RESOLUTION OENO 18/2003

ANALYTICAL AND CONTROL TECHNIQUES (Oenological Codex) - Chemical Section

THE GENERAL ASSEMBLY,

CONSIDERING Article 5, paragraph 4 of the International Convention of the Unification of Methods of Analysis and Appraisal of Wine of 13 October 1954,

UPON THE PROPOSAL of the Sub-commission of the Methods of Analysis and Appraisal of Wine,

DECIDES:

TO REPLACE AND COMPLETE Chapter II of the International Oenological Codex by the following analytical and control techniques

CHAPTER II: ANALYTICAL AND CONTROL TECHNIQUES

ANALYSES COMMON TO ALL MONOGRAPHIES DETERMINATION OF TOTAL NITROGEN

1. Apparatus

1.1. The apparatus used for separating NH_3 is either a distillation apparatus with a rectifying column or a distillation apparatus under a current of steam (diagram) made up of:

A 1 l flask **A** of borosilicate glass used as a boiler with a stopcock funnel for filling. It can be heated by a gas or electric furnace.

An adapter **C** which gathers the spent liquid from the bubbler **B**.

A bubbler **B** of 500 ml with an inclined neck; the supply tube must reach the lowest part of the flask. The out-going tube has an anti-entrainment ball that makes up the top part of the bubbler. A stop-cock funnel **E** allows to introduce the liquid to be treated and alkaline lye.

A cooler 30 to 40 cm long, vertical, with a ball with fine dowel bush on the tip.

A 250 ml conical flask for the distillate.

1.2. Mineralisation flask, 300 ml ovoid-shaped flask with a long neck.

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2. REAGENTS

Concentrated sulphuric acid (R).

Mineralisation catalyser (R).

Sodium hydroxide solution at 30% (m/m) (R).

Boric acid solution at 4% (R).

Hydrochloric acid solution 0.1 M.

Mixed-based indicator with methyl red (R) and methylene blue.

The boiler must contain acidulated water by 1 per 1 000 of sulphuric acid. It is advisable to boil this liquid before any operation, with the drain cock P open to let the CO2 escape.

3. PROCEDURE

In the mineralisation flask, introduce the test sample containing 4 to 50 mg of nitrogen. Add 5 g of mineralisation catalyser (R) and 10 ml of concentrated sulphuric acid (R), if the quantity of dry organic matter to be mineralised is below 500 mg. Increase these quantities if a higher quantity of organic matter must be used.

Heat in an open flame under a hood. The neck of the flask is maintained inclined until the solution becomes colourless and the walls of the flask are clear of carbonised products.

After cooling, dilute with 50 ml of water and cool; introduce this liquid in the bubbler **B** with the funnel **E**, then add 40 to 50 ml of sodium hydroxide solution at 30% (R) in order to obtain frank alkalinisation of the liquid. Entrain the ammoniac with the vapour by gathering the distillate in 5 ml of boric acid solution (R) placed beforehand in a receiving conical flask with 10 ml of water, with the tip of the ampoule plunged into the liquid. Add 1 or 2 drops of mixed-based indicator and gather 70 to 100 ml of distillate.

Titrate the distillate with the hydrochloric acid solution 0.1 M until the indicator turns pink violet.

1 ml of 0.1 M hydrochloric acid solution corresponds to 1.4 mg of nitrogen.

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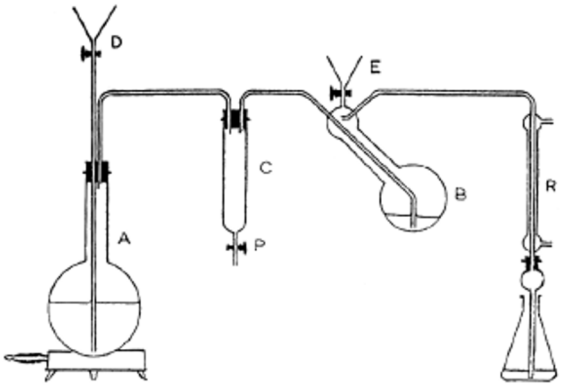

Apparatus for the distillation of ammoniac
in a current of steam
(PARNAS and WAGNER)

The cocks P and E can be replaced by a plastic pipe fitting with a Mohr pinch-clamp cock.

DETERMINATION OF MERCURY BY THE GENERATION OF VAPOUR AND ATOMIC FLUORESCENCE SPECTROMETRY

1. FIELD OF APPLICATION

This method is applied to the analysis of mercury in oenological products in the concentration range of 0 to 10 µg/l.

2. DESCRIPTION OF THE TECHNIQUE

2.1. Principle of the method

2.1.1. Mineralisation by the wet process of the oenological product to be analysed.

2.1.2. Reduction of the permanganate not consumed by hydroxylamine hydrochloride.

2.1.3. Reduction of mercury(II) into metal mercury by tin chloride (II).

2.1.4. Entrainment of mercury by a current of argon at room temperature.

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Detremining mercury in the state of monoatomic vapour by atomic fluorescence spectrometry, with the wave length at 254 nm: the mercury atoms are excited by a mercury vapour lamp; the atoms thus excited reemit fluorescent radiation that enables to quantify the mercury present using a photonic detector placed at 90° in relation to excitation beam; detection by atomic fluorescence enables to obtain good linearity and eliminates memory effects.

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

The peristaltic pump draws up the tin chloride (II) solution, the blank (demineralised water containing 1% nitric acid) and the mineralised sample or calibration.

The metal mercury is entrained in the gas-liquid separator by a current of argon.

After going through the membrane of a dessicator, the mercury is detected by fluorescence.

Then the gaseous current goes through a potassium permanganate solution in order to trap the mercury.

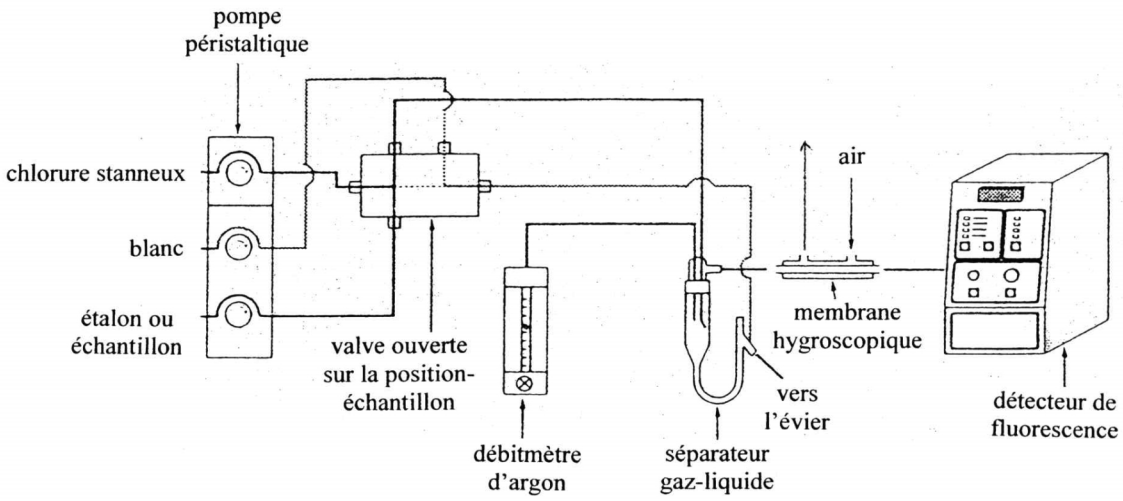

Figure n°1. Chaîne analytique pour doser le mercure

3. REAGENTS AND PREPARATION OF REAGENT SOLUTIONS

3.1. Ultra-pure demineralised water

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3.2. Ultra-pure nitric acid at 65%

3.3. **Blank: demineralised water** (3.1.) containing 1% nitric acid (3.2.)

3.4. **Nitric acid solution 5.6 M**: introduce 400 ml of nitric acid (3.2.) into a 1000 ml flask; complete to volume with demineralised water (3.1.).

3.5. **Sulphuric acid** (d = 1.84)

3.6. **Sulphuric acid solution 9 M**: introduce 200 ml of demineralised water (3.1.) in a 1000 ml flask, then 500 ml of sulphuric acid (3.5.); after cooling, complete to volume with demineralised water (3.1.).

3.7. **Potassium permanganate** $KMnO_4$

3.8. **Potassium permanganate solution at 5%**: dissolve with demineralised water (3.1.), 50 g of potassium permanganate (3.7.) in a 1000 ml flask; complete to volume with demineralised water (3.1.).

3.9. **Hydroxylamine hydrochloride** NH_2OH,HCl

3.10. **Reducing solution**: weigh 12 g of hydroxylamine hydrochloride (3.9.) and dissolve in 100 ml of demineralised water (3.1.).

3.11. **Tin chloride II** ($SnCl_2$,2 H_2O)

3.12. **Concentrated hydrochloric acid**

3.13. **Tin (II) chloride solution**: weigh 40 g of tin chloride (3.11.) and dissolve in 50 ml of hydrochloric acid (3.12.); complete to 200 ml with demineralised water (3.1.).

