Method OIV-MA-AS323-02A Type II method

Quantification of total nitrogen according to the Dumas method

(Musts and Wines)

(Resolution Oeno 13/2002)

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) ;
- 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 N_2 and trapping sulphur components and excess oxygen by copper at 700°C (5) ;
- Gases in helium include: N_2, CO_2 and H_2O (6) ;
- Trapping unmeasured elements: H_2O using anhydrone (granular anhydrous magnesium perchlorate) (7) and CO_2 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).

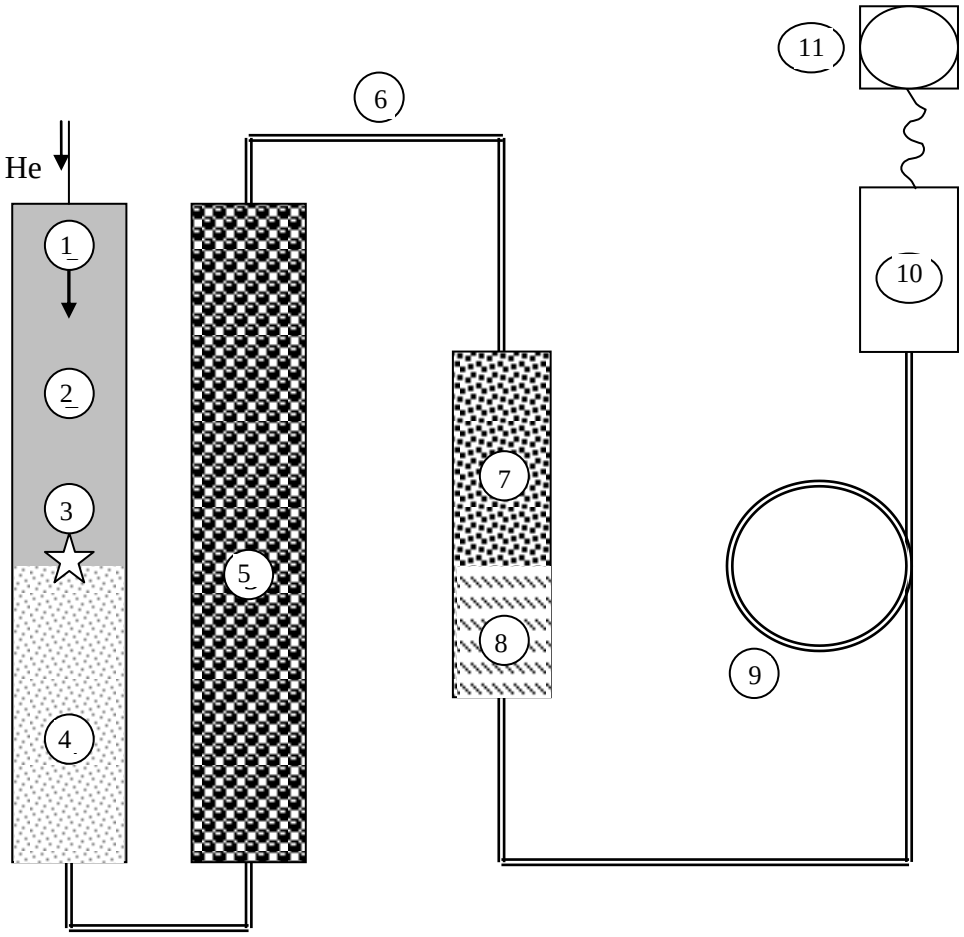

Figure 1 : Diagram of analysis principle

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) ;
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).

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

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

- 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 $\underline{\sim}$ 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

10 - BIBLIOGRAPHY

Dumas A. (1826) : Annales de chimie, 33,342.

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.