and plants testing has grown dramatically in recent years and many of the classical methods are now no longer suitable for routine analysis.

Analytical instruments based on the combustion of samples improve the reliability of the data available to the agronomist, without the use of hazardous chemicals.

The Thermo Scientific FLASH 2000 Sulfur Analyzer delivers all the requirements of modern laboratories such as accuracy, high sample throughput and low cost per analysis.

Description of the Analytical Method

The FLASH 2000 Analyzer operates according to the dynamic flash combustion technique. The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 200R Autosampler together with a proper amount of oxygen.

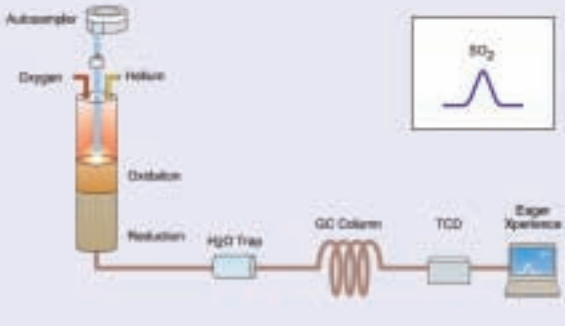
After combustion of the sample, the reaction gas products are carried by a helium flow to a layer filled with copper, then through an H_2O trap, a GC column and finally detected by a Thermal Conductivity Detector. Total run time is less than 4 minutes.

A complete sulfur report is automatically generated by the Thermo Scientific Eager Xperience dedicated software and displayed at the end of the analytical cycle.

Analytical Conditions

Combustion/Reduction reactor temperature: 950°C
Oven temperature: 65 °C
Helium flow rate:
Measurement: 140 ml/min
Reference: 100 ml/min
Oxygen flow rate: 250 ml/min
Total run time: 4 minutes
Sample weight: 1-20 mg
Calibration Method: K factor

Analytical Layout of FLASH 2000 Sulfur Analyzer



Results

To validate the system from trace levels to percent of sulphur, different types of soils, leaves and plant samples were chosen.

The data obtained when analyzing NIST Standard Reference Materials ten consecutive times is reported in Table 1.

The data shows excellent reproducibility and is in agreement with NIST certification.

Montana Soil – SRM 2711	
Weight (mg)	**S %**
12.136	0.0469
15.267	0.0433
10.824	0.0443
14.981	0.0427
15.139	0.0442
14.776	0.0422
13.645	0.0428
14.873	0.0440
11.229	0.0429
14.549	0.0458
Average	0.0439
Std. Dev.	0.0015
RSD %	3.3753
NIST Value	0.042 ± 0.001

Tomato Leaves – SRM 1573a	
Weight (mg)	**S %**
4.325	0.9779
4.982	0.9899
4.877	0.9642
4.367	0.9848
4.903	0.9983
4.255	0.9816
4.609	0.9938
4.025	0.9970
4.786	0.9776
4.865	0.9805
Average	0.9845
Std. Dev.	0.0105
RSD %	1.0648
NIST Value	0.96

Montana Soil – SRM 2711	
Weight (mg)	**S %**
4.546	0.1929
4.728	0.1907
4.091	0.1898
4.998	0.1913
4.482	0.1874
4.538	0.1973
4.906	0.1881
4.779	0.1908
4.965	0.1916
4.183	0.1980
Average	0.1918
Std. Dev.	0.0035
RSD %	1.8188
NIST Value	0.20

Table 1: Sulfur determination of NIST Standard Reference Materials.

Note: Sulfur NIST (National Institute of Standards and Technology, Maryland, USA) value is certified only for Montana Soil, for Tomato and Peach Leaves the sulfur value are provided for information only, they are not certified.

Table 2 shows the consistent data obtained when duplicating sulfur analysis in different soil samples. The range of sample weight was 10-15 mg, and samples were homogenized with a ball mill.

Sample	S %	Av. S %	RSD %	Sample	S %	Av. S %	RSD %
Soil 1	0.0492 0.0510	0.0501	2.5405	Soil 5	0.0330 0.0339	0.0334	1.9025
Soil 2	0.0185 0.0181	0.0183	1.5456	Soil 6	0.0209 0.0215	0.0212	2.0012
Soil 3	0.0367 0.0372	0.0369	0.9568	Soil 7	0.0426 0.0422	0.0424	0.6671
Soil 4	0.0219 020212	0.0215	2.2969	Soil 8	0.0233 0.0227	0.0230	1.8446

Table 2: Reproducibility of sulfur determination in soil samples

Table 3 shows the data obtained for sulfur determination in soya and corn. The samples were homogenized at 1 mm particle size with a rotor speed mill, and the sample weight used was 4-5 mg. Reproducibility was excellent and no memory effect was observed when changing sample matrix.

Sample	S %	Av. S %	RSD %	Sample	S %	Av. S %	RSD %
Soya 1	0.3558 0.3361 0.3381 0.3498 0.3430 0.3410	0.3439	2.1759	Corn 1	0.1152 0.1139 0.1164 0.1130 0.1126 0.1064	0.1129	3.0904
Soil 2	0.3508 0.3443 0.3432	0.3461	1.1867	Corn 2	0.1195 0.1176	0.1185	1.1332
Soya 3	0.3720 0.3728 0.3627	0.3691	1.5209	Corn 3	0.1043 0.1042	0.1042	0.0678
Soya 4	0.3657 0.3632 0.3645	0.3645	0.3430	Corn 4	0.1117 0.1095	0.1106	1.4065
				Corn 5	0.1024 0.0995	0.1009	2.0313

Table 3: Reproducibility of sulfur determination in Soya and Corn samples

Conclusion

- Excellent reproducibility.
- Large range of sulfur detection.
- No matrix effect when changing the type of sample and the content of Sulphur, indicating complete detection of the element.

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Application Note: 42157

Thermo Scientific FLASH 2000 Protein Analyzer for Cereals and Beans

Dr. Liliana Krotz, Luigi Ragaglia and Dr. Guido Giazzi, Thermo Fisher Scientific, Milan, Italy

Key Words

- Adopted by AACC, method 46-30
- Approved by AOAC, method 990.03
- Beans
- Cereal
- Soya
- Fast analysis: less than 5 minutes

Introduction

Cereals and beans are some of the most widely grown crops globally because they are the main component of the human diet and the principal part of feeding stock for domestic animals.

