OIV-MA-AS315-08 Examination of artificial colorants

Type IV method

1. Principle

The wine is concentrated to 1/3 its original volume, made alkaline with a solution of dilute sodium hydroxide and extracted with ether. The ether phase, after being washed with water, is extracted with a dilute acetic acid solution; this acetic solution, alkalinized with ammonia, is brought to boiling in the presence of a piece of wool thread treated with aluminum sulfate and potassium tartrate. The colorant, if any, is fixed on the wool. The wool on which it is fixed is then placed in a dilute acetic acid solution. After evaporation of the acetic solution, the residue is recovered with a water-alcohol solution and analyzed by thin layer chromatography for characterization of the colorant.

The aqueous phase remaining after the ether extraction contains the acid colorants that may be present. They are extracted by using their affinity for animal fibers that markedly absorb the color: they are fixed on a wool plug in a mineral acid medium.

To concentrate the coloring material, carry out a double fixation and/or several successive fixations on increasingly smaller wool plugs.

Coloring of the wool plug indicates that an artificial colorant was added to the wine; the colorant is then identified by thin layer chromatography.

2. Apparatus

2.1. 20 x 20 glass plates covered with cellulose powder,

2.2. Chromatography tank

3. Reagents

3.1. Ethyl ether

3.2. Sodium hydroxide solution, 5% (*m/v*)

3.3. Glacial acetic acid (ρ_{20}= 1.05 g/mL)

3.4. Dilute acetic acid, containing one part glacial acetic acid to 18 parts water

3.5. Dilute hydrochloric acid: to one part hydrochloric acid (ρ_{20} = 1.19 g/mL), add 10 parts distilled water

3.6. Ammonium hydroxide (ρ_{20} = 0.92 g/mL)

3.7. White wool threads, previously washed, degreased with ether and dried

3.8. White wool threads, previously washed, degreased with ether, dried and acidified

Acidulant: Dissolve 1 g crystallized aluminum sulfate $Al_2(SO_4)18H_2O$ and 1.2 g acid potassium tartrate in 500 mL water. Place 10 g of the white wool threads, previously washed, degreased with ether and dried in the solution and stir about 1 hour. Let stand 2 to 3 hours; drain, let dry at room temperature.

3.9. Solvent No.1 for chromatography of colorants with basic characteristics:

n-Butanol	50 mL
Ethanol	25 mL
Acetic acid (ρ_{20} = 1.05 g/mL)	10 mL
Distilled water	25 mL

3.10. Solvent No.2 for chromatography of colorants with acidic characteristics:

n-Butanol	50 mL
Ethanol	25 mL
Ammonium hydroxide (ρ_{20} = 0.92 g/mL)	10 mL
Distilled water	25 mL

4. Procedure

4.1. *Examination of colorants with basic characteristics*.

4.1.1. Extraction of the coloring materials.

Place 200 mL of wine in a 500 mL glass conical flask and boil until reduced to 1/3 its volume.

After cooling, neutralize with 5% sodium hydroxide solution until the natural color of wine shows a marked change.

Extract twice using 30 mL ether. The ether phases are recovered, containing basic colorants to be determined; the extraction residue must be saved for the analysis of acidic colorants.

Wash the extracted ether twice with 5 mL of water to eliminate the sodium hydroxide; mix with 5 mL dilute acetic acid. The acidic aqueous phase obtained is colored in the presence of a basic colorant.

The presence of the colorant may be confirmed by fixation on acidified wool. Make the acidic aqueous phase obtained alkaline using 5% ammonia. Add 0.5 g acidified wool and boil for about 1 minute. Rinse the wool under running water. If the wool is colored, the wine contains some basic colorant.

4.1.2. Characterization by thin-layer chromatography.

The aqueous acetic phase containing the basic colorant is concentrated to 0.5 mL. If the colorant is fixed on the acidic wool, the wool plug is treated by boiling with 10 mL distilled water and a few drops of acetic acid (ρ_{20} = 1.05 g/mL). Remove the wool fragment after wringing out liquid. Concentrate the solution to 0.5 mL.

Deposit 20 µL of this concentrated solution on the cellulose plate 3 cm from the lateral edge and 2 cm from the lower edge of the plate.

Place the plate in the tank containing solvent No.1 so that the lower edge is immersed in the solvent to a depth of 1 cm.

When the solvent front has migrated to a height of 15 to 20 cm, remove the plate from the tank. Allow to air dry.

Identify the colorant by means of a solution of known artificial colorants of basic characteristics deposited simultaneously on the chromatogram.

4.2. *Examination of colorants with acidic characteristics*

4.2.1. Extraction of the coloring material.

Use the residue from the wine used for examining colorants with basic characteristics, concentrated to 1/3 and neutralized after extraction with ether.

If the first part of the procedure has not been conducted, start with 200 mL wine, place in a conical flask, boil until reduced to 1/3.

In either case, add 3 mL of dilute hydrochloric acid and 0.5 g of white wool: boil for 5 minutes, decant the liquid and wash the wool under running water.

In the conical flask which contains the wool, add 100 mL water and 2 mL dilute hydrochloric acid; boil for 5 minutes, separate the acidic liquid and repeat this procedure until the liquid used to wash is colorless.

After the wool has been thoroughly washed to eliminate the acid completely, recover in a conical flask with 50 mL distilled water and a few drops of ammonium hydroxide (ρ_{20} = 0.92 g/mL): bring to a gentle boil for 10 minutes in order to dissolve any artificial coloring matter fixed on the wool.

Remove the wool from the flask, bring the liquid volume to 100 mL and boil until the ammonia completely evaporates. Acidify with 2 mL of dilute hydrochloric acid (check that the reaction of the liquid is definitely acidic by placing 1 drop of this liquid on

indicator paper).

Add to the flask 60 mg (about 20 cm of standard thread) of white wool and boil for 5 minutes; remove the wool and rinse it under running water.

If, after this procedure, the wool is colored red, when it involves red wine, or yellow if it pertains to white wine, the presence of artificial organic coloring matter of an acidic nature is proven.

If the color is weak or uncertain, repeat the ammonia treatment and do a second fixation using a 30 mg wool thread.

If, during the course of the second fixation a weak but distinct pink color is obtained, assume the presence of an acidic colorant.

If necessary for a more definite determination, carry out new fixations-elutions (up to 4 or 5) using a procedure identical to that used for the second fixation until a faint but distinct pink color is obtained.

4.2.2. Characterization by thin layer chromatography.

The plug of colored wool is treated by boiling with 10 mL distilled water and few drops of ammonium hydroxide (ρ_{20} = 0.92 g/mL). Recover the piece of wool after wringing.

Concentrate the ammonium hydroxide solution to 0.5 mL.

Deposit 20 µL of this solution on a cellulose plate to within 3 cm of the lateral edge and 2 cm of the lower edge of the plate.

Put the plate in place in the tank so that the lower edge is immersed in the solvent to a depth of 1 cm.

When the solvent front has migrated to a height of 15 to 20 cm, remove the plate from the tank and let dry in the air.

Identify the colorant by means of known artificial coloring solutions deposited simultaneously on the chromatogram.

Bibliography

- TERCERO C., *F.V., O.I.V.,* 1970, n° 356.
- Arata P., Saenz-Lascano-Ruiz, Mme I., *F.V., O.I.V.,* 1967, n° 229.