3.14 **Mercury reference solution at 1 g/l** prepared by dissolution of 1.708 g of $Hg(NO3)_2.H_2O$, in 1 l of HNO_3 solution at 12% (m/n).

3.15. **Mercury calibration solution at 10 mg/l**, containing 5 % of nitric acid and prepared from the reference solution at 1 g/l (3.14).

3.16. **Mercury solution at 50 µg/l**: place 1 ml of the solution at 10 mg/l (3.14.) in a 200 ml flask; add 2 ml of nitric acid (3.2.); complete to volume with demineralised water (3.1.).

4. APPARATUS

4.1. **Glassware**:

4.1.1. graduated flasks 100, 200 and 1000 ml (class A)

4.1.2. graduated pipettes 0.5; 1.0; 2.0; 5; 10 and 20 ml (class A)

4.1.3. precautions: before use, the glassware must be washed with nitric acid at 10%, left in contact for 24 hours, then rinsed with demineralised water.

4.2. **Mineralisation apparatus** (see Compendium of international methods of analysis

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of wines and musts)

4.3. Thermostatic **heating mantle**

4.4. **Peristaltic pump**

4.5. **Cold vapour generator**

4.5.1. gas-liquid separator

4.6. **Dessicator** (hygroscopic membrane) covered by an air current (supplied by a compressor) and placed before the detector

4.7. **Spectrofluorimeter**:

4.7.1. mercury vapour lamp, adjusted to the wave length of 254 nm

4.7.2. specific atomic fluorescence detector

4.8. **PC**:

4.8.1. software that adjusts the parameters of the vapour generator and atomic fluorescence detector and allows calibration and the analysis of results.

4.8.2. printer that archives results

4.9. **Bottle of neutral gas** (argon)

5. PREPARATION OF THE SET OF CALIBRATION SOLUTIONS AND SAMPLES

5.1. **Set of calibration solutions: 0; 0.25; 0.5 and 1.0 µg/l**

Introduce 0; 0.5; 1.0; 2.0 ml of the mercury solution at 50 µg/l (3.15.) in 4 100 ml flasks; add 1% nitric acid (3.2.); complete to volume with demineralised water (3.1.).

5.2. **Samples**

Mineralise the samples by wet process The test sample is introduced into the round-bottomed flask in borosilicate glass placed on a disc with a hole. The neck is inclined.

Add 5 ml of concentrated sulphuric acid (R) and 10 ml of concentrated nitric acid (R) and gently heat. When the mixture starts to turn brown, add a small quantity of nitric acid while continuing to heat and so forth until the liquid remains colourless and that the atmosphere of the flask fills with white smoke of SO3. Allow to cool, take 10 ml of distilled water and heat again to allow the nitrous fumes to escape until the release of the white smoke. This operation is repeated; after a third time, boil an instant, cool, stabilise with several drops (about 10) of potassium permanganate (aqueous sol.) at 5% (m/m) and add water to the liquid to reach 40 ml.

Filter on filters without cinders. Introduce 10 ml of filtrate into a 50 ml flask. Add potassium permanganate (3.8.) until persistence of coloration. Solubilise the

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precipitate (MnO_2) with the reducing solution (3.10.). Complete to volume with demineralised water (3.1.).

Do a blank test with demineralised water.

6. PROCEDURE

6.1. Analytical determination

Turn on the fluorimeter; the apparatus is stabilised after 15 minutes.

The peristaltic pump draws up the blank solution (3.3.), the tin chloride (II) solution (3.13.) and the calibrations or samples (5.1.) or (5.2.).

Check if there is a bubbling in the gas-liquid separator.

Present successively the calibration solutions (5.1.); start the programming of the vapour generator. The computer software sets up the calibration curve (percentage of fluorescence depending on the concentration of mercury in µg/l).

Then present the samples (5.2.).

6.2. Self-check

Every five determinations, an analytical blank solution and a calibration are analysed in order to correct a possible drift of the spectrofluorimeter.

7. EXPRESSION OF RESULTS

The results are given by the computer software and are expressed in p.p.b. (or µg/l).

The concentration of mercury in oenological products is calculated according to the test sample and the dilution of the mineralisate. It is expressed in µg/kg.

8. CONTROL OF RESULTS

The quality control is performed by placing, after the set of calibration solutions and all five samples, a reference material whose mercury content is known with certainty.

A control card is set up for each reference material used. The control limits are set at: +/- $2S_R$ intra (S_R intra: standard deviation for reproducibility).

9. BIBLIOGRAPHY

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SEARCH FOR HEAVY METALS

1. Principle of the method

Heavy metals react with the thiol function to form sulphurs. The coloration that results is compared to a standard.

2. Reagents

2.1. Ammonium acetate,
2.2. Lead nitrate (II),
2.3. Glycerol,
2.4. Methanol,
2.5. Sodium hydroxide, solution at 1 mole NaOH /l,
2.6. Hydrochloric acid at 37%,
2.7. Thioacetamide reagent (R):

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2.8. Standard lead solution:

2.8.1. Lead solution at 1000 µg/ml: dissolve 1.598 g of lead nitrate(II) in water and complete to 1000 ml.

2.8.2. Lead solution at 10 µg/ml. Add 10 ml of the solution 2.8.1 and complete to 1000 ml. To be prepared just before use.

2.9 **Buffer solution, pH = 3.5**: dissolve 6.25 g of ammonium acetate in 6 ml of water, add 6.4 ml of hydrochloric acid (2.6) and dilute with water until 25 ml.

3. Procedure

3.1. **Test solution**: pour 5 ml of buffer solution (2.9), 25.0 g of sample and about 15 ml of water into a 50 ml graduated flask. Complete with water up to the reference mark.

3.2. **Coloured solutions**:

3.2.1. Sample solution: mix 12.0 ml of test solution (3.1) and 2.0 ml of buffer solution (2.9) in a test tube.

3.2.2. Comparative solution: mix 2.0 ml of test solution (3.1), 2.0 ml of buffer solution (2.9), 0.5 ml of standard lead solution (2.8.2), 4.5 ml of water and 5.0 ml of methanol in a test tube.

3.2.3. Control solution: mix 12.0 ml of test solution (3.1), 2.0 ml of buffer solution (2.9) and 0.5 ml of standard lead solution (2.8.2) in a test tube.

3.2.4 Comparison of colorations:

add 1.2 ml of thioacetamide reagent (2.7) in the 3 test tubes (3.2.1 to 3), mix and wait 2 minutes. Compare the coloration vertically in the light of day.

- The sample solution must not be darker than the comparative solution.
- The control solution must not be lighter than the comparative solution.

4. Results

The conditions described in 3.2.4 are obtained if the heavy metal content is less than 10 mg/l expressed in lead and with a precision of 1 mg/l.

SEARCH FOR SULPHATES

In a 160 □ 16 mm test tube, place the volume prescribed of the solution obtained by the means indicated in each monography; add 1 ml of diluted hydrochloric acid (R); adjust

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to 20 ml with water and add 2 ml of barium chloride solution at 10% (R).

Compare the opalescence or any cloudiness to the control sample prepared with 1 ml of solution at 0.100 g of sulphuric acid per litre (i.e. 0.10 mg of H_2SO_4,) with 1 ml of diluted hydrochloric acid (R) and water until volume of 20 ml and 2 ml of barium chloride solution (R). This tube contains 100 µg of H_2SO_4.

BROMINE INDEX

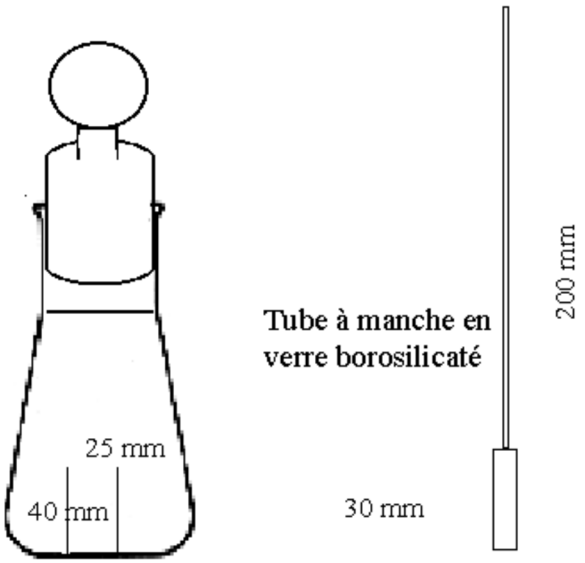
The bromine index is the quantity of bromine expressed in grammes, that 100 g of the substance can set.

1. Apparatus

A graduated flask of 300 to 400 ml with an interior tube welded at the bottom, an emery stopper and a tube with a handle, compliant with the following diagram

Bromination flask 300 ml in borosilicate glass.