One of the most important nutrients is protein and the monitoring of the amount of Nitrogen, must be accurate to determine the nutritional quality of these products. In addition to its dietary importance, protein content also has become a quality guideline for some cereals trade transactions.

For this reason, modern advances in instrumentation have greatly improved the capabilities of the combustion method making it faster, safer and more reliable than the traditional Kjeldahl method.

As a direct consequence of these advantages the combustion method was approved and adopted by the Association of Official Analytical Chemists (AOAC method 990.03) and American Association of Cereal Chemists (AACC method 46-30).

The Thermo Scientific FLASH 2000 Protein Analyzer copes with a wide array of additional important requirements of modern laboratories such as accuracy, high sample throughput and low cost per analysis.

Description of the Analytical Method

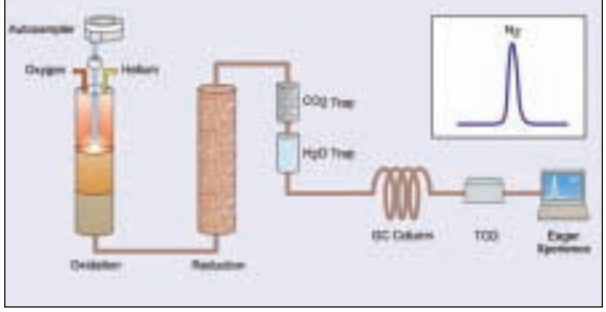
The FLASH 2000 Analyzer is based on the dynamic flash combustion technique. The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 200R Autosampler together with a proper amount of oxygen determined by the Thermo Scientific OxyTune® function.

After combustion of the sample, the produced gases are carried by a helium flow to a second reactor filled with copper, then through CO_2 and H_2O traps, a GC column and finally detected by a Thermal Conductivity Detector. A complete report is automatically generated by the Thermo Scientific Eager Xperience dedicated software.

Analytical Conditions

Combustion temperature: 950°C
Reduction temperature: 840°C
Oven temperature: 50 °C
Helium flow rate:
Measurement: 140 ml/min
Reference: 100 ml/min
Oxygen flow rate: 300 ml/min
Total run time: less than 5 minutes
Nominal sample weight: 200-300 mg
Standard: 50-100 mg Aspartic acid
Calibration Method: K factor

Analytical Layout of FLASH 2000 Protein Analyzer



Results

The samples were homogenized with a rotor speed mill (particle size 1 mm) and dried at 130°C for 1 hour. The calibration was performed with Aspartic acid using the K factor as calibration method. The protein content is calculated using the protein factor 6.25.

The reproducibility obtained analyzing a soya sample is reported in Table 1. No significant differences in nitrogen and protein values were observed when changing the weight of sample from 100 to 300 mg.

Weight (mg)	N %	Protein %
103.4	7.89	49.34
205.9	7.96	49.74
296.6	7.92	49.49
217.4	7.95	49.67
189.8	7.92	49.50
200.2	7.85	49.06
232.4	7.88	49.28
230.2	7.95	49.70
188.5	7.85	49.06
211.8	7.88	49.26
178.9	7.86	49.13
216.0	7.94	49.61
188.0	7.93	49.55
185.1	7.86	49.12
242.5	7.84	49.00
186.7	7.87	19.19
202.9	7.89	49.33
218.0	7.99	49.97
198.7	7.97	49.82
204.0	7.86	49.11
220.1	7.98	49.85
206.2	7.85	49.05

Table 1: Nitrogen/Protein reproducibility of Soya sample

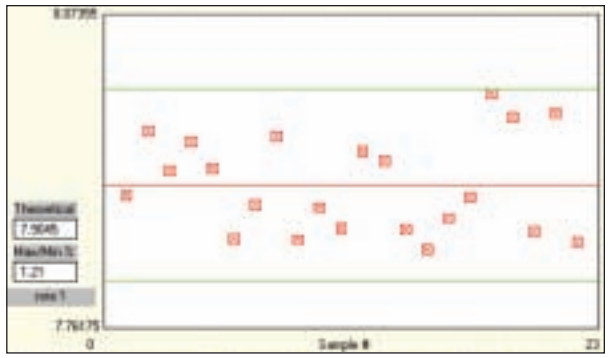


Figure 1: Statistical plot of Nitrogen data

Figure 1 shows the statistical plot of the data. The excellent fluctuation obtained demonstrates the stability of the system and the reproducibility of the results.

Statistical Data:
Number of runs: 22

Nitrogen:
Average %: 7.90
Std. Dev.: 0.048
RSD %: 0.610

Protein:
Average %: 49.40
Std. Dev.: 0.301
RSD %: 0.610

Table 2 shows the Nitrogen and Protein determination in various cereal and bean samples to validate the system at different content of nitrogen and protein. The data shows an excellent reproducibility.

In all cases the relative standard deviation was less than 2 %, according to the AOAC method 990.03 for animal feed.

No memory effect was observed when changing the type of sample, indicating the complete detection of the nitrogen present in the sample.

Sample	W (mg)	N %	Prot. %	RSD %
Corn	276.7	1.38	8.62	8.60
	240.8	1.40	8.77	
	247.5	1.39	8.71	
	272.2	1.43	8.93	
	285.7	1.38	8.61	
	272.9	1.37	8.57	
	247.5	1.37	8.57	
	256.8	1.37	8.58	
	241.3	1.37	8.58	
	259.2	1.38	8.60	
Wheat HRS	259.2	2.99	18.70	0.40
	255.7	2.99	18.68	
	258.2	3.01	18.82	
Wheat CPS-W	213.9	2.24	14.00	0.44
	250.9	2.26	14.12	
	250.2	2.25	14.09	
Wheat SWS	254.4	2.28	14.23	0.49
	233.4	2.29	14.28	
	230.9	2.30	14.37	\
Lentils	296.0	3.99	24.96	0.43
	300.7	4.00	25.01	
	307.5	3.98	24.89	
	297.4	3.96	24.75	
	310.7	4.01	25.08	
	309.1	3.97	24.82	
	310.5	3.99	24.94	
	265.1	3.98	24.90	
	270.0	3.97	24.84	
	327.9	4.01	25.08	
Green Peas	297.0	3.92	24.49	0.53
	301.0	3.88	24.25	
	323.0	3.91	24.45	
Brown Peas	320.0	4.48	28.00	0.45
	312.4	4.45	27.81	
	284.9	4.48	28.03	
	315.0	4.46	27.89	
	290.5	4.44	27.73	

Table 2: Nitrogen / Protein determination in cereals and beans

A comparison of results of different cereals obtained by the FLASH 2000 Protein Analyzer and the Kjeldahl method is reported in Table 3.

