OIV-MA-AS312-04 Glycerol and 2,3-Butanediol

Type IV method

1. Principle

Glycerol and 2,3□butanediol are oxidized by periodic acid after treatment through an anion exchange resin column to fix the sugars and a large proportion of mannitol and sorbitol. The product obtained by the action of phloroglucinol on formaldehyde (by glycerol oxidation) is determined colorimetrically at 480 nm. The product formed by the action of piperidine solution and sodium nitroferricyanide solution with the ethanol (by oxidation of 2,3□butanediol) is determined colorimetrically at 570 nm.

2. Apparatus

2.1. Glass column 300 mm long and approximately 10-11 mm internal diameter fitted with a stopcock.

2.2. Spectrophotometer allowing measurement to be made between 300 and 700 nm and glass cells with optical path length of 1 cm.

3. Reagents

3.1. Glycerol, $C_3H_8O_3$

3.2. 2,3-Butanediol, $C_4H_{10}O_2$

3.3. A strongly basic anion exchange resin e.g. anion exchange resin of Merck strength III or Amberlite IRA 400.

3.4. Polyvinylpolypyrrolidone (PVPP) (see *International Oenological Codex*).

3.5. Periodic acid, 0.1 M, in sulfuric acid, 0.05 M.

Weigh 10.696 g of sodium periodate, $NaIO_4$, place into a 500 mL volumetric flask, dissolve with 50 mL of sulfuric acid, 0.5 M, and make up to 500 mL with distilled water.

3.6. Periodic acid, 0.05 M, in sulfuric acid, 0.025 M.

The above solution (3.5) is diluted 1 : 1 with distilled water.

3.7. Sulfuric acid, 0.5 M.

3.8. Sodium hydroxide solution, 1 M.

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

3.10. Ethanol, 96% (*v/v*).

3.11. Phloroglucinol solution, 2% (*m/v*), to be prepared fresh daily.

3.12. Sodium acetate solution, 27% (*m/v*), prepared from anhydrous sodium acetate, CH_3COONa.

3.13. Sodium nitroferricyanide solution, $Na_2Fe(CN)_5NO.2H_2O$, 2% (m/v), to be prepared fresh daily

3.14. Piperidine solution, $C_5H_{11}N$ 25% (*v/v*), to be prepared fresh daily.

3.15. Standard glycerol solution

Prepare a solution containing 250 g glycerol in 100 mL and determine the glycerol content by the enzymatic or periodimetric method (see section 6).

Prepare the standard glycerol solution as follows: weigh in a 100 mL volumetric flask a mass "*m*" corresponding to 250 mg of pure glycerol, make up to 100 mL with water.

3.16. Standard 2,3□butanediol solution

Prepare a solution containing 250 mg of 2,3□butanediol sample in 100 mL and determine the 2,3-butanediol content by the periodimetric method (see section 6).

Prepare the standard solution of 2,3□butanediol by weighing in a 100 mL volumetric flask a mass "*m*" corresponding to 250 mg of pure 2,3□butanediol; make up to 100 mL with water.

3.17. Alkaline copper solution:

Copper Solution A

Copper sulfate,$CuSO_45H_2O$: 40 g

Sulfuric acid (r=1.84 g/mL): 2 mL

Make up to 1000 mL with water

Alkaline tartaric solution B

Potassium sodium tartrate tetrahydrate

$KNaC_4H_4O_{6.}4H_20$: 200 g

Sodium hydroxide: 150 g

Make up to 1000 mL with water

The copper alkaline solution is obtained by mixing solution A and B in equal quantities at the time of use.

4. Procedure

4.1. *Preparation of an anion exchange column*

The anion exchange resin (Cl-) must be kept in a flask filled with decarbonated distilled water.

Put 30 mL of anion exchange resin (3.3) in the column (2.1), place a wool plug on the top of the column to stop air contact with the resin. Pass 150 mL of 5% sodium hydroxide (3.9) through the column at a flow rate of 3.5 to 5 mL per minute followed by a similar quantity of decarbonated distilled water at the same flow rate until the eluent is neutral or slightly alkaline to phenolphthalein. The resin is then ready for use.

The anion exchange resin can only be used once. It can be regenerated by treating with 5% hydrochloric acid for a few hours and then rinsed with water until free of chloride. (Check for absence of chloride).

4.2. Preparation of sample

The wine sample is diluted 10/50.

In case of strongly colored wines, first decolorize with PVPP (3.4): place 10 mL wine in a 50 mL volumetric flask, dilute with water (20 mL) and add 300 mg of PVPP (3.4). Leave for 20 min. stirring occasionally, make to the mark and filter through fluted filter paper. Take 10 mL of diluted wine (treated or untreated with PVPP) and place on the anion exchange column. Allow to drain, drop by drop, at flow rate not exceeding 2 mL per minute. When the level of diluted wine reaches 5□10 mm above the glass wool plug, add decarbonated distilled water to bring the volume of the eluent to 100 mL at a flow rate 2□3 mL per minute. The eluate must be free of sugars. To ensure this, boil rapidly 5 mL of eluate with 5 mL of alkaline copper solution (3.17). There should not be any discoloration or precipitation.

4.3. Determination of glycerol

4.3.1. Photometric determination

Place into a 100 mL conical ground necked vessel:

- 10 mL eluate and add successively
- 10 mL distilled water and
- 10 mL periodic acid solution, 0.05 M (3.6).

Stir carefully; leave exactly 5 min. for the oxidation to take place. Add 10 mL sodium hydroxide solution, (3.8), and 5 mL 96% ethanol (*v/v*) (3.10).