Stopper with ground-glass joints standardised 24/40.



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2. Solutions

2.1. Potassium bromate solution 0.016 M

This solution contains for 1000 ml:

Potassium bromate $KBrO_3$: 2.783 g

Weigh exactly 2.783 g of potassium bromate and introduce into a 1000 ml graduated flask containing about 500 ml of distilled water; shake in order to dissolve and complete to 20°C with distilled water the volume of 1000 ml of solution. Mix and store in a flask with a glass stopper.

2.2. Iodine solution 0.05 M

Iodine I : 12.69 g

Potassium iodide de KI: 18 g

Water q.s.p. 1000 ml

Weigh exactly 12.69 g of iodine, then 18 g of potassium iodide and introduce into a 1000 ml graduated flask with about 200 ml of distilled water. Allow the dissolution to operate in cold conditions with the flask being sealed. Add about 500 ml of distilled water, then shake to absorb the iodine in a vapour state and complete to 20°C with distilled water, the volume to 1000 ml of solution. Mix and store in a coloured glass flask with a glass stopper.

2.3. Sodium thiosulphate solution 0.1 M

The 0.1 M sodium thiosulphate solution contains for 1000 ml:

Sodium thiosulphate $Na_2S_2O_3.5H_2O$: 24.82 g

Weigh exactly 24.82 g of sodium thiosulphate and introduce into a 1000 ml graduated flask containing about 600 ml of boiled distilled water. Shake to dissolve and complete to 20°C with boiled distilled water, the volume to 1000 ml of solution. Mix. Store away from light. Control the titre of this solution using the 0.05 M iodine solution.

3. Technique

Using a tube with a handle, put about 0.50 g of potassium iodide in the recipient inside the flask; (it is convenient to make a circular mark on the tube corresponding to the salt's weight so as not to have to weigh each dosage). Caution has to be taken so as not to introduce iodide on the external part of the flask. Then introduce the measured

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volume of the solution of the product to be measured, dissolved in neutral or alkaline water, in the external part of the flask, then 25 ml of potassium bromate solution 0.016 M measured with a pipette, and 2 g of pure potassium bromide. Rinse the sides with water to come to a total volume of about 100 ml, then add 5 ml of concentrated hydrochloric acid (R); quickly close the flask with the stopper, the joint being humid with distilled water; by a circular movement homogenise the content and allow to stand the prescribed time. Shake the flask *vigorously* so as to put the potassium iodide in contact with the liquid so as to enable the vapour bromine to react; open the flask while rinsing the joint and the stopper with a spray of distilled water, and determine iodine using 25 ml of sodium thiosulphate solution 0.1 M; titrate the excess of sodium thiosulphate with the iodine solution 0.05 M in the presence of starch paste;

Let *n* be the volume used:

Quantity of bromine (in mg) set by the substance to be dosed = *n* □ 0.008

SEARCH FOR CHLORIDES

In a 160 □ 16 mm test tube, place the volume prescribed of the solution obtained by the means indicated in each monography; add 5 ml of diluted nitric acid (R); complete to 20 ml and add 0.5 ml of silver nitrate solution at 5% (R).

Compare the opalescence or any cloudiness to the control sample prepared with 0.5 ml of hydrochloric acid at 0.10 g per litre (0.05 mg of HCl) with 5 ml of diluted nitric acid (R), and adjust to 20 ml with distilled water. Add 0.5 ml of silver nitrate solution at 5% (R). This tube contains 50 µg of HCl.

MINERALISATION METHODS OF SAMPLES BEFORE DETERMINATION BY ATOMIC ABSORPTION SPECTROMETRY

1. MINERALISATION BY DRY PROCESS

Method applicable for determining the following elements: calcium, magnesium, sodium, iron, copper, zinc.

1.1. Obtaining cinders

Weigh with precision 5 g of oenological product (or 1 g in the case of products rich in mineral matters), in a platinum or silice capsule cleaned and tared beforehand.

Gently burn the sample with the flame of a Bunsen burner under a hood.

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Put the capsule in a muffle oven at 525°C □ 25°C for 12 hours.

Take up the residue with a few ml of demineralised water.

Evaporate water over a water bath at 100°C.

Replace the capsule containing the sample in the oven.

The mineralisation is over when the cinders are white.

1.2. Putting the cinders in a solution

The cinders are solubilised with 2 ml of concentrated hydrochloric acid (R), bring to volume at 100 ml with demineralised water

Complementary dilutions:

Re-dilute the cinders solution in hydrochloric acid in order to be compatible with the sensitivity of the apparatus; see separately the method of each cation.

For the determination of calcium and magnesium, add lanthanum chloride during this dilution.

Do a blank test.

2. MINERALISATION BY WET PROCESS

Method applicable for determining the following elements: arsenic, cadmium, lead in oenological products containing water.

2.1. Case of aqueous products

Weigh with precision in a 50 ml polypropylene tube 3 grammes of pulverised oenological product, add 5 ml of nitric acid at 65%; close with a screw cap; leave 12 hours at room temperature then after unscrewing the cap place the tube in a water bath at 90°C for 3 hours under a hood; allow to cool; adjust the volume to 20 ml with demineralised water; shake; filter on an ashless filter paper (if necessary).

Do a blank test in the same conditions.

2.2. Case of dry products

The mineralisation is similar as for aqueous products but by using a test sample of 0.5 gramme of oenological product.

TANTALISATION OF PLATFORMS OF L'Vov IN GRAPHITE

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PREPARATION OF TANTALUM SOLUTION AT 6% (m/v) ACCORDING TO THE ZATKA PROCESS

Three grammes of tantalum powder are put in a 100 ml Teflon □ cylindrical vase.

Add 10 ml of hydrofluoric acid diluted to a half, 3 g of dehydrated oxalic acid and 0.5 ml of hydrogen peroxide at 30 vol.

Heat carefully to dissolve the metal.

Add a few drops of hydrogen peroxide as soon as the reaction slows down; when the dissolution is complete, add 4 g of oxalic acid and 30 ml of water.

The acid is dissolved and the solution is brought to 50 ml with ultra pure demineralised water.

Store this solution in a plastic flask.

TREATMENT OF GRAPHITE PLATFORMS

The platform is placed inside the graphite tube or used pyrolytic graphite tube. It is set to the unit of atomisation of the spectrophotometer.

A volume of 10 µl of tantalum solution is injected on the platform using an automatic distributor of samples;

Put the tantalum solution in the blank's position on the sample holder.

The temperature cycle is set according to the following programme:

- drying at 100°C for 40 seconds
- mineralisation at 900°C for 60 seconds
- atomisation at 2600°C for 2.5 seconds
- argon is used as an inert gas.

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MEASURING ARSENIC BY HYDRIDE GENERATION AND ATOMIC ABSORPTION SPECTROMETRY

1. FIELD OF APPLICATION

This method applies to the analysis of arsenic in the concentration range of 0 to 200 µg/l with prior mineralisation for oenological products.

2. DESCRIPTION OF THE TECHNIQUE

2.1. Principle of the method

After reducing arsenic (V) into arsenic (III), arsenic is determined by hydride generation and atomic absorption spectrometry.

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

The peristaltic pump draws up the borohydride solution, hydrochloric acid solution and calibration or sample.

The hydride formed in the gas-liquid separator is entrained by a neutral gas (argon).

The gaseous current passes in a dessicator made up of calcium chloride.

The arsenic hydride is analysed in an quartz absorption cell in the flame of a air-acetylene burner.

The optical path of the hollow-cathode lamp of the atomic absorption spectrometer passes in the quartz cell.

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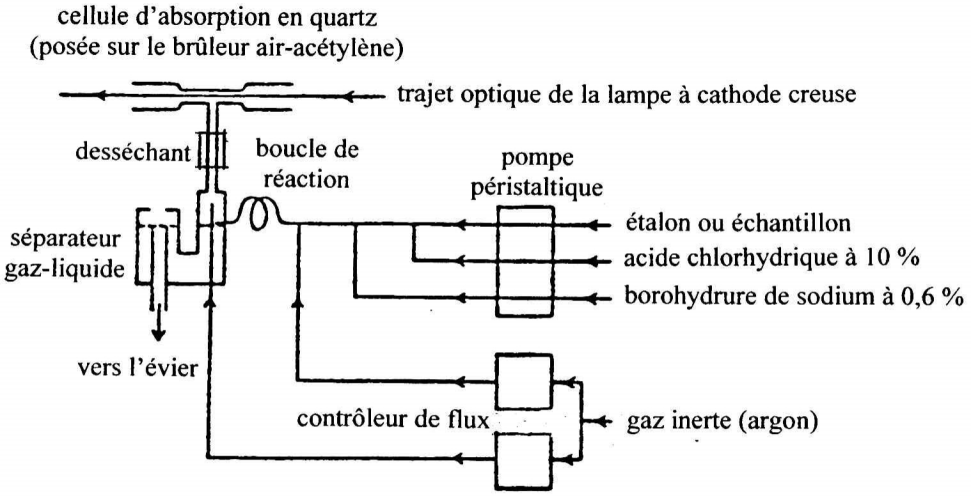

Figure n°1. Générateur d'hydrure

3. REAGENTS AND PREPARATION OF REAGENT SOLUTIONS

3.1. Ultra-pure demineralised water

3.2. Ultra-pure nitric acid at 65%

3.3. Potassium iodide KI

3.4. Potassium iodide at 10% (m/v)

3.5. Concentrated hydrochloric acid

3.6. Hydrochloric acid at 10% (m/v)

3.7. Sodium borohydride $NaBH_4$

3.8. Sodium hydroxide NaOH in patches

3.9. Sodium borohydride solution at 0.6% (containing 0.5% of NaOH)

3.10. Calcium chloride $CaCl_2$ (used as a dessicator)

3.11. Silicone antifoam

3.12. **Arsenic calibration solution at 1 g/l** containing 2% of nitric acid and prepared from the following acid: H_3AsO_4½ H_20

3.13. **Arsenic solution at 10 mg/l**: place 1 ml of the calibration solution (3.12.) in a 100 ml flask; add 1% of nitric acid (3.2.); complete to volume with demineralised water

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(3.1.).