The data shows that the two methods are perfectly comparable, demonstrating the validity of the combustion method for N/Protein analysis and that the FLASH 2000 Protein Analyzer represents the best alternative to the traditional wet method.

Sample	Kjeldahl Method Protein %	FLASH 2000 Protein Analyzer Protein %
Soya	39.18	39.20
Lentils	27.19	27.17
Rice	7.00	7.08
Wheat	10.89	10.91
Beans	23.38	23.35

Table 3: FLASH 2000 Protein Analyzer vs Kjeldahl method

Conclusion

- Excellent reproducibility
- Stability of the system
- No memory effect was observed when changing the type or weight of sample, indicating the complete detection of the nitrogen.

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Application Note: 42158

Organic Elemental Characterization of Soils and Plants by Thermo Scientific FLASH 2000 Elemental Analyzer

Dr. Liliana Krotz, OEA Product Specialist, and Dr. Guido Giazzi, OEA Product Manager, Thermo Fisher Scientific, Milan, Italy

Key Words

- NC
- Plants
- Soils
- Total Organic Carbon
- Sulfur

Introduction

Elemental characterization of materials in agricultural and environmental research is important for knowing flows of elements between system components and also for management purposes. Nitrogen (N) analyses are useful to evaluate the quality of food and feed crops, and to set up fertilization plans based either on the balance method or on some indicators of N nutritional status of plants. Nitrogen and carbon analysis in soils gives information pertaining to the deficiency or excess of nutritional elements. The differentiation of Total Carbon and Total Organic Carbon is also useful to evaluate the quality of soils while the interest in determination of sulfur (S), is rising as a consequence of recurrent S-deficiency in rainy regions on sandy soils. Sulfur is an essential components of living matter. The deficiency of sulfur has a negative influence in the growth of vegetables, in the quality of proteins through the synthesis of amino acids such methionine, cysteine and cystine, and in the synthesis of vitamins.

The importance of soil and plant testing has grown in the last years as many of the classical methods are now no longer suitable for routine analysis due to time consuming preparation and the use of environmentally hazardous reagents. For this reason a simple and automatic technique which allows the fast analysis with an excellent reproducibility from low to high level is required. Thermo Scientific Elemental Analyzers, based on the dynamic combustion of the sample, requires no sample digestion or toxic chemicals, while providing important advantages in terms of time, automation and quantitative elemental determination in a wide range of concentration. The Thermo Scientific FLASH 2000 NC Soil Analyzer (Figure 1), permits the nitrogen and carbon determination of soils and plants samples in an automatic way. The same analytical conditions can be used for the differentiation between the Total Carbon and Total Organic Carbon determination after an acid pre-treatment of the sample. Through its flexibility, the analyzer allows also the simultaneous NCS analysis while for trace Sulfur determination, the analyzer has been coupled with the flame photometric detector (FPD). This method combines the advantages of the elemental analyzer with the sensitivity, selectivity and robustness of the FPD.

Figure 1 – FLASH 2000 NC Soil Analyzer

Methods

The FLASH 2000 Analyzer operates according to the dynamic flash combustion technique. Samples are weighed in tin capsules and introduced into the combustion reactor via the MAS 200R Autosampler together with a proper amount of oxygen. In NC configuration, after combustion the resultant gases are carried by a helium flow to a second reactor filled with copper, then through a water trap, then through a GC column and finally, detected by a thermal conductivity detector (TCD) (Figure 2). In NCS configuration, after combustion of the sample the resultant gases are carried by a helium flow to a layer filled with copper, then through a water trap, then in a GC column and finally, detected by a thermal conductivity detector (TCD) (Figure 3), while for trace sulfur analysis, after the water trap, the gases are carried by a helium flow through a short GC column and finally, detected by the flame photometric detector (Figure 4). A complete report is automatically generated by the Thermo Scientific Eager Xperience dedicated software and displayed at the end of the analysis.

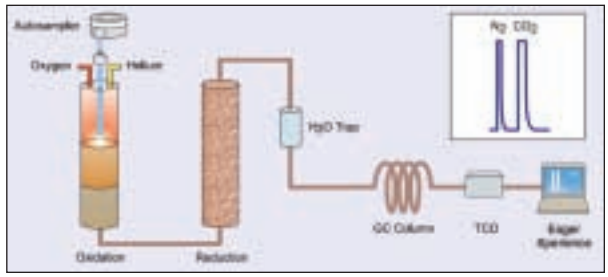

Figure 2: NC configuration

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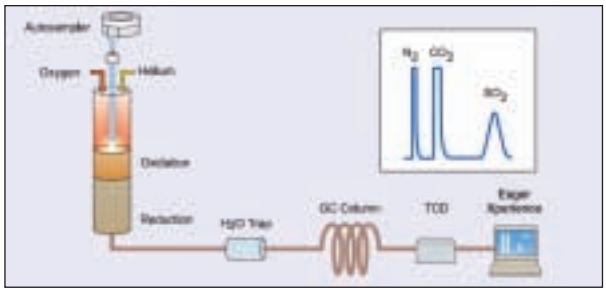

Figure 3: NCS configuration - TCD detector

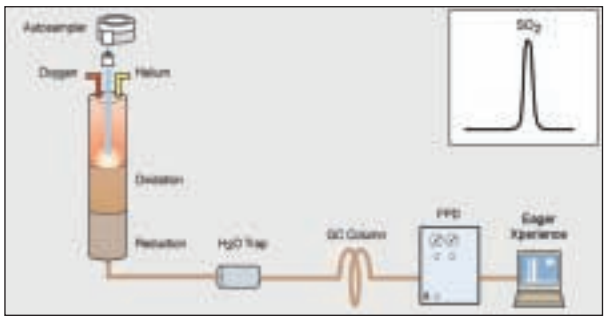

Figure 4: NCS configuration - FPD detector

Results

The soils and plants samples analyzed were chosen on the basis of the different nature and different content of nitrogen, carbon and sulfur. The data obtained demonstrate the no-matrix effect indicating complete combustion for all type of samples.

