RESOLUTION OENO 13/2002

QUANTIFICATION OF TOTAL NITROGEN

THE GENERAL ASSEMBLY,

HAVING CONSIDERED Article 5 of the October 13, 1954 International Convention on Unification of the Methods of Analysis and Appraisal of Wines,

WITH THE PROPOSAL of the Sub Commission on Methods of Analysis and Appraisal of Wines

DECIDES to complement Appendix A of the Compendium on International Methods of Analysis of Wines and Musts, with the following method:

QUANTIFICATION OF TOTAL NITROGEN ACCORDING TO THE DUMAS METHOD

(Musts and Wines)

1. FIELD OF APPLICATION

This method can be applied to the analysis of total nitrogen in musts and wine within the range of 0 to 1000 mg/l.

2. DESCRIPTION OF THE TECHNIQUE

2.1. Principle of the Dumas method

The analysis of total nitrogen in an organic matrix can be carried out using the Dumas method (1831). This involves a total combustion of the matrix under oxygen. The gases produced are reduced by copper and then dried, while the CO_2 is trapped. The nitrogen is then quantified using a universal detector.

2.2. Principle of the analysis (Figure n° 1)

- Injection of the sample and oxygen in the combustion tube at 940°C (1) ;
- « Flash » Combustion (2) ;

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- The combustion of the gathering ring (3) brings the temperature temporarily up to 1800°C ;
- Complementary oxidation and halogen trappings on silver cobalt and granular chromium sesquioxide (4) ;
- Reduction of nitrogen oxides in N2 and trapping sulphur components and excess oxygen by copper at 700°C (5) ;
- Gases in helium include: N2, CO2 and H2O (6) ;
- Trapping unmeasured elements: H2O using anhydrone (granular anhydrous magnesium perchlorate) (7) and CO2 by ascarite (sodium hydroxide on silica) (8) ;
- Chromatography separation of nitrogen and methane possibly present following very large trial uptake (9) ;
- Catharometer detection (10) ;
- Signal gathering and data processing (11).

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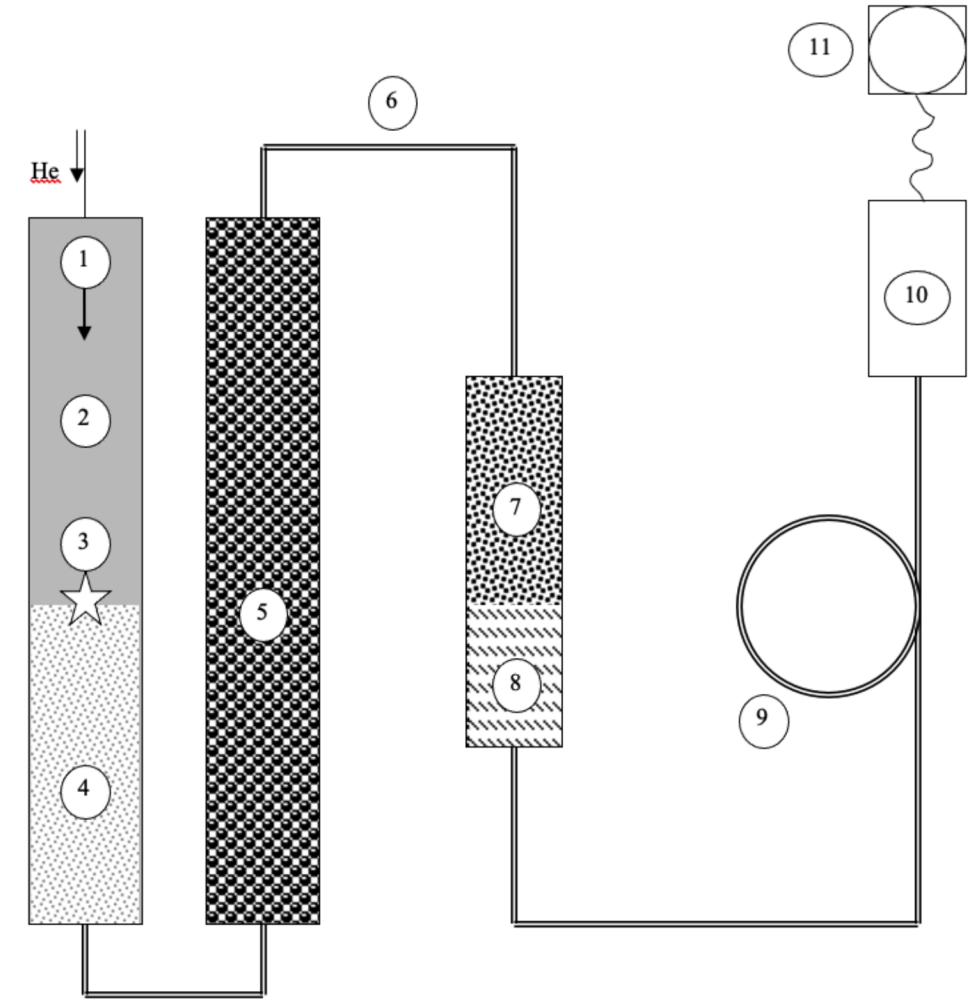