3.14. **Arsenic solution at 100 µg/l**: place 1 ml of the arsenic solution at 10 mg/l (3.13.) in a 100 ml flask; add 1% of nitric acid (3.2.); complete to volume with demineralised water (3.1.).

4. APPARATUS

4.1. **Glassware**:

4.1.1. graduated flasks 50 and 100 ml (class A)

4.1.2. graduated pipettes 1, 5, 10 and 25 ml (class A)

4.1.3. cylindrical vases 100 ml

4.2. **Hot plate** with thermostat

4.3. Ashless **filter paper**

4.4. **Atomic absorption spectrophotometer:**

4.4.1. air-acetylene burner

4.4.2. hollow-cathode lamp (arsenic)

4.4.3. deuterium lamp

4.5. **Accessories:**

4.5.1. vapour generator (or gas-liquid separator)

4.5.2. quartz absorption cell placed on the air-acetylene burner

4.5.3. bottle of neutral gas (argon)

5. PREPARATION OF THE SET OF CALIBRATION SOLUTIONS AND SAMPLES

5.1. **Set of calibration solutions 0, 5, 10, 25 µg/l**

Place successively 0, 5, 10, 25 ml of the arsenic solution at 100 µg/l (3.14.) in 4, 100 ml flasks; add to each flask 10 ml potassium iodide at 10% (3.4.) and 10 ml of concentrated hydrochloric acid (3.5.); complete to volume with demineralised water (3.1.); allow to stand at room temperature for one hour.

5.2. **Samples of oenological products**

The sample is mineralised by wet process (cf. mineralisation methods of samples before determination by atomic absorption spectrometry) then filtered. Transfer 10 ml of filtered mineralisate to a 50 ml flask; add 5 ml of potassium iodide at 10% (3.4.) and 5 ml of concentrated hydrochloric acid (3.5.); add a drop of anti-foam (3.11.); adjust to volume with demineralised water (3.1.). Allow to stand at room temperature for one

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hour. Filter on an ashless filter paper.

6. PROCEDURE

6.1. Instrumental parameters of the atomic absorption spectrophotometer (given as an example)

6.1.1. oxidant air-acetylene flame

6.1.2. wave length: 193.7 nm

6.1.3. width of the monochromator's slit: 1.0 nm

6.1.4. intensity of the hollow-cathode lamp: 7 mA

6.1.5. correction of the non specific absorption with a deuterium lamp

6.2. Analytical determination

The peristaltic pump draws up the reagent solutions (3.6.) and (3.9.) and the calibrations or samples (5.1.) or (5.2).

Present successively the calibration solutions (5.1.); wait long enough so that the hydride formed in the gas-liquid separator, passes in the absorption cell; perform an absorbance reading for 10 seconds; perform two measurements; the spectrometer's computer software sets up the calibration curve (absorbance depending on the concentration of arsenic in µg/l).

Then present the samples (5.2.). Perform two measurements.

6.3. Self-check

Every five determinations, an analytical blank solution and a calibration are analysed in order to correct a possible deviation of the spectrometer.

7. EXPRESSION OF RESULTS

The results are directly printed by the printer connected to the computer.

The concentration of arsenic in oenological products is expressed in µg/kg while taking into account the test sample.

8. CONTROL OF RESULTS

The quality control is performed by placing, after the set of calibration solutions and every five samples, a reference material whose content in arsenic is known with

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certainty.

A control card is set up for each reference material used. The control limits were set at: +/- $2S_R$ intra (S_R intra : standard deviation of reproductibility).

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DETERMINATION OF CADMIUM BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The cadmium is determined in solid oenological products after mineralisation by wet process or directly for liquid oenological products or put in a solution.

The determinations are performed by atomic absorption without a flame (electro-thermal atomisation in a graphite oven).

2. APPARATUS

2.1. Instrumental parameters (given as an example)

Spectrophotometer equipped with an atomiser with a graphite tube.

- wave length: 228.8 nm
- hollow-cathode lamp (cadmium)
- width of slit: 1 nm
- intensity of the lamp: 3 mA
- correction of continuum by the Zeeman effect

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- graphite oven with a tantalised platform
- (tantalisation procedure of the platform described above)
- adjusting the oven for an analysis:

step	temperature (°C)	time (s)	gas flow rate (/ mn	type of gas	reading of signal
1	100	35	3.0	argon	no
2	500	10	3.0	argon	no
3	500	45	1.5	argon	no
4	500	1	0.0	argon	no
5	2250	1	0.0	argon	yes
6	2250	1	0.0	argon	yes
7	2500	2	1.5	argon	no
8	1250	10	3.0	argon	no
9	75	10	3.0	argon	no

2.2. Adjustments of the automatic sampler (given as an example)

	volumes injected in µl		
	solution of Cd at 8 µg/l	blank	matrix modifier
blank	0	10	2

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calibration N° 1 at 8 µg / l	1	9	2
calibration N° 2 at 16 µg / l	2	8	2
calibration N° 3 at 24 µg / l	3	7	2
calibration N° 4 at 32 µg / l	4	6	2
Sample to be dosed	5	5	2

3. REAGENTS

Demineralised water

Pure nitric acid for analysis at 65%

Anhydrous palladous chloride (59% in Pd)

Magnesium nitrate with 6 water molecules (ultra pure)

Ammonium dihydrogenophosphate

Matrix modifier: palladous chloride and magnesium nitrate mixture (dissolve 0.25 g of $PdCl_2$ and 0.1 g of $Mg(NO_3)_2.6H_2O$ in 50 ml of demineralised water) or ammonium dihydrogenophosphate at 6% (dissolve 3 g of $NH_4H_2PO_4$ in 50 ml of demineralised water).

Cadmium reference solution at 1 g/l, commercial or prepared as follows: dissolve 2.7444 g $Cd(NO_3)_2.4H_2O$ in a solution of HNO_3 0.5 M, adjust to 1 l with HNO_3 0.5 M.

Cadmium solution at 10 mg/l: place 1 ml of the reference solution in a 100 ml graduated flask, add 5 ml of pure nitric acid and complete to volume with demineralised water.

Cadmium solution at 0.8 g/l: place 4 ml of the diluted solution in a 50 ml graduated flask, add 2.5 ml of pure nitric acid and complete to volume with demineralised water.

Calibration range at 0, 8, 16, 24 and 32 µg/l of cadmium.

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4. PREPARATION OF SAMPLES

No preparation is necessary for liquid oenological products or in solution form; solid products are mineralised by wet process.

The blank solution is made up of a pure nitric acid solution for analysis at 1%.

5. PROCEDURE

Each calibration solution is passed right after the blank solution. Perform 2 successive absorbance readings and establish the calibration curve.

Calculate the cadmium content of the samples while taking into account the test sample of different dilutions.

DETERMINATION OF CALCIUM BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The calcium is directly determined in the liquid oenological product (or in the mineralisation solution) suitably diluted by atomic absorption spectrometry by air-acetylene flame after the addition of spectral buffer.

2. APPARATUS

Instrumental parameters (given as an example)

Atomic absorption spectrophotometer

Reducing air-acetylene flame

Hollow-cathode lamp (calcium)

- wave length: 422.7 nm
- width of slit: 0.2 nm
- intensity of the lamp: 5 mA

No correction of non specific absorption.

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3. REAGENTS

3.1. **demineralised water**

3.2. **calcium reference solution at 1 g/l**, commercial or prepared as follows: dissolve 5.8919 g of $Ca(NO_3)_2.4H_2O$ in a solution of HNO_3 0.5 M, adjust at 1 l with HNO_3 0.5 M.

