

STANDARD OPERATION PROCEDURE

Faculty of Biosciences, NMBU

Method name: CHNS (DUMAS / total-nitrogen)

BIOVIT No: Arb1039

1 Introduction

Simultaneous CHNS analysis requires high-temperature combustion in an oxygen-rich environment and is based on the classical Pregl-Dumas method. The samples are combusted with oxygen in a combustion chamber ($t = 1150\text{ }^{\circ}\text{C}$), and the combustion products are then passed through a reduction tube ($t = 850\text{ }^{\circ}\text{C}$) of an inert carrier gas (helium), before being separated on 3 different columns. CHNS is detected as CO_2 , H_2O , N_2 and S_2 , respectively, with a thermal conductivity detector (TCD).

2. Reagents

Standard: Sulfanilamide Art.nr: B15.00-0062 (Elementar)

N: 16,25 % C: 41,81 % H: 4,65 % S: 18,62 %

Checks:

BBOT (CRM-certified reference material, Elementar Microanalysis B2044)

N: 6,54 % C: 72,59 % H: 6,06 % S: 7,43 %

- Feed control (For various feed samples)
- Soy Control (For Liquid Samples)

3. Risk assessment

There is high temperature ($1150\text{ }^{\circ}\text{C}$) and high pressure inside the instrument. When replacing the combustion and reduction columns, the temperature must be turned down to below 200 degrees and the gases must be turned off. Wear heat-protective gloves and loosen the clips carefully. Remember that the glass bridge under the column is very hot. This also applies to the entrance of the detector. The gas should be turned off before the drying column is replaced. (This happens automatically if you put the system in "maintenance mode").

4. Equipment

-Tin capsules - solid samples: 0.12 mL , $8 * 5\text{ mm}$ Art. No.: 05 003 394 (Elementar)

-Tin capsules - liquid samples: 0.05 mL , $3.5 * 9 * 0.1\text{ mm}$ Art. No.: B03 951 619 (Elementar)

BIOVIT/NMBU						ARB:
Prepared by Elin Follaug Johnsen	Approved by Hanne Kolsrud Hustoft	Valid from 21.08.2017	Revision 03.2020	Replaced 06.2018	Document name Arb 1039 CHNS.docx	Page 1/5

- liquid samples: 0.1 mL. Art.nr: S05 000 481 (Elementar)
- Mettler toledo XP6 Microbalance
- Capsule press for liquid samples
- Tweezers
- Vario El Cube elemental analyzer

5. Special remarks

- The columns must be replaced with a new one after approximately 400 samples. When the reduction tube is used up, N% becomes too high. This is observed on the sulfanilamide sample.
- A new column should be tested for leaks, especially the drying column. The instrument does not detect leaks, even when using “leak check”.
- If the «daily factor» for N% and C% deviates from the normal range of 0.9-1.1, the instrument must be calibrated.
- H% is somewhat unstable, and the information is only provided if requested.
- Another detector (IR) must be used to detect low levels of S%.
- Our detector gives acceptable results as low as 0.2% S.
- For liquid samples, blank values must be determined with both an empty capsule, a capsule filled with air and a capsule filled with water (possibly the requester's own blank sample). The lower detection limit for N% in liquid samples is 0.05%.

6. Sample material

The analysis can be done on almost all types of samples; dry samples (as finely ground as possible), raw samples and liquid samples. Weigh small amounts of sample (5 mg - solid samples, 20 µL liquid samples), sample homogeneity is important.