Table 1 shows the reproducibility of NC determination using the FLASH 2000 NC Soil configuration. The calibration was performed with aspartic acid using K factor as calibration method. The performance of the system was evaluated with the analysis of the Thermo Scientific Soil Reference Material (0.186 %N, 1.697 %C).

Sample	N %	RSD %	C %	RSD %
Soil Ref Mat	0.1846	0.6415	1.7000	0.1310
	0.1865		1.7008	
	0.1868		1.7042	
Soil A	0.0537	0.8455	1.9939	0.8281
	0.0543		1.9932	
	0.0546		1.9651	
Humus A	1.7476	0.5993	36.6962	0.1176
	1.7654		36.6918	
	1.7663		36.6194	
Humus B	1.4094	0.1748	32.5212	0.4014
	1.4139		32.3690	
	1.4099		32.6287	
Barley	1.6988	0.4914	41.9268	0.0288
	1.6833		41.9220	
	1.6859		41.9039	
Hay	1.1948	0.6076	41.8792	0.1055
	1.2057		41.9459	
	1.1919		41.9629	

Table 1: Reproducibility of NC determination

Table 2 shows the reproducibility of the simultaneous NCS determination. The calibration was performed with BBOT* using K factor as calibration method. The performance of the system was evaluated with the analysis of the Thermo Scientific Soil Reference Material (0.20 %N, 2.01 %C, 0.032 %S).

** BBOT: 2,5 bis (5-ter-butyl-benzoxazol-2-yl)thiophene*

Sample	N %	RSD %	C %	RSD %	S %	RSD %
Soil Ref Mat	0.2027	0.3828	2.0451	0.4673	0.0313	1.1300
	0.2006		2.0513		0.0309	
	0.2038		2.0316		0.0318	
Soil B	0.2301	0.4157	4.6274	0.1649	0.2062	1.5810
	0.2320		4.6278		0.2091	
	0.2313		4.6144		0.2026	
Soil C	0.0894	1.2358	1.0176	1.6551	0.0195	2.8785
	0.0875		1.0421		0.0185	
	0.0894		1.0096		0.0194	
Plant 1	1.0512	0.3824	49.5572	0.1166	0.1823	0.1553
	1.0323		49.6275		0.1878	
	1.0569		49.5129		0.1819	
Plant 2	0.3594	0.9769	44.7434	0.5088	0.0359	1.5584
	0.3634		44.2922		0.0343	
	0.3644		44.5536		0.0367	
Plant 3	2.1075	0.5107	47.7879	0.1126	0.1084	1.6387
	2.0897		47.8755		0.1052	
	2.1090		47.7775		0.1056	

Table 2: Reproducibility of NCS determination in soils and plants

The differentiation of Total Carbon (TC) and Total Organic Carbon (TOC) was performed by sample manipulation prior to analysis. TOC was determined after removing carbonates by acidification of the sample with HCl 1:1. The two analyses TC and TOC were performed consecutively using the same analytical conditions of the instrument. Figure 5 shows the sample pre-treatment technique for Total Organic Carbon determination while Table 4 show the data of Total Carbon and the relative Total Organic Carbon obtained after acidification of samples.

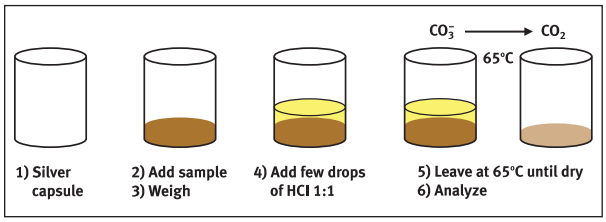

Figure 5: Method for TOC determination

Kit for Total Organic Carbon (TOC) in Solid Sample (PN 190 041 28)

Sample	TC %	RSD %	TOC %	RSD %
Soil D	4.4184	0.2626	0.4119	0.3269
	4.4150		0.4162	
	4.4366		0.4100	
Soil E	0.8289	0.4573	0.5094	0.2503
	0.8215		0.5089	
	0.8265		0.5076	
Humus C	36.6962	0.1176	34.7956	0.2261
	36.6918		34.5334	
	36.6194		34.6845	
Humus D	32.5212	0.4014	31.0916	0.1074
	32.3690		31.0238	
	32.6287		31.0444	

Table 3: TC and TOC determination in soils and humus samples

Table 4 shows the reproducibility of Sulfur determination by FPD detector. The calibration of the system was performed with the Thermo Scientific Soil Reference Material (0.032 %S) using Quadratic Fit as calibration method.

Sample	Sulfur by FPD	RSD %
Soil F	31	6.24
	30	
	27	
	30	
	32	
Soil G	44	2.60
	41	
	42	
	42	
	42	
Soil H	138	3.53
	129	
	129	
	137	
	129	
Soil I	272	1.51
	269	
	264	
Soil J	661	2.08
	637	
	660	

Table 4: Reproducibility of Sulfur determination by FPD detector

Figure 6: FPD detector (PN 432 101 45)

The accuracy and precision of the FLASH 2000 Analyzer was evaluated through the participation in International Round Robin Tests **WEPAL** (Wageningen Evaluating Programs for Analytical Laboratories, Wageningen University, Netherlands). For soil samples, data were compared with the range accepted by WEPAL statistic studies including all methods for nitrogen, carbon and sulfur determination. For plant samples the results were compared with the range accepted for carbon and sulfur while for nitrogen the results were compared with both Kjeldahl and Total Nitrogen methods which include the combustion method.

Table 5 shows the NCS results of **Soil WEPAL samples** and while Table 6 shows the NCS of **Plant WEPAL samples**. The NCS data obtained are inside in the range of concentration approved by the WEPAL statistic studies.