3.3. **calcium solution at 100 mg/l**:

place 10 ml of the reference solution in a 100 ml graduated flask and 1 ml of pure nitric acid.

complete to volume with demineralised water

3.4. **concentrated hydrochloric acid (R)**: 35% minimum

3.5. **lanthanum solution at 25 g/l**:

- weigh 65.9 g lanthanum chloride ($LaCl_3.6H_2O$) in a 250 ml cylindrical vase, transfer to a 1000 ml graduated flask with demineralised water; add to the test tube 50 ml of concentrated hydrochloric acid (R); after solubilisation, allow to cool, complete to volume with demineralised water.

3.6. **set of calibration solutions**: 0, 2, 4, 6, 8 mg/l of calcium

place successively 0, 1,0, 2,0, 3,0 and 4.0 ml of the solution at 100 mg/l of calcium in 5, 50 ml graduated flasks, add 10 ml of lanthanum solution at 25 g/l, complete to volume with demineralised water.

4. PREPARATION OF SAMPLES

4.1. Case of liquid or solution oenological products

In a 50 ml graduated flask place 10 ml of the lanthanum solution and a volume of sample as after having being completed to volume with demineralised water; the concentration is below 8 mg/l.

4.2. Case of solid oenological products

Proceed with mineralisation by dry process;

Put in each solution of the set the same quantity of acid used for putting cinders in solution or mineralisation (see chapter “Mineralisation”).

1. Take up cinders and 2 ml of concentrated hydrochloric acid (35% minimum) in a

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100 ml flask; add 20 ml of lanthanum solution at 25 g/l and complete to volume with demineralised water.

2. Perform a blank test in the same conditions.

5. PROCEDURE

Pass each solution of the set in ascending order of the concentration of calcium.

For each solution, perform 2 absorbance readings when they are perfectly stabilised (integration time of signal: 10 seconds).

Pass each sample twice and calculate the calcium content.

DETERMINATION OF CHROME BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The chrome is determined by atomic absorption spectrophotometer without flame.

2. APPARATUS

2.1. Experimental parameters (given as an example)

- Atomic absorption spectrophotometer
- wave length: 357.9 nm
- hollow-cathode lamp (Chrome)
- width of slit: 0.2 nm
- intensity of the lamp: 7 mA
- correction of continuum by the Zeeman effect
- introduction in hot conditions of the samples in the graphite oven
- measurement of the signal: peak height
- time of measurement: 1 second

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- number of measurements per sample: 2
- pyrolytic graphite tube:
- pyrolytic graphite oven containing a platform L'Vov tantalised
- tantalisation of platform (see above)
- inert gas: argon - hydrogen mixture (95%; 5%)
- parameters for oven:

step	temperature (°C)	time (s)	gas rate flow (l / mn)	type of gas	reading of signal
1	85	5	3.0	argon + hydrogen	no
2	95	40	3.0	argon + hydrogen	no
3	120	10	3.0	argon + hydrogen	no
4	1000	5	3.0	argon + hydrogen	no
5	1000	1	3.0	argon + hydrogen	no
6	1000	2	0.0	argon + hydrogen	no
7	2600	1.2	0.0	argon + hydrogen	yes
8	2600	2	0.0	argon + hydrogen	yes
9	2600	2	3.0	argon + hydrogen	no
10	75	11	3.0	argon + hydrogen	no

2.2. Adjustments of the automatic sampler

(given as an example)

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	volumes injected in µl		
	chrome solution at 50 µg/l	blank	matrix modifier
blank	0	17	3
calibration N° 1 at 50 µg/l	5	12	3
calibration N° 2 at 100 µg/l	10	7	3
calibration N° 3 at 150 µg/l	15	2	3
sample to be measured	5	12	3

3. REAGENTS

3.1. **pure demineralised water for analysis**

3.2. **pure nitric acid for analysis at 65%**

3.3. **anhydrous palladous chloride** (59% in Pd)

3.4. **pure hexahydrated magnesium nitrate** for analysis

3.5. **ammonium dihydrogenophosphate**

3.6. **matrix modifier**: mixture of palladium chloride and magnesium nitrate (dissolve 0.25 g of $PdCl_2$ and 0.1 g of $Mg(NO_3)_2.6H_2O$ in 50 ml of demineralised water) ammonium dihydrogenophosphate at 6% (dissolve 3 g of $NH_4H_2PO_4$ in 50 ml of demineralised water).

3.7. **reducing agent**: L-ascorbic acid in solution at 1% m/v.

3.8. **chrome reference solution at 1 g/l**, commercial or prepared as follows: dissolve 7.6952 g of $Cr(NO_3)_3.9H_2O$ in a solution of HNO_3 0.5 M, adjust at 1 l with HNO_3 0.5 M

3.9. **chrome solution at 10 mg/l**: place 1 ml of the reference solution in a 100 ml graduated flask, add 5 ml of nitric acid at 65% and complete to volume with

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demineralised water.

3.10. **set of calibration solutions**: 0, 50, 100 and 150 µg/l of chrome (see table: adjustments of the automatic sampler).

4. PREPARATION OF SAMPLES

4.1. Case of liquid or solution oenological products

The preparations are performed manually or automatically by the diluter by following the data from the table “adjustments of the automatic sampler”.

4.2. Case of solid oenological products

Proceed with mineralisation by wet process. Do a blank test.

5. PROCEDURE

Pass each solution of the set in ascending order of the concentration of chrome;

Pass each sample twice and calculate the chrome content while taking into account the test sample.

DETERMINATION OF COPPER BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The copper is determined by atomic absorption spectrometry by flame by using the method of measured additions.

2. APPARATUS

Instrumental parameters: (given as an example)

- Atomic absorption spectrophotometer
- flame: oxidant air-acetylene
- wave length: 324.7 nm
- hollow-cathode lamp (copper)

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- width of slit: 0.5 nm
- intensity of the lamp: 3.5 mA
- no correction of non specific absorption.

3. REAGENTS

3.1. **pure demineralised water for analysis**

3.2. **pure nitric acid for analysis at 65%**

3.3. **reference solution copper at 1 g/l**, commercial or prepared as follows: dissolve 3.8023 g of $Cu(NO_3)_2.3H_2O$ in a solution of HNO_3 0.5M, adjust at 1 l with HNO_3 0.5M.

3.4. **copper solution at 10 mg/l**: place 2 ml of the reference copper solution in a 200 ml graduated flask, add 2 ml of nitric acid at 65% and complete to volume with demineralised water.

Adjust apparatus using a calibration solution at 0.4 mg/l (2 ml of the copper solution at 10 mg/l in a 50 ml graduated flask, complete to volume with pure demineralised water for analysis).

4. PREPARATION OF SAMPLES (METHOD OF MEASURED ADDITIONS)

- Addition of 02 mg/l of copper:

place 5 ml of liquid oenological product or mineralisate of oenological product obtained by dry process in a flask and add 100 µl of the copper solution at 10 mg/l

- Addition of 0.4 mg/l of copper:

place 5 ml of liquid oenological product or mineralisate in a flask and add 200 µl of the copper solution at 10 mg/l

- Dilution of the sample

Dilution of the sample: the dilution is only necessary if the copper content is more than 0.5 mg/l of copper.

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5. PROCEDURE

For each sample, pass in order:

- blank solution (demineralised water)
- sample with 0.2 mg/l of copper
- sample with 0.4 mg/l of copper
- sample without addition
- the results are obtained automatically or by manual graph.

DETERMINATION OF IRON BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The iron is determined by atomic absorption spectrophotometry by flame.

2. APPARATUS

2.1. Instrumental parameters: (given as an example)

- atomic absorption spectrophotometry
- flame: oxidant air-acetylene
- hollow-cathode lamp (iron)
- wave length: 248.3 nm
- width of slit: 0.2 nm
- intensity of the lamp: 5 mA
- no correction of non specific absorption.

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3. REAGENTS

3.1. **pure demineralised water for analysis**

3.2. **iron solution at 1 g/l**, commercial or prepared as follows: dissolve 7.2336 g of $Fe(NO_3)_2,9H_2O$ in a solution HNO3 0.5 M adjust at 1 l avec HNO_3 0.5 M.

3.3. **iron solution at 100 mg/l**

place 10 ml of the reference iron solution in a 100 ml graduated flask, complete with demineralised water pure for analysis

3.4. **set of calibration solution**: 2, 4, 6, 8 mg/l of iron

place successively 1.0, 2.0, 3.0 and 4.0 ml of the solution at 100 mg/l of iron in 4, 50 ml graduated flasks; complete to volume with pure demineralised water for analysis

Perform a blank without iron in the same conditions.

4. PREPARATION OF SAMPLES

4.1. Case of liquid or solution oenological products

Each sample is diluted with demineralised water in order to have a concentration of iron between 0 and 8 mg/l.

4.2. Case of solid oenological products

Proceed with mineralisation by dry process.

Put in each solution of the set of calibration the same quantity of acid used for putting of cinders in solution; each sample is diluted with demineralised water in order to have a concentration of iron between 0 and 8 mg/l.