Stir after each addition, then add 10 mL phloroglucinol solution (3.11)

Mix rapidly and transfer the solution into a 1 cm cell. The purple coloration is

obtained very quickly. Its intensity reaches a maximum after 50 to 60 seconds, then decreases. Note the maximal absorbance. The measurement is carried out at 480 nm using air as a reference.

4.3.2. Preparation of the calibration curve

Pipette into 100 mL volumetric flasks:

3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mL glycerol standard solution (3.15) and make up to volume with distilled water.

These solutions correspond, according to the conditions in 4.2, to the following concentrations:

3.75, 5.00, 6.25, 7.50, 8.75 and 10.00 g/L of glycerol.

Proceed with the determination as described in 4.3.1, replacing the eluate by the same volume of each of the standard solutions.

4.4. Determination of 2,3□butanediol

4.4.1. Photometric determination

Place into a conical 100 mL ground stoppered vessel:

- 20 mL eluate and add successively
- 5 mL sodium acetate solution (3.12) and
- 5 mL 0.1 M periodic acid solution (3.5).

Stir to mix, leave for 2 min exactly for oxidation to take place

Add:

- 5 mL sodium nitroferricyanide solution (3.13) and
- 5 mL piperidine solution (3.14).

Transfer the solution into a 1 cm cell. The purple color is obtained very rapidly; its intensity reaches a maximum after 30□40 sec then diminishes. Note the maximal absorbance. The measurement is carried out at 570 nm using air as a reference.

4.4.2. Preparation of the calibration curve

Put 10.0 mL of 2,3-butanediol standard solution (3.16) in a 100 mL volumetric flask and make up with distilled water. From this solution prepare standard solutions by pipetting respectively into 100 mL volumetric flasks:

2.0, 4.0, 6.0, 8.0 and 10.0 mL, make up with distilled water

These solutions correspond, according to the conditions described in 4.2 to the following concentrations: 0.25, 0.50, 0.74, 1.00 and 1.25 g/L of 2,3-butanediol.

Proceed with the determination as described in 4.4.1, replacing the eluate by the same volume of each of the standard solutions. The straight line of the calibration graph should pass through the origin.

5. Calculation and expression of results

1. *Glycerol*

    1. Method of calculation

Read the glycerol content from the calibration curve. The result is expressed in g/L to one decimal place.

5.1.2. Repeatability

5.1.3. Reproducibility

5.2. 2,3-Butanediol

5.2.1. Method of calculation

Read the 2,3-butanediol content on the calibration. The result is expressed in g/L to two decimal places.

5.2.2. Repeatability

5.2.3. Reproducibility

6. Glycerol and 2,3-butanediol by periodimetric titration

1. *Reagents*

    1. Sodium hydroxide solution, 1 M.
    2. Sulfuric acid solution, 0.5 M.
    3. Periodic acid solution, 0.025 M.
    4. Sodium bicarbonate solution, $NaHCO_3$, 8% (*m/v*).
    5. Sodium arsenate solution, 0.025 M.

In a 1000 mL volumetric flask, dissolve 2.473 g of arsenic III oxide, As_2O_3 , with 30 mL 1 M sodium hydroxide, (6.1.1) add 35 mL 0.5 M sulfuric acid (6.1.2), and make up to the mark with distilled water.

6.1.6. Iodine solution, 0.025 M.

6.1.7. Potassium iodide, 10% (*m/v*).

6.1.8. Starch paste, 2% (*m/v*).

6.2. *Procedure*

In a 300 mL conical flask add:

- 5 mL glycerol sample solution (3.15)
- 45 mL distilled water

or

- 25 mL 2,3-butanediol sample solution (3.16)
- 25 mL distilled water

Add:

- 20 mL periodic acid, 0.025 M (6.1.3), leave for 15 min, shaking from time to time
- 10-20 mL sodium bicarbonate solution (6.1.4)
- 20 mL sodium arsenate solution (6.1.5)

Leave for 15 min shaking from time to time and add:

- 5 mL potassium iodide solution (6.1.7)
- 2 mL starch paste (6.1.8)

Titrate the excess sodium arsenate with 0.025 M iodine solution (6.1.6).

Prepare at the same time a blank test using 50 mL distilled water and the same quantity of reagents.

6.3. *Method of calculation*

6.3.1. Glycerol

1 mL periodic acid, 0.025 M, oxidizes 1.151 mg glycerol.

The glycerol content in g/L of the glycerol standard solution (3.15) is:

$$G = \frac{(X - B) \times 1{,}151}{\alpha}$$

The percentage of glycerol used in the standard glycerol solution (3.15) is:

$$\frac{G}{2{,}5} \times 100$$

- X = mL of the iodine solution, 0.025 M, used up by the standard solution (3.15)
- B = mL of the iodine solution, 0.025 M, in the blank test

- a = mL of the solution test (3.15) (equal to 5 mL)

2. 2,3-Butanediol

1 mL periodic acid, 0.025 M, oxidizes 2.253 mg of 2,3-butanediol.
The 2,3-butanediol content in g/L of the 2,3-butanediol standard solution (3.16) is:

$$BD = \frac{(X' - B') \times 2{,}253}{b}$$

The percentage of 2,3-butanediol used in the 2,3-butanediol standard solution (3.2) is:

$$\frac{BD}{2{,}5} \times 100$$

- X' = mL of iodine solution, 0.025 M, used up by the standard solution (3.16)
- B' = mL of iodine solution, 0.025 M, used in blank test
- b = mL of the solution test (3.16) (equal to 25 mL)

Bibliography

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