Sample	Nitrogen %		Carbon %		Sulfur %	
Name	Wepal Range	FLASH 2000	Wepal Range	FLASH 2000	Wepal Range	FLASH 2000
Sandy Soil-90	0.200 - 0.242	0.216	2.41 - 2.85	2.43	0.0449–0.0550	0.0549
Riverclay	0.158 - 0.195	0.177	1.66 - 1.80	1.68	0.0285-0.0338	0.0310
Sandy Soil	0.177 - 0.230	0.210	3.13 - 3.62	3.32	0.0184-0.0252	0.0228
Moist Clay	0.066 - 0.091	0.077	0.835 - 0.940	0.881	0.0080-0.0120	0.0112
Braunerde pseudoclay	0.174 - 0.195	0.185	1.72 - 1.89	1.80	0.0273-0.0286	0.0285

Table 5: NCS determination in Soil WEPAL samples

Sample	Nitrogen %		Carbon %		Sulfur %	
Name	Wepal Range	FLASH 2000	Wepal Range	FLASH 2000	Wepal Range	FLASH 2000
Oil Palm leaf	2.59 - 2.93	2.71	45.1 - 51.3	47.3	0.1725 - 0.2240	0.1854
Lucerne	2.87 – 3.33	2.94	41.4 – 47.4	43.8	0.2342 - 0.2816	0.2512
Cherry Laurel	1.50 – 1.78	1.62	44.9 – 47.7	45.8	0.0646 - 0.0854	0.0709
Pepper	1.76 – 2.16	1.92	38.5 – 43.2	39.6	0.5760 – 0.6912	0.5885
Carnation (Straw)	1.55 – 1.94	1.73	38.1 – 41.5	39.5	0.2489 – 0.2880	0.2589

Table 6: NCS determination in Plant WEPAL samples

Conclusions

- The data obtained show excellent reproducibility.
- No memory effect was observed when changing the type of sample, indicating the complete detection of the nitrogen, carbon and sulfur present in the sample.
- Thermo Scientific Analyzers are able to analyze nitrogen, carbon and sulfur in a wide range from low to high content without matrix effect.
- The nitrogen, carbon and sulfur data obtained by FLASH 2000 are inside the tolerance accepted by WEPAL International Round Robin Tests demonstrating the high performance of the instrument.

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Application Note: 42159

Reproducibility of Nitrogen/Protein determination with the Thermo Scientific FLASH 2000 Protein Analyzer

Luigi Ragaglia, Dr. Liliana Krotz and Dr. Guido Giazzi, Thermo Fisher Scientific, Milan, Italy

Key Words

- Large sample weight
- Easier sample handling
- Automated, unattended analysis
- Fast analysis: less than 5 minutes
- Outstanding day to day reproducibility

Introduction

In a typical production process within the food industry, the protein content is periodically monitored and tested for quality control.

The determination of total nitrogen is the simplest way to measure protein content in various matrices as the direct protein determination is very difficult due to the complexity and variety of protein molecules.

Therefore the reproducibility of data, measured as deviation of results from their mean value, is one of the first objectives in all analytical tests to have alternative techniques accepted .

In this light the Thermo Scientific FLASH 2000 Protein Analyzer has proven to be reliable and copes with a wide array of additional important requirements of modern laboratories such as accuracy, high sample throughput and low cost per analysis.

Description of the Analytical Method

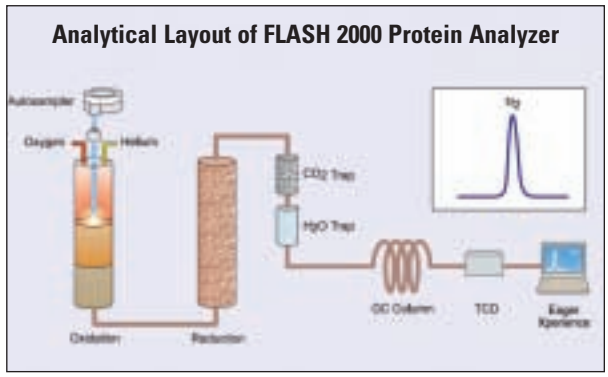
The FLASH 2000 Protein Analyzer is based on the dynamic flash combustion technique. The sample is weighed in a tin capsule and introduced into the combustion reactor via the Thermo Scientific MAS 200R Autosampler together with a proper amount of oxygen by Thermo Scientific OxyTune® function. After combustion of the sample, the produced gases are carried by a helium flow to a second reactor filled with copper, then through CO_2 and H_2O traps, a GC column and finally detected by a significant. A complete N/Protein report is automatically generated by the Thermo Scientific Eager Xperience dedicated software and displayed at the end of the analytical cycle.

Eager Xperience software also allows the sample weight transfer from the balance to the sample table, and the complete control of the analytical parameters of the instrument.

Analytical Conditions

Combustion temperature: 950°C
Reduction temperature: 840°C
Oven temperature: 50°C
Helium flow rate:
Measurement: 140 ml/min
Reference: 100 ml/min
Oxygen flow rate: 300 ml/min
Total run time: less than 5 minutes
Nominal sample weight: 200-300 mg
Standard: 50-100 mg Aspartic acid
Calibration Method: K factor

Analytical Layout of FLASH 2000 Protein Analyzer



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Results

To validate the system, a variety of sample types were analyzed. The protein content is calculated using the protein factor 6.25.

The reproducibility obtained analyzing pasta, flour and couscous 5 times consecutively is reported in Table 1.

Table 2 shows the day to day data. The sample was analyzed at different times throughout the day and for 3 consecutive days.

The data shows excellent reproducibility and no effect on the results was observed when changing the weight of the sample.

No recalibration of the system was necessary during the 3 days of testing, indicating the stability of the system.

Flour			Pasta			Couscous		
W (mg)	N %	Prot.%	W (mg)	N %	Prot.%	W (mg)	N %	Prot.%
267.1	1.27	7.92	256.70	1.97	12.29	343.4	2.04	12.76
219.9	1.27	7.92	230.40	1.96	12.26	295.5	2.04	12.72
305.8	1.27	7.93	249.70	1.96	12.24	303.9	2.06	12.90
249.1	1.26	7.86	247.10	1.96	12.27	337.2	2.06	12.85
273.8	1.27	7.92	245.70	1.96	12.28	258.7	2.05	12.79
Average	1.27	7.91	Average	1.96	12.27	Average	2.05	12.80
RSD %	0.35	0.35	RSD %	0.23	0.16	RSD %	0.49	0.55

Table 1: Reproducibility of Nitrogen/Protein determination

Day 1			Day 2			Day 3		
W (mg)	N %	Prot.%	W (mg)	N %	Prot.%	W (mg)	N %	Prot.%
102.5	2.06	12.89	202.2	2.08	12.98	217.7	2.10	13.14
167.6	2.07	12.95	208.7	2.06	12.90	221.1	2.07	12.93
216.9	2.06	12.91	207.3	2.09	13.06	215.2	2.08	12.98
126.7	2.09	13.07	199.7	2.07	12.95	314.8	2.08	13.02
184.9	2.08	13.01	199.7	2.09	13.09	294.9	2.07	12.96
239.2	2.07	12.95	296.1	2.08	12.99	285.4	2.10	13.14
296.6	2.05	12.81	294.2	2.09	13.05	280.8	2.09	13.10
			304.7	2.09	13.08	287.1	2.09	13.07
			295.7	2.09	13.09	108.8	2.05	12.83
			300.0	2.10	13.13	103.6	2.03	12.69
Statistical Data:	Number of runs: 27;							
	Average N %: 2.08;		RSD %: 0.83					
	Average Protein %:12.99;		RSD %: 0.83					

Table 2: Day to Day reproducibility of Semolina sample

Conclusion

- Excellent reproducibility
- Stability day by day
- No memory effect observed.