5. PROCEDURE

Pass successively the calibration solutions and the blank which will be demineralised water or a water-acid solution with concentrations used for samples of solid oenological products mineralised by dry process and perhaps diluted.

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DETERMINATION OF NICKEL BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The nickel is directly determined by atomic absorption spectrometry without flame (electro-thermal atomisation).

2. APPARATUS

2.1. Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer equipped with an atomiser with a graphite tube.

- wave length: 232.0 nm
- hollow-cathode lamp (nickel)
- width of the slit: 0.2 nm
- intensity of the lamp: 4 mA
- correction of continuum by the Zeeman effect
- introduction in hot conditions of the samples in the graphite oven with an automatic distributor
- rinsing water contains 2 drops of Triton per litre.
- measurement of signal: peak height.
- Time of measurement: 1 second.
- pyrolytic graphite tube:
- pyrolytic graphite oven containing a platform of L'Vov tantalised.
- tantalisation of a platform: see above.
- inert gases: argon and argon + hydrogen mixture (95%: 5%).
- parameters for oven:

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Parameters for oven for determining nickel

step n°	temperature (°C)	time (s)	gas flow rate (l/min)	type of gas	reading of signal
1	85	5.0	3.0	argon	no
2	95	40.0	3.0	argon	no
3	120	10.0	3.0	argon	no
4	800	5.0	3.0	argon	no
5	800	1.0	3.0	argon	no
6	800	2.0	0	argon	no
7	2 400	1.1	0	argon + hydrogen	yes
8	2 400	2.0	0	argon + hydrogen	yes
9	2 400	2.0	3.0	argon	no
10	75	11.0	3.0	argon	no

2.2. Adjustment of automatic sampler (given as an example)

- Parameters of automatic sampler

	volume injected in µl		
	solution of Ni	blank	matrix modifier

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	at 50 µg/l		
blank		17	3
calibration 1	5	12	3
calibration 2	10	7	3
calibration 3	15	2	3
sample	5	12	3

3. REAGENTS

3.1. Pure demineralised water for analysis

3.2. Pure nitric acid for analysis at 65%

3.3. Anhydrous palladium chloride (59% in Pd)

3.4. Pure hexahydrated magnesium nitrate for analysis

3.5. Ammonium dihydrogenophosphate

3.6. Matrix modifier: mixture of palladium chloride and magnesium nitrate (dissolve 0.25 g of $PdCl_2$ and 0.1 g of $Mg(NO_3)_2.6H_2O$ (3.4) in 50 ml of demineralised water) ammonium dihydrogenophosphate at 6% (dissolve 3 g de $NH_4H_2PO_4$ in 50 ml of demineralised water), (3.1).

3.7 L-ascorbic acid

3.8 Analytical blank solution: L-ascorbic acid solution at 1% (m/v).

3.9 Nickel reference solution at 1 g/l (1000 µg/ml) off the shelf or prepared as follows: dissolve 4.9533 of $Ni(NO_3)_2.6H_2O$ in a solution of HNO_3 0.5 M, adjust at 1 l with HNO_3 0.5 M.

4. PROCEDURE

Nickel solution at 10 mg/l: place 1 ml of the reference solution (3.8) in a 100 ml graduated flask, add 5 ml of nitric acid (3.2); complete to volume with demineralised water.

Nickel solution at 50 µg/l: place 1 ml of the nickel solution at 10 mg/l in a 200 ml

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graduated flask, 10 ml of nitric acid (3.2) and complete with demineralised water.

Set of calibration solution: 0, 50, 100 and 150 µg/l of nickel.

The automatic distributor cycle enables to perform this calibration on the platform from a nickel solution at 50 µg/l.

5. PREPARATION OF SAMPLES

5.1. Case of liquid or solution samples

No preparation or sample dilution is necessary; the samples are placed directly in the cups of the automatic injector.

5.2. Case of solid samples

The solid samples are mineralised by dry process.

6. DETERMINATIONS

The calibration graph (absorbance depending on the concentration of nickel) gives the concentration of nickel in the samples.

DETERMINATION OF POTASSIUM BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The potassium is determined by mineralisation by dry process by atomic absorption spectrometry.

The addition of a spectral buffer (cesium chloride) to avoid the ionisation of the potassium is necessary.

2. APPARATUS

2.1. Glassware

100 and 200 ml graduated flasks (class A)

1, 2, 4 and 10 ml graduated pipettes (class A)

100 ml cylindrical vase

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2.2. Instrumental parameters (given as an example)

- atomic absorption spectrophotometer
- oxidant air-acetylene flame (flow rate-air: 3 l/min, flow rate-acetylene: 1.8 l/min.)
- Hollow-cathode lamp (potassium)
- wave length: 769.9 nm
- width of the slit: 0.5 nm
- intensity of the lamp: 7 mA
- no correction of non specific absorption.

3. REAGENTS

3.1. Pure demineralised water for analysis

3.2. Cesium chloride (CsCl)

3.3. **Cesium chloride solution at 5% in cesium**: Dissolve 6.330 g of cesium chloride in 100 ml of demineralised water.

3.4. **Potassium reference solution at 1 g/l** commercial or prepared as follows: dissolve 2.5856 g KNO_3 in water, adjust to 1 l.

3.5. **Diluted potassium solution at 100 mg/l**: Place 10 ml of the potassium reference solution at 1 g/l in a 100 ml graduated flask and 1 ml of pure nitric acid; complete to volume with pure demineralised water for analysis.

3.6. **Set of calibration solution** at 0, 2, 4, 6 and 8 mg of potassium per litre:

In a series of 100 ml graduated flasks, introduce 0; 2.0; 4.0; 6.0; 8.0 ml of the potassium solution at 100 mg/l ; add 2 ml of the cesium chloride solution to all the graduated flasks; adjust the volume to 100 ml with pure demineralised water for analysis.

The calibration solutions prepared contain 1 g of cesium per litre.

4. PREPARATION OF SAMPLES

4.1. Liquid or solution oenological products

In a 50 ml graduated flask, place 1 ml of the cesium chloride solution at 5% and a

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volume of a sample as is after having completed to volume with demineralised water; the concentration of potassium to be measured is below 8 mg/l.

4.2. Solid oenological products

Proceed with mineralisation by dry process (take cinders in 2 ml of hydrochloric acid in a 100 ml flask, add 2 ml of cesium chloride at 5% and complete to volume with demineralised water).

Perform a blank test with demineralised water.

5. DETERMINATIONS

Present successively the calibration solutions.

Perform an absorbance reading for 10 seconds; perform two measurements.

Set up the calibration curve (absorbance depending on the concentration in mg/l of potassium).

Then present the samples, perform an absorbance reading for 10 seconds; perform two measurements.

Calculate the concentration of potassium in the oenological products in mg/kg.

DETERMINATION OF LEAD BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

After mineralisation of the sample in an acid medium, the lead is determined by spectrometry without flame (electro-thermal atomisation).

2. APPARATUS

2.1. Instrumental parameters: (given as an example)

- Atomic absorption spectrophotometer equipped with an atomiser with a graphite tube
- wave length: 283.3 nm
- hollow-cathode lamp (lead)
- width of slit: 0.5 nm

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- intensity of the lamp: 5 mA
- correction of continuum: by Zeeman effect
- introduction in hot conditions of the samples in the graphite oven by an automatic distributor (rinsing water contains 2 drops of Triton per litre)
- measurement of signal: peak height
- time of measurement: 1 second
- number of measurements per sample: 2
- pyrolytic graphite tube
- pyrolytic graphite oven containing a platform of L'Vov tantalised
- (tantalisation of a platform: see above).
- parameters for oven

temperature (°C)	time (s)	gas flow rate (l / min)	type of gas	Reading of signal
150	20.0	3.0	argon	no
150	35.0	3.0	argon	no
800	15.0	3.0	argon	no
800	30.0	3.0	argon	no
800	2.0	0.0	argon	no
2250	0.8	0.0	argon	yes
2250	1.0	0.0	argon	yes
2500	1.0	1.5	argon	no
1200	9.0	3.0	argon	no

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75	10.0	3.0	argon	no

2.2. Adjustments of the automatic sampler

(given as an example)

	volumes injected in µl		
	lead solution at 50 µg / l	blank	matrix modifier
blank	0	10	2
calibration N° 1	1	9	2
calibration N° 2	2	8	2
calibration N° 3	3	7	2
calibration N° 4	4	6	2
calibration N° 5	6	4	2
Sample to be measured	10	0	2

3. REAGENTS

3.1. Pure demineralised water for analysis

3.2. Pure nitric acid for analysis at 65%

3.3. Ammonium dihydrogenophosphate

3.4. Matrix modifier: ammonium dihydrogenophosphate at 6%.

Introduce 3 g of ammonium dihydrogenophosphate in a 50 ml graduated flask, dissolve and complete to volume with demineralised water.

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Lead reference solution at 1 g/l commercial or prepared as follows: dissolve 1.5985 g of pure $Pb(NO_3)_2$ for analysis in a solution of HNO_3 0.5 M, adjust at 1 l avec HNO_3 0.5 M.