BIOVIT/NMBU						ARB:
Prepared by Elin Follaug Johnsen	Approved by Hanne Kolsrud Hustoft	Valid from 21.08.2017	Revision 03.2020	Replaced 06.2018	Document name Arb 1039 CHNS.docx	Page 2/5

7. Job description

Startup instrument:

- Wake up the instrument → *Options - settings - Sleep / wake up - wake up now*
- Check off "*sleeping deactivated*"
- Wait until the temperatures reach 1150 °C and 850 °C (changes from turquoise to green)
- Check that the gas velocity is approximately equal for He and TCD (approx. 230 mL / min)

If these do not become equal, there is a leak in the system, and this must be fixed before the analysis can be done (see manual - troubleshooting)

Start by creating the following analysis sequence:

nr	Weight	name	Method
1	1	RunIn	Blank without O
2	1	RunIn	Blank without O
3	1	RunIn	Blank without O
4	1	RunIn	Blank with O
5	1	RunIn	Blank with O
6	1	Blnk	Blank with O
7	1	Blnk	Blank with O
8	Sulfanilamide	5mg90s
9	CRM	5mg90s
10	Control	5mg90s
11	Req XX – nr 1	5mg90s
12	Req XX – nr 2	5mg90s
13	Etc. etc.	

After the tenth test, a sulfanilamide sample should be run and then CRM to check that the calibration is satisfactory, for example for humidity, (daily factor = daily calibration). «5mg90s» is the standard method for solid samples. If the sample has a low specific gravity (low density), more sample is needed. Then the method must be changed to, for example, 10mg120s.

«20mg150s» is the standard method of fluid testing. Change if the weight differs much from 20 mg.

Remember to save the sequence (YYMM.DD_reqXX) and save regularly as there is no auto-save.

BIOVIT/NMBU						ARB:
Prepared by Elin Follaug Johnsen	Approved by Hanne Kolsrud Hustoft	Valid from 21.08.2017	Revision 03.2020	Replaced 06.2018	Document name Arb 1039 CHNS.docx	Page 3/5

Weighing of samples

Dry samples must be enclosed in tin (Sn) capsules for analysis. (0.12 mL)

Weigh an empty capsule.

- Tare the weight and weigh in approximately 5 mg (3-6 mg using standard method).
- Transfer the weight to PC.
- Fold the capsule and place it in the marked box.

Liquid samples are pipetted into tin capsules (0.05 mL or 0.1 mL).

- Weigh an empty capsule. Tare the weight.
- Insert the capsule into the appropriate adapter in the capsule, then press.
- Pipette 20 µL into the capsule (the volume can be adjusted to the type of liquid).
- Seal the capsule as soon as possible (flush if necessary, with O₂ first to remove air).
- *If it does not flush with O₂ it is important to analyze several blank capsules in order to correct for contributions from air.*
- Weigh in the capsule and transfer the weight to the PC.
- Place the capsule in the marked box.
- *The capsule press pressure can be adjusted as needed (too loose = the sample evaporates, too hard = there is a hole in the capsule).*
 - Check by pipetting Acetone into the capsule and see if the weight remains stable over time.*

Analysis of samples

- Press: System - Carousel position- check off that the samples are removed -position: 80-ok
 - autosampler starts on hole 1.
- Transfer samples from a weighing well to autosampler.
 - The first 7 holes must be empty (RunIns and blank samples)
 - The sulfanilamide sample must be in hole 8, CRM in hole 9, and so on.
- When all the samples have been loaded in the auto-sampler: press the long green button in the toolbar (start auto analysis).
- Alternatively, one sample at a time can be analyzed by clicking on “start a single analysis”.

Stop

- Put the instrument in sleep mode → *Options - settings - Sleep / wake up – sleep.*
- If the instrument is to continue to run overnight: check off «*sleep after end of samples*».

BIOVIT/NMBU						ARB:
Prepared by Elin Follaug Johnsen	Approved by Hanne Kolsrud Hustoft	Valid from 21.08.2017	Revision 03.2020	Replaced 06.2018	Document name Arb 1039 CHNS.docx	Page 4/5

8. Calculation

- Correct for blank value:
 - Math - Blank values - calculate- YES (sequential)

- Correct for «daily factor»:
 - Math - Factor - YES (sequential)

- If the calculations are to be done manually: Options - Settings - Calculations

BIOVIT/NMBU						ARB:
Prepared by Elin Follaug Johnsen	Approved by Hanne Kolsrud Hustoft	Valid from 21.08.2017	Revision 03.2020	Replaced 06.2018	Document name Arb 1039 CHNS.docx	Page 5/5