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Thermo Scientific FLASH 2000 Series Organic Elemental Analyzers

Carbon, Hydrogen, Nitrogen, Sulfur and Oxygen analyzers


Organic Chemistry & Pharmaceuticals


Petrochemistry & Energy


Environmental Analysis


Material Characterization


Agronomy & Marine Science

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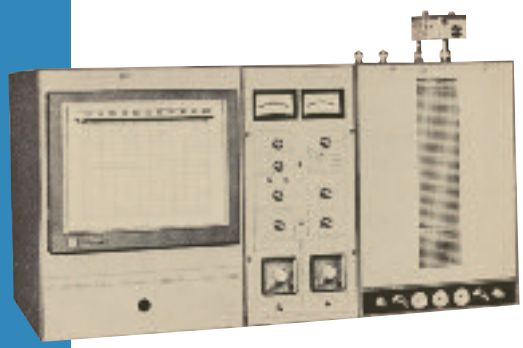
Tradition and Innovation

The Thermo Scientific FLASH 2000 Series makes Organic Elemental Analysis (OEA) a simple, precise and cost-effective tool for any laboratory. With a long and successful history in OEA starting back in 1968 with the first automated analyzer (trading as Carlo Erba), you can be confident that your FLASH 2000 Series OEA is from a knowledgeable and dedicated team.



EA 1102 (1968)

Utilizing our experience in OEA, Thermo Fisher Scientific offers a sophisticated package of benefits with the FLASH 2000 Series including:

- **Wide analytical range, allowing extensive applications**
  - *The FLASH 2000 Series OEA is now a standard in Elemental Analysis in terms of accuracy, precision and versatility; as confirmed by the over 1,000 installations worldwide.*
- **Versatility and Modularity**
  - *Versatility and modularity are key design aspects of the FLASH 2000 Series OEA, ensuring performance that meets your requirements, no matter how often those requirements change.*
- **Accuracy and Precision**
  - *A high precision integrated electronic mass flow controller achieves extensive stability in flow and temperature parameters thus ensuring the highest levels of precision and accuracy of results for both homogeneous and non-homogeneous samples from trace to high amounts.*
- **Ease of use**
  - *The FLASH 2000 Series makes OEA one of the simplest methods of analysis. With unique functions such as Auto-Start, Auto-Standby, Auto-Ready and Automatic Leak Test, the demands on operators are significantly reduced. The Eager Xperience software also simplifies operation by minimizing user involvement in setting up the analyzer.*
- **Comprehensive and user-friendly software**
  - *A useful and powerful tool tailored for every lab requirement.*

All these benefits lead to cost effective analysis

Wide range of applications

The FLASH 2000 Series OEA allows a variety of configurations tailored to the application they serve:

Organic/Inorganic Chemistry & Pharmaceuticals

- Fine Chemicals
- Pharmaceuticals Products
- Organo-metallic compounds
- Polymers
- Plastic
- Synthetic rubbers
- Fibers
- Explosives
- Catalysts
- Textiles
- Pesticides
- Detergents
- Fluorine-compounds

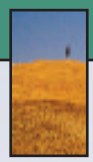
Petrochemistry & Energy

- Coals
- Cokes
- Crude oils
- Gasoline/Diesel
- Alternative fuels
- Petroleum derivates
- Lubricants
- Oil additives
- Graphite

Material Characterization

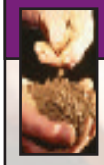
- Glue/Resins
- Papers
- Rubbers
- Cement
- Ceramics
- Carbon/Glass Fibers
- Tires
- Pigments & Dyes
- Refractory materials
- Building materials
- Inorganic materials
- Metals
- Textile fibers
- Wood powders

Environmental Analysis



- Soils, sediments, and rocks
- Composts
- Wastes
- Sewage/sludge
- Pesticides
- Water solution
- Waste Water
- Particulates in Air by Filters
- Particulates in Water by Filters
- Woods

Agronomy & Marine Science



- Soil
- Plants (leaves, roots, fruit)
- Sediments
- Humus
- Algae
- Plankton
- Particulate matter in water by filters
- Water
- Fertilizer

Food

- Human and animal food
- Beverages (beer, juice, milk, wine, soft drinks ..)

Human & Animal Samples

- Blood
- Hairs
- Nails
- Serum
- Urine
- Faeces

Isotope Analysis

- Soil and plant research
- Forensic
- Bio Oceanography
- Food and goods control

FLASH 2000 configurations:

- FLASH 2000 CHN
- FLASH 2000 CHN/O
- FLASH 2000 CHNS
- FLASH 2000 CHNS/O
- FLASH 2000 N org
- FLASH 2000 N Lubricants
- FLASH 2000 NC org
- FLASH 2000 NCS
- FLASH 2000 NC Soils/Sediments/Filters
- FLASH 2000 N/Protein
- FLASH 2000 N Brew
- FLASH 2000 IRMS & HT

High flexibility to meet any requirement

Flash Combustion

The FLASH 2000 Series analyzer operates according to the dynamic flash combustion (modified Dumas method) of the sample for the determination of Carbon, Hydrogen, Nitrogen and Sulfur. Samples - organic or inorganic, solid or liquid – are weighed in a tin capsule and introduced into the combustion reactor by an autosampler. When the sample enters the reactor, inserted in the special furnace heated at 900 – 1000°C, a small volume of pure Oxygen is added to the system and helps to burn the organic or inorganic material, converting the sample into elemental (simple) gases. A separation column and TCD detector allows the user to determine element concentrations without using a complex splitting system, aliquote dosing device or purge & trap adsorbers. On the same instrument, but working in a different analytical condition, the Oxygen determination can be obtained when operating in pyrolysis mode. Utilizing the Flash Dynamic Combustion method, the FLASH 2000 Series analyzer achieves accurate and precise sample characterization within a few minutes.