Lead solution at 10 mg / l: place 1 ml of the reference lead solution at 1 g/l in a 100 ml graduated flask; add 1 ml of nitric acid at 65% complete to volume with pure demineralised water for analysis.

Lead solution at 0.1 mg/l: place 1 ml of the lead solution at 10 mg/l in a 100 ml graduated flask,

add 1 ml of nitric acid at 65%; complete to volume with pure demineralised water for analysis.

Set of calibration solutions: 0, 50, 100, 150, 200, 300 µg/l of lead.

The automatic distributor cycle allows to directly inject these quantities of lead on the platform from the lead solution at 0.050 mg/l.

4. PREPARATION OF SAMPLES

The liquid or solution samples must have concentrations between 0 and 300 µg/l of lead.

The solid samples will be mineralised by wet process (attack by nitric acid).

The blank is made up of pure water for analysis containing 1% of nitric acid at 65%.

5. PROCEDURE

The calibration curve represents the variations of absorbencies depending on the concentrations enabling to calculate the lead content of the samples.

DETERMINATION OF SELENIUM BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

After mineralisation of the sample by wet process, the selenium is determined by atomic absorption spectrometry without flame (electro-thermal atomisation in the graphite oven).

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2. APPARATUS

2.1. Glassware

Graduated flasks 50, 100 ml (class A)
Graduated pipettes 1, 5 and 10 ml (class A)
Polypropylene tubes 50 ml with screw top.

2.2. Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer equipped with an atomiser

- with a graphite tube.
- wave length: 196.0 nm
- hollow-cathode lamp (selenium)
- width of slit: 1.0 nm.
- intensity of the lamp: 10 mA
- correction of continuum by the Zeeman effect
- introduction in hot conditions of the samples in the graphite oven with an
- automatic distributor (rinsing water contains 2 drops of Triton
- per litre).
- measurement of signal: peak height
- time of measurement: 1 second
- number of measurements per sample: 2

Pyrolytic graphite tube:

- Pyrolytic graphite oven containing a platform of L'Vov tantalised.
- tantalisation of a platform: see given procedure beforehand.
- inert gas: argon.
- parameters for oven: table I

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Table I - Parameters for oven for determining selenium

step	temperature (°C)	time (s)	gas flow rate (l/min)	type of gas	reading of signal
1	85	5	3.0	argon	no
2	95	40	3.0	argon	no
3	120	10	3.0	argon	no
4	1 000	5	3.0	argon	no
5	1 000	1	3.0	argon	no
6	1 000	2	0	argon	no
7	2 600	0.8	0	argon	yes
8	2 600	2	0	argon	yes
9	2 600	2	3.0	argon	no

2.3. Automatic sampler parameters (table II)

(given as an example)

Table II - Parameters de automatic sampler.

	volumes injected in µl		
	solution	blank	matrix modifier
blank		17	3

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calibration n°1 50 µg/l	5	12	3
calibration n°2 100 µg/l	10	7	3
calibration n°3 150 µg/l	15	2	3
sample	15	2	3

3. REAGENTS

3.1. Pure demineralised water for analysis

3.2. Pure nitric acid for analysis at 65%

3.3. Anhydrous palladium chloride (59% in Pd)

3.4. Pure hexahydrated magnesium nitrate for analysis

3.5. Ammonium dihydrogenophosphate

3.6. **Matrix modifier**: mixture of palladium chloride and magnesium nitrate (dissolve 0.25 g of $PdCl_2$ and 0.1 g of $Mg(NO_3)_2.6H_2O$ in 50 ml of demineralised water) ammonium dihydrogenophosphate at 6% (dissolve 3 g de $NH_4H_2PO_4$ in 50 ml of demineralised water).

3.7. **Selenium reference solution at 1 g/l**, off the shelf or prepared as follows: dissolve 1.4052 g SeO_2 in a solution of HNO_3 0.5 M, adjust at 1 l avec HNO_3 0.5 M.

3.8. **Selenium solution at 10 mg/l**: place 1 ml of the reference solution at 1 g/l in a 100 ml graduated flask; add 5 ml of nitric acid at 65%; complete to volume with pure demineralised water for analysis

3.9. **Selenium solution at 50 µg/l**: place 0.5 ml of the selenium solution at 10 mg/l, 5 ml of nitric acid at 65% in a 100 ml graduated flask; complete to volume with pure demineralised water for analysis.

3.10. **Set of calibration solutions**: 0, 50, 100 and 150 µg/l of selenium.

The automatic distributor cycle enables to perform this calibration on the platform from the selenium solution at 50 µg/l.

4. PREPARATION OF SAMPLES

Weigh with precision a test sample of 1 to 3 g in the graduated tube; add 5 ml of nitric acid at 65%; close with the screw cap; leave 12 hours at room temperature;

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place the tube in a water bath at 90°C for 3 hours (the caps are unscrewed during the heating); allow to cool; adjust the volume to 20 ml with pure demineralised water for analysis.

5. DETERMINATIONS

Set up the calibration graph (absorbance depending on the concentration in µg/l of selenium); determine the concentration of selenium in the samples.

Calculate the concentration of selenium in the mineralisate, then in the sample in µg/kg.

DETERMINATION OF SODIUM BY ABSORPTION ATOMIC SPECTROMETRY

1. PRINCIPLE

The sodium is determined after mineralisation by dry process by atomic absorption spectrometry.

The addition of a spectral buffer (cesium chloride) to avoid ionisation of sodium is necessary.

2. APPARATUS

2.1. Glassware

Graduated flasks 50 and 100 ml (class A)

Graduated pipettes 2.0; 5.0; 10.0 ml (class A)

Automatic pipette 1000 µl

Cylindrical vase 100 ml.

2.2. Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer

- oxidant air-acetylene flame (rate-air: 3.1 l/mn; rate-acetylene: 1.8 l/mn)
- wave length: 589.0 nm
- hollow-cathode lamp (sodium)

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- width of slit: 0.2 nm
- intensity of the lamp: 5 mA
- no correction of non specific absorption

3. REAGENTS

3.1. Pure demineralised water for analysis

3.2. Pure nitric acid for analysis at 65%

3.3. **Cesium chloride solution at 5% in cesium**: Dissolve 6.330 g of cesium chloride in 100 ml of pure demineralised water for analysis.

3.4. **Sodium reference solution at 1 g/l** commercial or prepared as follows: dissolve 3.6968 g NaNO3 in water, adjust at 1 l.

3.5. **Diluted sodium solution at 10 mg/l**:

Place 1 ml of the reference solution at 1 g/l in a 100 ml graduated flask, 1 ml of nitric acid at 65%, complete to volume with pure demineralised water for analysis.

3.6. **Set of calibration solutions** 0; 0.25; 0.50; 0.75; 1.00 mg of sodium

per litre:

In a series of 100 ml graduated flasks, place 0; 2.5; 5.0; 7.5; 10 ml of the diluted sodium solution; in all the graduated flasks add 2 ml of the cesium chloride solution and adjust the volume at 100 ml with pure demineralised water for analysis.

The calibration solutions prepared contain 1 g of cesium per litre; they are stored in polyethylene flasks.

4. PREPARATION OF SAMPLES

4.1. Liquid or solution oenological products

In a 50 ml graduated flask, place 1 ml of the cesium chloride solution at 5% and a volume of sample after having been completed to volume with demineralised water, the concentration of sodium to be measured is below at 1 mg/l.

4.2. Solid oenological products

Proceed with a mineralisation by dry process (take up the cinders in 2 ml of hydrochloric acid in a 100 ml flask, add 2 ml of cesium chloride at 5% and complete to volume with demineralised water).

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Perform a blank test with demineralised water.

5. DETERMINATIONS

Present successively calibration solutions.
Perform an absorbance reading for 10 seconds; perform two measurements.
Set up the calibration curve (absorbance depending on the concentration in mg/l of sodium).
Then present the samples; determine the concentration of sodium of the diluted samples in mg/l.
Calculate the concentration of sodium in the oenological products in mg/kg.
The dosages of air-acetylene flame are performed manually.

DETERMINATION OF ZINC BY ATOMIC ABSORPTION SPECTROMETRY

1. PRINCIPLE

The zinc is determined directly by atomic absorption spectrometry by flame.

2. APPARATUS

2.1. Instrumental parameters: (given as an example)

- atomic absorption spectrometer
- oxidant air-acetylene flame
- wave length: 213.9 nm
- hollow-cathode lamp (zinc)
- width of slit: 0.5 nm
- intensity of the lamp: 3.5 mA
- correction of the non specific absorption with a deuterium lamp.

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3. REAGENTS

3.1. Pure demineralised water for analysis

3.2. Pure nitric acid for analysis at 65%

3.3. **Zinc reference solution at 1 g/l** commercial or prepared as follows: dissolve 4.5497 g of $Zn(NO_3)_2$. $6H_2O$ in a solution of HNO3 0.5 M, adjust at 1 l with HNO_3 0.5 M.

