AK0008_IFU_EN_V2302

Acetic acid, UV method

Catalogue number	Presentation
AK00081	53 tests

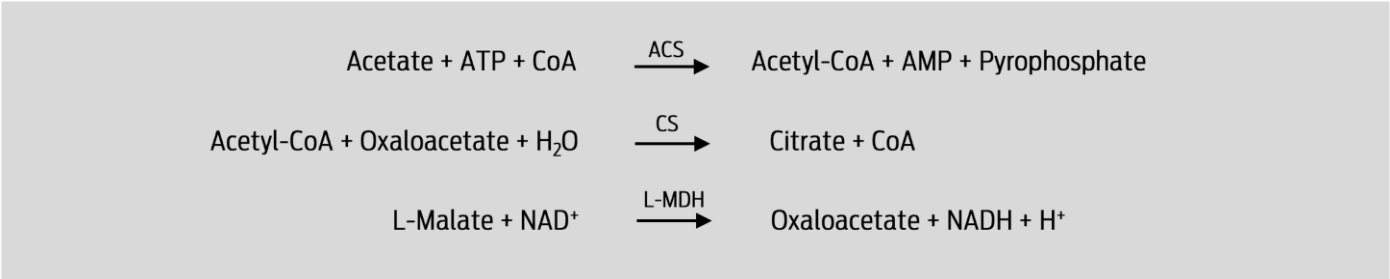
Application

This rapid and simple specific enzymatic method is used for the determination of acetic acid (acetate) in foodstuffs such as wine, vinegar, beer, dairy products, bread, fruit, fish, meat and vegetable, as well as in paper, animal feed (silages), pharmaceuticals and biological samples.

Introduction

Acetic acid is a very important metabolite since it is the end product of fermentation processes and the oxidation product of acetaldehyde and ethanol. Acetic acid is the main component of the "volatile acids" in wine and one of the most important parameters of its quality control. A high concentration of acetic acid in wine results in spoilage of the product. Thus, there may be legal limitations in the acetic acid content. Acetic acid is used in food production as a preservative and a taste improver. Acetic acid is the compound determining the monetary value of vinegar.

Principle



The amount of NADH formed through the combined action of acetyl-CoA synthetase (ACS), citrate synthase (CS) and L-malate dehydrogenase (L-MDH) is measured at 340 nm. Since the previous reaction is an equilibrium reaction, the amount of acetate existing in the sample must be calculated using the equation on page 2.

Specificity

The determination is specific for acetic acid.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 AU and a sample volume of 2.00 mL. This corresponds to an acetic acid concentration of 0.07 mg/L sample solution when measured at 340 nm. The detection limit of 0.14 mg/L results from the absorbance difference of 0.010 (340 nm) and a maximum sample volume of 2.00 mL.

Linearity and precision

Linearity of the determination exists from 0.3 to 20 µg acetic acid per assay (v = 2.00 mL). In a double assay using one sample solution, a difference of 0.005 to 0.010 AU may occur (0.07-0.14 mg/L of acetic acid, v = 2.00 mL). The CV is approx. 1 to 3% in the measuring range.

Kit composition

Solution 1. TEA buffer (30 mL, 0.8 M, pH 8.4), L-malic acid (60 mM), $MgCl_2$ (20 mM) and sodium azide (0.02% w/v) as a preservative. Store at 2 °C to 8 °C.

Solution 2 (x2). NAD^+ (67 mg), ATP (137 mg), PVP (30 mg) and CoA (9.8 mg). Store at 2 °C to 8 °C (Long term storage: -30 °C to -15 °C).

Dissolve in 5.5 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -30 °C to -15 °C between use (stable for 2 years) and keep cool during use. The content of the second bottle should be dissolved only when required.

Suspension 3. L-Malate dehydrogenase (L-MDH) and citrate synthase (CS) in 3.2 M ammonium sulphate (1.1 mL). Store at 2 °C to 8 °C.

Suspension 4. Acetyl-CoA synthetase (ACS) in 3.2 M ammonium sulphate (1.1 mL). Store at 2 °C to 8 °C.

Solution 5. Acetic acid standard solution (5 mL, 0.10 mg/mL). Stable for 2 years at 2 °C to 8 °C. This standard can be used when there is doubt about the method accuracy ($\varepsilon_{NADH,340\ nm}$ = 6300 L×mol^{-1}×cm^{-1}).

Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 °C

Final volume: 2.84 mL

Sample solution: 0.3-20 µg of acetic acid per cuvette (in 0.10 2.0 mL sample volume)

Read against air (without a cuvette in the light path) or against water

PIPETTE INTO CUVETTES (mL)	**BLANK**	**SAMPLE**
Distilled water (at ~25 °C)	2.10 mL	2.00 mL
Sample (can vary 0.10-2.00 mL)	-	0.10 mL
Solution 1 (TEA buffer)	0.50 mL	0.50 mL
Solution 2 (NAD^++ATP+CoA+PVP)	0.20 mL	0.20 mL
Mix, measure the absorbance of the solutions (A0) after ~3 min and start the reaction by addition of		
Suspension 3 (L-MDH/CS)	0.02 mL	0.02 mL
Mix, measure the absorbance of the solutions (A1) after ~4 min and start the reaction by addition of		
Suspension 4 (ACS)	0.02 mL	0.02 mL
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 12 min). If the reaction has not stopped after 12 min, continue measuring absorbance until 20 min (4 min intervals). Use the *NZYKit Calculator* helping file to consider the "creep" rate.		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

Calculation

For a simplified calculation, use the *NZYKit Calculator* helping file, which download could be done from the additional information of the product on NZYtech website (www.nzytech.com). If "creep" reactions occur (slowly increase of absorbance (A2) of sample after the initial rapid reaction), take this linear rate into account to obtain a corrected A2 value. Read absorbance at 16 and 20 minutes and use *NZYKit Calculator* helping file.

If you do not use the *NZYKit Calculator*, determine the absorbance differences for both blank and sample (A1-A0 and A2-A0). Since there is not a linear relationship between the measured absorbance difference and the acetic acid concentration, the value of $\Delta A_{Acetic\ acid}$ must be calculated using the following equation:

$$\Delta A_{Acetic\ acid} = [(A2\text{-}A0)_{sample} - \frac{(A1\text{-}A0)^2_{sample}}{(A2\text{-}A0)_{sample}}] - [(A2\text{-}A0)_{blank} - \frac{(A1\text{-}A0)^2_{blank}}{(A2\text{-}A0)_{blank}}]$$

The concentrations of acetic acid (g/L), based on the ε of NADH at 340 nm (6300 L×mol^{-1}×cm^{-1}), are calculated as follows:

$$C\ (Acetic\ acid) = 0.2707 \times \Delta A_{Acetic\ acid} \quad [g/L]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the

standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the “Alternative Procedures”, available on the NZYtech website.

Interferences

Acetic acid esters hydrolyse slowly under these reaction conditions. The acetic acid formed is responsible for sample-dependent “creep” reactions. This effect may be eliminated by extrapolating the A2 value to the time of ACS addition. As referred previously, this correction might be easily achieved by taking additional absorbance measurements at 16 and 20 minutes and using the NZYKit Calculator file. The corrected A2 will originate the acetic acid concentration.

However, the complete conversion of acetic acid within the time specified in the assay (approx. 10-12 min) indicates, in general, that no interference has occurred.

Total acetate concentration, in free and in esterified form, can be determinate by allowing the reaction to reach the end-point (until absorbance value becomes stable).

General information on sample preparation

The amount of acetic acid present in the cuvette should range between 0.3 and 20 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield an acetic acid concentration between 3 and 200 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 0.15 to 200 mg/L).

To implement this assay use clear, colourless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8 - 9 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 8 - 9 and incubate for approx. 15 min; measure "coloured" samples (if necessary, adjust to pH 8 - 9) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Examples of sample preparation

Determination of acetic acid in white and red wines

For white wine, use 0.10 mL in the assay. For samples containing low acid content, volumes up to 2.0 mL can be used.

For red wine containing approx. 0.2 g of acetic acid/L, use 0.10 mL of sample without decolorizing in the assay. For red wine containing less than 0.1 g of acetic acid/L, samples should be decolorized by adding 0.2 g of PVPP per 10 mL of sample and stirring for 5 min. Filter an aliquot through Whatman No. 1 filter paper, and adjust the pH to approx. 8.4. Adjust the volume to twice that of the original volume of sample used. A sample volume up to 2.0 mL may be used and dilution and sample volume must be taken into account in calculations.

When large sample volumes are used, the high alcohol concentration of wine samples may delay enzymes activities used in the quantification of acetate. In such cases, increase incubation times to 20 min, and take subsequent measurements of absorbance values to confirm the reaction has finished. Normally, a dilution of 1:5 and a sample volume of 0.1 mL are satisfactory.

Determination of acetic acid in fruit juices

For fruit juices containing a high level of acetic acid (approx. 0.3 g/L), dilute an aliquot of the sample with an equal volume of water and use 0.1 mL for assay. If a large volume of sample is required, adjust the pH of the solution to approx. 8.4 before analysis. Coloured juices should be decolourised as described above. Use 0.10 to 2.00 mL of sample for the assay. Typically, no dilution is required and a sample volume of 0.1 mL is satisfactory.

Determination of acetic acid in beer

Degas the beer sample by filtration or by stirring for 5 min with a glass rod. Typically, no dilution is required and a sample volume of 0.2 mL is satisfactory.

Determination of acetic acid in vinegar

Dilute the sample according to fit the range defined in the in general sample preparation. Usually, a dilution of 1:500 and sample volume of 0.1 mL is adequate.

References

Beutler, H.O. (1984). Acetic acid. In Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., vol. VI, pp. 639-645, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel.

AOAC Official Methods of Analysis (2002). 17th ed., Chapter 32, pp. 47-48.

Recommendations

This method is recommended/approved by the:

- European, German and International standards (EN, DIN, ISO);

- Recommended by the International Federation of Fruit Juice Producers (IFU) and by the Central European Commission for Brewing Technology.

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications