OIV-MA-AS4-02F Detection of preservatives and fermentation inhibitors

Type IV method

1. Sodium Azide

1.1. Method by high performance liquid chromatography

1.1.1. Principle

Hydrazoic acid isolated in wine using double distillation is identified after derivatization with 3,5-dinitrobenzoyl chloride, by high performance liquid chromatography. Detection is carried out by ultraviolet absorption spectrophotometry at 240 nm.

1.1.2. Apparatus

1.1.2.1. Distillation apparatus (distillation apparatus for determination of alcoholic strength); the end of the condenser terminating in a tampered tube

1.1.2.2. 500 mL spherical flasks with ground glass necks

1.1.2.3. 10 mL flask with a ground glass stopper

Operating conditions:

- Column: C_{18}, 25 cm long.
- Mobile Phase: acetonitrile-water (50:50)
- Flow rate: 1 mL/min.
- Volume injected: 20 µL
- Detector: ultraviolet absorption spectrophotometer at 240 nm
- Temperature: ambient

3. Reagents

1. Sodium hydroxide, 5% (*m/v*).
2. Sulfuric acid solution, 10% (*m/v*).
3. Indicator reagent: methyl red 100 mg, and methylene blue 50 mg, 100 mL alcohol, 50% (*v/v*).
4. Acetonitrile for chromatography.

5. Derivatizing reagent: 3,5-dinitrobenzoyle chloride, 10% (*m/v*), in acetonitrile.
6. Buffer solution of sodium acetate, pH 4.7: mix 1 volume of sodium acetate solution, Na $C_2H_3O_2.3H_2O$, 1 M, with 1 volume acetic acid solution, 1 M.
7. Sodium azide, NaN_3.

4. Procedure

1. Preparation of the sample.

Into a spherical flask with a ground glass neck, place 100 mL of wine, distill by plunging the end of the condenser in 10 mL of 5% sodium hydroxide solution (1.1.3), to which are added a few drops of reagent indicator. Distill until 40□50 mL of distillate is recovered.

Transfer the distillate into another spherical flask (1.1.2.2), rinse the flask twice with 20 mL of water and add water to bring to 100 mL. To eliminate the ethanol, attach the flask to the distillation apparatus and eliminate about 50 mL of distillate (reduce the volume by half).

Cool the flask completely. Acidify with 10% sulfuric acid. Distill, recover the distillate into a 10 mL flask with a ground glass stopper containing 1 mL of water, and immerse in an iced bath. Stop the distillation when the total volume reaches 10 mL.

1.1.4.2. Derivitization

Mix 1 mL distillate (1.1.4.1), 0.5 mL of acetonitrile, 0.2 mL buffer solution and 30 µL of derivatizing reagent and stir vigorously; leave for five minutes.Chromatography

1.1.4.3. Inject 20 µL in accordance with the conditions specified, the hydrazoic acid derivative has a retention time of about 11 minutes. Detection limit: 0.01 mg/L.

Note : Sometimes another substance not derivatized can simulate hydrazoic acid. It is necessary to verify a positive result as follows: inject 20 µL of distillate directly; a disappearance of the peak indicates the presence of hydrazoic acid.

1.1.5. Calculation

To determine the concentration of sodium azide, compare the sample response to that of the standard solution after derivatization. Take into account the concentration

factor 10 of the sample of wine at the time of analysis.

1.2. Colorimetric method

1.2.1. Principle

Hydrazoic acid, which is very volatile, is separated by double distillation, permitting the elimination of ethanol, acetic acid and sulfur dioxide. Then the amount is determined colorimetrically after forming a colored complex with ferric chloride (maximum absorbance at 465 nm).

1.2.2. Apparatus

1.2.2.1. Simple distillation apparatus, consisting of a 500 mL flask with a ground glass neck and a condenser ending in a pointed tube

1.2.2.2. Spectrophotometer with optical glass cells 1 cm path length

1.2.3. Reagents

1.2.3.1. Sodium hydroxide solution, 1 M

1.2.3.2. Sulfuric acid, 1 M

1.2.3.3. Hydrogen peroxide, 3% (*v/v*), whose strength must be adjusted just before use using a solution of potassium permanganate, 0.02 M; where *p* mL equals the volume which oxidizes 1 mL of the hydrogen peroxide solution, 3%

1.2.3.4. Ferric chloride solution at 20 g per liter of Fe III: (weigh 96.6 g of $FeCl_3.6H_2O$, or more as this salt is very hygroscopic; control the concentration of Fe III of the solution and adjust if necessary to 20 $\pm$ 0.5 g per liter)

1.2.3.5. Stock solution of sodium azide, NaN_3, at 1 g per liter in distilled water

1.2.3.6. 200 mg per liter sodium azide solution prepared by dilution of the solution at 1 g per liter

1.2.4. Procedure

a) Into a 500 mL flask with a ground glass neck, place 200 mL of wine, distill, recover the distillate in a 50 mL volumetric flask, containing 5 mL water, which is immersed in an iced bath. Stop the distillation when the total volume reaches about 50 mL.

b) Transfer quantitatively the distillate into another 500 mL flask with a stopper and rinse the 50 mL flask twice with 20 mL of water.

Neutralize using 1 M sodium hydroxide solution (1.2.3.1) (using pH indicator paper).

Acidify using 10 mL 1 M sulfuric acid (1.2.3.2), mix, then oxidize the sulfur dioxide by adding 3% hydrogen peroxide solution (1.2.3.3.).

If the wine contains S mg per liter of sulfur dioxide, and if *p* mL is the volume of

0.02 M potassium permanganate solution necessary to oxidize 1 mL of 3% hydrogen peroxide solution, then for 200 mL of wine use the following calculation:

$$\frac{S}{5 \times 3.2p} = \frac{S}{16p}\ mL\ of\ H_2O_2\ solution$$

Bring the volume to about 200 mL by addition of distilled water.

Distill, recover the distillate in a 50 mL glass flask containing 5 mL distilled water, which is immersed in an ice bath; stop the distillation before the measurement line, bring back to ambient temperature and adjust the volume to 50 mL.

c) Add 0.5 mL (measured exactly) of ferric chloride solution, mix and measure immediately (maximum delay 5 min.) the absorbance at 465 nm in a 1 cm

cell; the zero of the apparatus is set using a blank composed of 50 mL of water added to 0.5 mL of ferric chloride solution.

d) Preparation of the standard curve.

Into each of five 50 mL volumetric flasks add 1, 2, 3, 4, and 5 mL of 200 mg/L sodium azide solution respectively, bring the volume to 50 mL with distilled water, add 0.5 mL of ferric chloride solution and measure the absorbance at 465 nm.

These solutions contain 4, 8, 12, 16, 20 mg of sodium azide per liter. The corresponding concentrations are 1, 2, 3, 4, and 5 mg per liter of wine.

The typical curve of absorbance variation as a function of concentration is a straight line passing through the origin.

1.2.5. Calculation

Plot the absorbance read for the sample analyzed on the straight line and interpolate the concentration of sodium azide in mg/L of wine.

Bibliography

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