Sample Introduction, tailored to your needs

Whether you require high throughput or a cost-effective solution, the FLASH 2000 Series OEA is available with a choice of autosamplers, to ensure that your analysis starts efficiently

- Thermo Scientific MAS 200R universal autosampler
  - *The MAS 200R autosampler is a mechanically driven, reliable workhorse suitable for both liquid and solid samples. The samples loaded in tin capsules are automatically dropped into the combustion reactor sequentially by electronically controlled movements. As standard, the MAS 200R includes a 31- position sample carousel, but up to three more carousels can be added during the analytical process for an uninterrupted analysis of up to 124 samples.*

FLASH 2000 CHNS/O Analyzer with MAS 200R 4 drum universal autosampler

MAS 200R and AS3000 Autosamplers

- Thermo Scientific AI/AS 3000 autosamplers
  - *The AI and AS 3000 autosamplers mount effortlessly to the top of the FLASH 2000 Series analyzers. The AI autosampler is ideal for those analyzing lower sample numbers with an 8 position tray. The AS 3000, with a 105 sample capacity is more suited for high throughput laboratories. Both are easy to mount and feature an automatic alignment system with optical sensors to ensure safe and reproducible syringe positions, which ultimately result in more accurate and reproducible data. Furthermore, the autosamplers are controlled by the Eager Xperience software which provides users with a Help routine.*

New Horizons for OEA – The FPD Option for Sulfur trace analysis

Determination of Sulfur content in trace analysis is becoming more and more important due to the wide presence of this element in numerous organic and inorganic compounds.

By coupling the FLASH 2000 Series to an OEA / FPD (Flame Photometric Detector) system, it is possible to reach as low as 5-10 ppm of Sulfur, which opens a new horizon for OEA applications.

Approved by official organizations

The Dynamic Flash Combustion technique is endorsed by a large array of renowned international official organizations including AOAC (Association of Official Analytical Chemists), AOCS (American Oil Chemists Society), AACC (American Association of Cereal Chemists), ASTM (American Society for Testing and Materials) and ASBC (American Society of Brewing Chemists). The simplicity of the method, unparalleled data reproducibility and truly quantitative results are the essence of this wide acceptance.

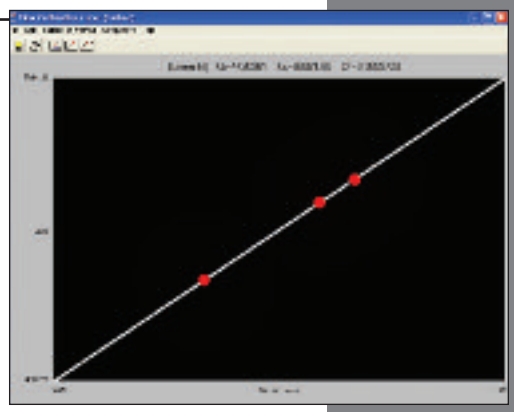
Thermo Scientific Eager Xperience software:

The most comprehensive software dedicated to OEA

Our dedicated software controls the operation, data acquisition and data evaluation capabilities of the FLASH 2000 Series OEA, enabling quick reference to method parameters and instrument status readout. Users can configure this flexible platform to gain access to either all available features or alternatively to a customized and simplified user interface incorporating pre-set methods. Eager Xperience is the most advanced, complete and flexible dedicated software for OEA applications.

Calibration

For easy instrument calibration either K factor, linear regression or quadratic fit response can be selected, according to the type of analysis requested or to the detection range evaluated.

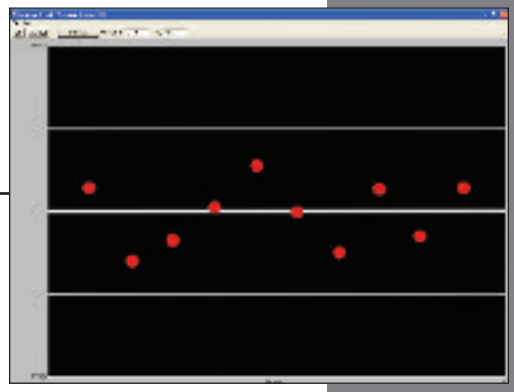


Average Visualization

Users often prefer visual aids when performing a Quality Control of the results: Average Visualization allows users to control the data variation and the precision and accuracy of the results at-a-glance. This is useful for preparing a complete and personalized analytical report.

Green / Red light indicators

This simple yet ingenious function enables users to evaluate at-a-glance whether the Nitrogen, Carbon, Hydrogen and Sulfur percentage value is within the expected acceptable range or control limits. The acceptable range can be a default value or user defined according to the characteristics of the compound, the sample nature and the precision required.

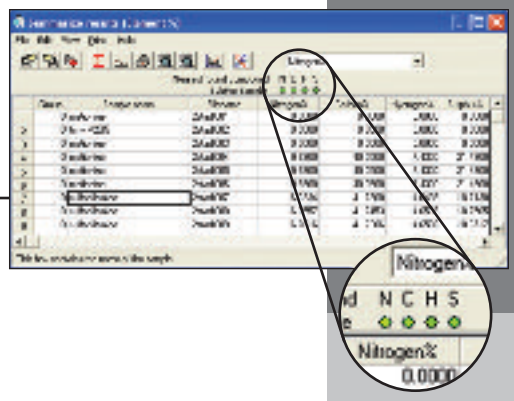


Maintenance

Eager Xperience software allows users to pre-program the maintenance of the instrument and monitor the status of catalysts, filters and adsorbers in real time. A color change from green to yellow indicates the catalysts usage while red indicates that maintenance needs to be performed.

Consumables Catalog

An electronic Consumables Catalog with information on chemicals, spare parts and accessories is available within our Eager Xperience software. User-friendly pathways guide users through the catalog offering an easy access to the various sections and providing the required information in a straightforward, quick and simple way.