Figure 1 : Diagram of analysis principle

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3. Reagents and preparation of reactive solutions

3.1 - Nitrogen (technical quality) ;

3.2 - Helium (purity 99.99994%) ;

3.3 – Chromium oxide (chromium sesquioxide me in granules) ;

3.4 – Cobalt Oxide (silver granule cobalto-cobaltic oxide) ;

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3.5 – Quartz wool ;
3.6 - Copper (reduced copper in strings) ;
3.7 - Ascarite (sodium hydroxide on silica) ;
3.8 - Anhydrone (granular anhydrous magnesium perchlorate) ;
3.9 - Oxygen (purity 99.995%) ;
3.10 - Atropine ;
3.11 – Glumatic-hydric chloride acid;
3.12 – Demineralised water;
3.13 – Tin boat.

4. Apparatus

4.1 - Centrifuge with 25 ml pots;
4.2 – Nitrogen analyser;
4.3 – Metallic crucible;
4.4 - Quartz reaction tube (2) ;
4.5 – Precision balance between 0.5 mg and 30 g at □ 0.3 mg ;
4.6 – Boat carrier;
4.7.- Furnace;
4.8 – Apparatus for folding boats;
4.9 – Sample changer;
4.10 – Computer and printer.

5. SAMPLING

Degas by nitrogen bubbling (3.1) for 5 to 10 mn, sparkling wine. The musts are centrifuged (4.1) for 10 mn at 10°C, at 4200 g.

6. OPERATING INSTRUCTIONS

- Open the apparatus programme (4.2 and 4.10) ;
- Put the heating on the apparatus (4.2).

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6.1. Principle analytical parameters

Nitrogen analyser (4.2) under the following conditions:

- gas carrier: helium (3.2) ;
- metallic crucible (4.3) to be emptied every 80 analyses ;
- oxidation tube (4.4), heated to 940° C, containing chromium oxide (3.3) and cobalt oxide(3.4) held back by quartz wool (3.5). The tube and reagent set must be changed every 4000 analyses ;
- reduction tube (4.4), heated to 700° C, containing copper (3.6) held back by the quartz wool (3.5). The copper is changed every 450 analyses;
- absorption tube, containing 2/3 of ascarite (3.7) and 1/3 anhydrone (3.8). the ascarite which is taken in block is eliminated and replaced every 200 analyses. The absorbers are completely changed once a year.
- The more organic matter to be burned, the more oxygen is needed: the oxygen sampling valve (3.9) is 15 seconds for musts and 5 seconds for wine.

NOTE : The metals are recuperated and sent to a centre for destruction or specialised recycling.

6.2. Preparation of standard scale

Prepare two samples of atropine (3.10) between 4 to 6 mg. Weigh them (4.5) directly with the boat. The calibration scale goes through 3 points (origin = empty boat).

6.3. Preparation of internal standards

Internal standards are used regularly in the beginning and in the middle of analyses.

- Internal checks are carried out using glumatic acid in the form of hydrochloride at 600 mg N/l in demineralised water (3.12).

Molar mass of glumatic acid = 183.59

Molar mass of nitrogen = 14.007

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$$\frac{183.59 \times 0.6}{14.007} = 7.864\ g/l$$

- Weigh (4.5) 7.864 g of glumatic acid (3.11) and dilute in demineralised water (3.12) qsp/l, to obtain a 600 mg N/l solution. This solution is diluted by 50% to obtain a 300 mg N/l solution, which is diluted by 50% again to obtain 150 mg/l solution.

6.4. Preparation of samples:

6.4.1 – In a boat (3.13), weigh (to the nearest 0.01 mg) 20 µl of must or 200 µl of wine with a precision balance (4.5). Repeat this procedure three times per sample;

6.4.2 – Write down the mass

6.4.3 – Place the boats progressively in the boat carrier (4.6) ;

6.4.4 – Place the boats in the furnace (4.7) set at ~ 60° C, until the liquid has completely evaporated (this requires at least one hour) ;

6.4.5 – Fold and crush the boats with an appropriate apparatus (4.8), put them in the changer (4.9) in number order.

7. EXPRESSION OF RESULTS

Results are expressed in g/l to the fourth decimal.

8. CHECKING RESULTS

Splicing by mass, temperature, and volume.

9. PERFORMANCE CHARACTERISTICS OF THE METHOD

Number of laboratories	Average contents	Repeatability	Reproductibility
11	591 mg/l	43 mg/l	43 mg/l

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10. BIBLIOGRAPHY

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2. Buckee G.K. (1994) : Determination of total nitrogen in Barley, Malt and Beer by Kjeldahl procedures and the Dumas combustion method. Collaborative trial. J. Inst. Brew., 100, 57-64.

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