3.4. **Zinc solution at 10 mg/l**:

place 1 ml of the zinc reference solution in a 100 ml graduated flask, 1 ml of nitric acid (3.2) and complete to volume with pure demineralised water for analysis.

3.5. **Set of calibration solution**: 0.2; 0.4; 0.6; 0.8; 1.0 mg/l: place successively 1, 2, 3, 4, 5 ml of the zinc solution at 10 mg/l in 5, 50 ml graduated flasks, complete to volume with pure demineralised water for analysis.

4. PREPARATION OF SAMPLES

The liquid or solution samples must have concentrations between 0 and 1 mg/l of zinc.

The solid samples are mineralised by dry process.

The blank solution is made up of pure water for analysis containing 1% of nitric acid at 65%.

5. PROCEDURE

Pass successively the blank, the calibration solutions and the samples of oenological products.

The absorbency readings are performed for 10 seconds and the measurements are duplicated.

The concentrations of zinc in the samples are obtained from absorbency values.

ANALYSES OF GAS CONTROL BY GASEOUS CHROMATOGRAPHY

1. PRINCIPLE

The gases are controlled by chromatography in gaseous phase using a "molecular sieve" type column and detection by catharometer or flame ionisation.

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2. SAMPLING

Either use

- a stainless steel flask for sampling gas
- a Teflon sampling bag for gas.

3. INJECTION METHOD

Use of a unheated gas valve with a 250 µl ring.

4. SEPARATION OF LIGHT GASES, H_2, O_2, N_2, CO, CH_4.

4.1. Column (for example)
Phase: Molecular sieve Chromosorb 101, Porapak Q

- diameter of particles 5µm
- granulometry: 80 to 100 mesh

Dimensions: length: 2 m, internal diameter: 2 mm.
4.2. Vector Gas
Helium (He), flow: 3 ml/mn
4.3. Oven temperature: 40°C isotherm
4.4. Detector: Catharometer, Intensity 190 µA

5. SEPARATION OF LIGHT HYDROCARBONS

5.1. Column (for example)

Wide bore
Phase: apolar, diameter of particles: 5 µm
Length: 30 m, internal diameter: 0.53 mm

5.2. Vector gas

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Nature: Helium, Flow: 3 ml/mn

Oven temperature 35°C to 200°C rise: 10°C/mn

5.3. Detector:

Flame ionisation, temperature 220°C.

AROMATIC POLYCYCLIC HYDROCARBONS DETERMINATION OF BENZO[a]PYRENE IN OENOLOGICAL CHARBONS BY HPLC

1. PRINCIPLE

Polycyclic aromatic hydrocarbons including benzo□a□pyrene are extracted by hexane; the solvent is evaporated and the residue is taken up by the methanol-tetrahydrofuran for analysis by HPLC.

2. APPARATUS AND REAGENTS

2.1. Reagents and calibrations

- Acetonitrile for HPLC
- Hexane for pesticide residues
- Tetrahydrofuran for HPLC (THF)
- Deionised and microfiltered water
- Benzo[a]pyrene for HPLC.

2.2. Apparatus and chromatographic conditions

- octadecyl type HPLC column
- fluorimetric detector adjusted to the following detection conditions:
- excitation wave length: 300 nm,
- emission wave length: 416 nm.

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Mobile phase:

- solvent A: Deionised and microfiltered water
- solvent B: acetonitrile
- variations in the composition of the solvent

TIME in min	% solvent A	% solvent B
0	50	50
15	20	80
40	0	100
45	50	50

Flow 1.0 ml/mn

2.3. Preparation of reference solutions

Benzo□a□pyrene reference solution at about 100 mg/l in a methanol/THF mixture (50/50) stored for 3 years maximum in cold conditions.

Daughter solution at about 20 µg/l, prepared extemporaneous (0.5 ml of reference solution in 50 ml of methanol/THF then 1 ml of this intermediate solution in 50 ml de methanol/THF).

2.4. Preparation of samples

2 g of oenological charbon are mixed in a 50 ml volumetric flask with 30 ml of hexane.

The polycyclic aromatic hydrocarbons are extracted for 5 min using a magnetic stirrer. The organic phase recovered by filtration is gathered in a evaporating flask and evaporated. The extract is taken up by 2 ml of a methanol/THF mixture (1/1, v/v) and injected.

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3. RESULTS

The benzo□a□pyrene content must not be higher than 1 µg/kg.

REMARK: It is also possible to determine benzo[a]pyrene by chromatography in gaseous phase by an apolar capillary column with detection by mass spectrometry.

DETERMINATION OF 5-(HYDROXYMETHYL)FURFURAL

1. PRINCIPLE

The 5-(hydroxymethyl)furfural (HMF) is determined by HPLC (sharing liquid chromatography in reverse phase).

2. APPARATUS AND SOLUTIONS

2.1. Instrumental parameters (for example)

Chromatograph in liquid phase

- UV/visible detector
- column: octadecyl type grafted silica (C18), (length: 20 cm; internal diameter: 4.6 mm; granulometry of phase: 5 µm)
- mobile phase: ultra filtered demineralised water - methanol - acetic acid (80, 10, 3: v/v/v)
- flow: 0.5 ml/mn
- detection wave length: 280 nm
- injected volume: 20 µl

2.2. Preparation of calibration solutions

Solution HMF at 20 mg/l:

In a 100 ml graduated flask, introduce 20 mg of HMF weighed within 0.1 mg and complete to the graduated line with ultra filtered demineralised water,

introduce 10 ml of this solution in a 100ml graduated flask and complete with ultra

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filtered demineralised water;
the solution HMF at 20 mg/l is to be prepared each day.

3. PREPARATION OF SAMPLES

The samples and the calibration solution HMF are injected after filtration on a 0.45 µm membrane.

4. PROCEDURE

The chromatographic column is stabilised with the mobile phase for about 30 min.
Calculate the concentration of HMF of the sample from the peak surfaces.

GRAPE SUGAR:

DETERMINATION OF SACCHAROSE BY HPLC

1. PRINCIPLE

The samples diluted or put in solution are analysed by high performance liquid chromatography: Separation on column of grafted silica NH_2 and detection using a differential refractometer.

2. APPARATUS AND ANALYTICAL CONDITIONS (for example)

2.1. Chromatograph

- Grafted silica column NH_2 (length 20 cm, internal diameter 4 mm granulometry 5 µm)
- A pumping system
- An auto-sampler (maybe
- Microfiltres with porosity 0.45 µm
- Differential refractometry detector

2.2. Chromatographic conditions (given as an example)

The water used is deionised and microfiltered.

The acetonitrile is of HPLC quality

The composition of the mobile phase is the following:

- If the column is new: acetonitrile/water (75/25)
- When the fructose - glucose resolution starts to deteriorate, the mobile phase is then a acetonitrile/water 80/20 mixture.

The flow is 1 ml/min.

3. REAGENTS AND CALIBRATION SOLUTIONS

3.1. Preparation of the reference solution

The chemicals used for the reference solution preparation are of "pure for analysis" quality.

The composition of this solution is about 10 g/l for each sugar (fructose, glucose and saccharose).

The reference solution is prepared every two weeks (maximum) and stored in the refrigerator in the 100 ml graduated flask used for the preparation.

SULPHURIC CINDERS

The sulphuric cinders result from the calcination after being in contact with air after being attacked by sulphuric acid.

Heat a silica or platinum crucible of low form for 30 min until red; allow to cool in a vacuum dessicator and tare the crucible. Place the exactly weighed test sample in the crucible and wet it with a sufficient quantity of concentrated sulphuric acid (R) diluted beforehand by an equal volume of water. Heat until dry evaporation, then in a muffle oven, first carefully until red without exceeding the temperature of 600°C □ 25°C. Maintain calcination until the black particles disappear, allow to cool, add 5 drops of sulphuric acid diluted to half to the residue, then evaporate and calcinate as previously until constant weight; weigh after cooling in the desiccator.

Calculate the rate of sulphuric cinders referring to 100 g of substance.

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TOTAL CINDERS

The total cinders result from the calcination of the product after contact with air.

Heat a silica or platinum crucible of low form for 30 min until red. Allow to cool in a vacuum dessicator and tare the crucible. Dispose homogenously the exactly weighed test sample in the crucible. Desiccate for an hour in the incubator at 100°C-105°C. Incinerate in the muffle oven, first carefully to avoid that the sample catches fire, then until red at a temperature of 600°C □ 25°C. Maintain the calcination until the black particles disappear. For 30 min allow to cool in a vacuum desiccator. Weigh. Continue the calcination until constant mass.

If the black particles persist, take up the cinders in hot distilled water. Filter these cinders on an ashless filter paper (porosity 10 µm). Incinerate the filter and residue until constant mass. Group the new cinders with the filtrate. Evaporate the water. Incinerate the residue until constant mass.

Calculate the rate of total cinders by referring to 100 g of substance.

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