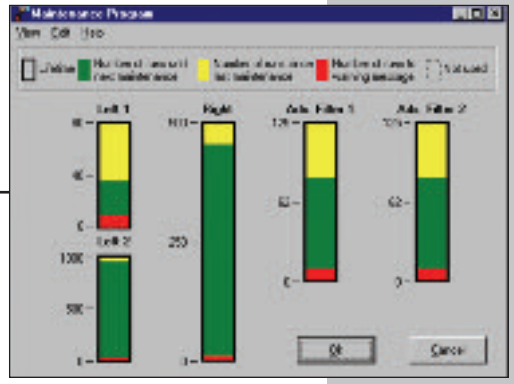


Powerful Report Publisher

Users can customize an analytical report format to contain information such as chromatograms, analytical conditions, statistical evaluation of results and relative graphics, operator nominative and company logos.

Interfacing Analytical Balance

Eager Xperience software provides a direct interface to the most common analytical balances. The direct connection allows users to transfer sample weights to the software eliminating the transcription errors.



21 CFR part 11 Compliance

Eager Xperience software supports compliance to strict FDA regulations (21 CFR part 11) for a closed analytical system included data security, method authorization, electronic signature etc.

Optimized operation for lower cost

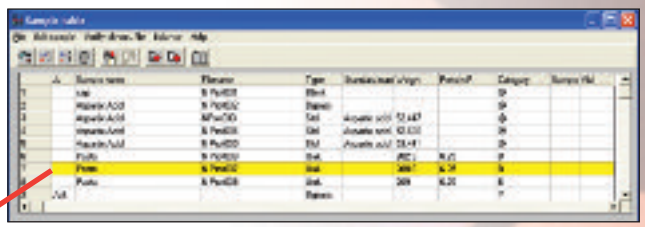
Thermo Scientific OxyTune® – Automatic Oxygen Dosing System

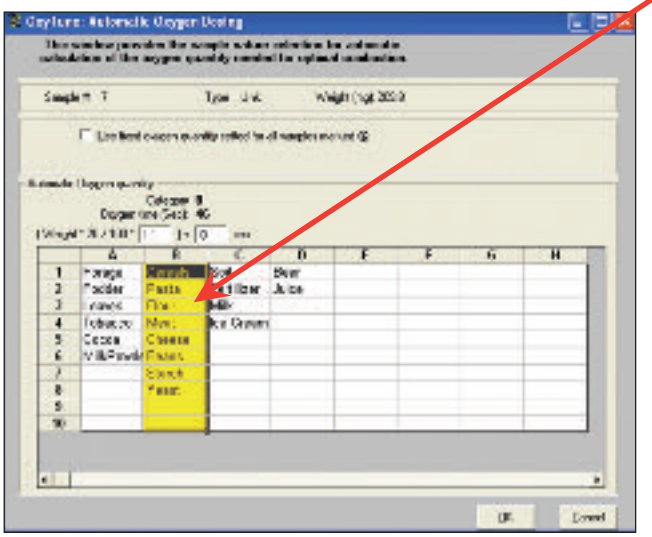
This capability enables the FLASH 2000 Series OEA to supply the precise volume of Oxygen needed for the optimized combustion of each sample in an easy and simple way. This process significantly reduces the quantity of Oxygen needed, dramatically extends the lifetime of the catalyst and minimizes user involvement in setting-up the analyzer.

Additionally, organic and inorganic matter is completely combusted, providing quantitative results over a wide analytical range. In case the sample does not appear within the family of compounds preloaded, it is possible for the user to edit the list by adding their own family. This provides a simple solution to reduce costs per analysis, whatever the sample.

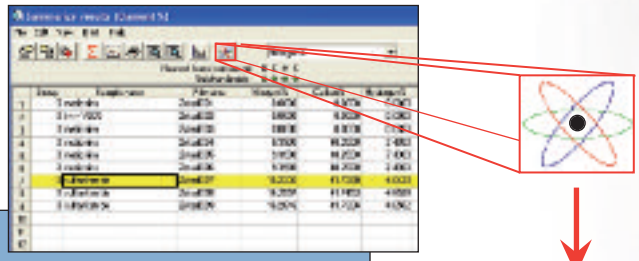
Minimized maintenance downtime

The simple analytical layout of the FLASH 2000 Series analyzer ensures fast and easy maintenance. The combustion and reduction reactors are plumbed through 'Fast Connectors' making them easily accessible from the front of the instrument and simplifying their replacement. The fast heating capability of the reactors reduce unnecessary waiting time hence maximizing sample throughput. Leak check of the analytical gas flow path after each maintenance procedure is automatically performed under the control of the Eager Xperience software without the need for laborious user intervention. Furthermore, Auto-Start, Auto-Standby and Auto-Off functions of the Eager Xperience software drastically decrease the need of instrument downtime for maintenance. The end result is substantial operating and analytical cost savings.





Automatic Oxygen Dosing System



Empirical Formula

Eager Xperience software is able to automatically calculate the Empirical Formula and the relative Molecular Weight in within only a few seconds, without the need to transfer results into any external software. Molecules with even up to 7 unknown elements and up to 2000 amu can be calculated. For humid compounds and if the percentage of water is known, the suitable correction can be easily inserted in the calculation system.

Unique capabilities

FLASH 2000 & Isotope Ratio Mass Spectrometry (IRMS) – a powerful combination

The accurate determination of Nitrogen, Carbon, Sulfur, Oxygen and Hydrogen Isotope ratio offers a powerful tool in many research areas from environmental and agronomy to nutritional and marine biology. Connection between the FLASH 2000 Series OEA and IRMS takes advantage of the extremely simple FLASH 2000 analytical layout, whereby gas splitting is not required and therefore highly quantitative results are easily obtained regardless of the complexity of the determination.

There are two dedicated models of the FLASH 2000 for use with IRMS.

- FLASH 2000 IRMS – to determine the isotope analysis of N and C by combustion
- FLASH 2000 HT (High Temperature) – In addition to NC (or Sulfur) analysis it is possible to evaluate H and O using a HT furnace (1450 °C).

FLASH 2000 / Delta IRMS

FLASH 2000 OEA Validation

A comprehensive Validation Kit ensures quick and efficient validation to meet the stringent prerequisites required for the different analytical industrial areas. The Kit consists of a Validation Folder that collates the IQ (Installation Qualification), OQ (Operational Qualification) and PQ (Performance Qualification) procedures and a dedicated Configuration Pack, which includes all the items needed for validating the instrument. A certified Thermo Scientific engineer performs the validation.

Thermo Scientific FLASH 4000 N/Protein analyzer

For dedicated N/Protein analysis, the FLASH 4000 completes the Thermo Scientific range of organic elemental analyzers. Visit www.thermoscientific.com/flash4000 for information.

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