UV method for approx. 32 assays

For laboratory use only
Store between +2 and +8°C

The method is contained in the German and Dutch laws. Recommended e.g. by IFU, AIJN and MEBAK. Standardized by DIN and EN.

Principle

Acetate + ATP + CoA ——— ACS ⟶ Acetyl-CoA + AMP + PPi

Acetyl-CoA + oxaloacetate + H_2O —— CS ⟶ citrate + CoA

L-Malate + NAD^+ ⟵ L-MDH ⟶ oxaloacetate + NADH + H^+

Ref.: Beutler, H.-O. (1964) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 3rd ed., vol. VI, pp. 639-645, Verlag Chemie, Weinheim, Deerfield Berach/Florida, Basel

Assay performance

Wavelength:	340 nm (NADH) ε = 6.3 l x $mmol^{-1}$ x cm^{-1}
Light path:	1.00 cm (glass or plastic cuvettes)
Temperature:	+20 to +25°C
Assay volumes:	3.230 ml
Measurement:	against air or against water
Sample solution:	0.3 to 30 µg acetic acid in 0.100 to 2.000 ml sample solution

Reagents

1: Approx. 32 ml *triethanolamine* buffer, pH approx. 8.4; approx. 134 mg L-malic acid; approx. 67 mg magnesium sulfate hexa-hydrate (for stability see pack label). *The solution is ready for use.*

2: *Lyophilizate* with approx. 175 mg ATP, approx. 18 mg CoA and approx. 86 mg NAD (for stability see pack label). *Dissolve contents of bottle # 2 with 7 ml redist. water.* The solution is stable for 1 month at +2 to +8°C, resp. for 2 months at -15 to -25°C.

3: Approx. 0.4 ml L-malate dehydrogenase / citrate synthase (L-MDH / CS) suspension (approx. 1100 U / 270 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use*. Swirl bottle carefully before the suspension is pipetted.

4: Approx. 0,7 ml Acetyl-CoA-synthetase (ACS) suspension (approx. 16 U) in ammonium sulphate (for stability see pack label). *The suspension is ready for use*. Swirl bottle carefully before the suspension is pipetted.

In addition (not contained in the kit): standard solution acetic acid, 0.15 g/l for test control only.

The reagents for the determination of acetic acid are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Procedure

Pipette into cuvettes:	**Blank**	**Standard[1]**	**Sample[2]**	**Rerun assay[3]**	**Assay with internal standard[4]**	**High sensitive assay[5]**
Tea buffer solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
ATP, CoA, NAD solution # 2	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml	0.200 ml
Sample solution[6] (e.g. 0.015 to 0.15 g acetic acid/l)	**-**	**-**	**0.100 ml**	**0.200 ml**	**0.100 ml**	**2.000 ml**
Standard solution[6] (e.g. 0.15 g acetic acid/l)	-	0.100 ml	-	-	0.100 ml	-
Redist. Water	2.000 ml	1.900 ml	1.900 ml	1.800 ml	1.800 ml	-
Mix[7], read the absorbances (A_0). Add:						
L-MDH/CS suspension # 3	0.010 ml	0.010 ml	0.010 ml	0.010 ml	0.010 ml	0.010 ml
Mix[7], after approx. 3 min read the absorbances (A_1). Add:						
ACS solution # 4	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix[7], after approx. 10 to 15 min read the absorbances (A_2). Repeat absorbance reading after another 2 min[8].						

Notes:

1 Run a "standard" to see "accidents" in analysis. The measurement of the standard is not necessary for calculating results.
2 This assay together with the blank is a single determination.
3 In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes. Calculate with the resp. v.
4 Recovery = $[(\Delta A_{sample+standard} - \Delta A_{sample}) / \Delta A_{standard}] \times 100$ [%]
5 Assay recommended in the case of trace level compound analysis, with sample volume increased up to 2.000 ml (0.00015 to 0.015 g acetic acid/l).
6 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
7 e.g. with a plastic spatula, or after closing the cuvette with Parafilm (trademark of American Can Co., Greenwich Ct., USA).
8 The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until they increase constantly over e.g. 2 min. Extrapolate absorbances to the time of the addition of ACS (solution # 4).

Calculation

Determine the absorbance differences (A_1-A_0) and (A_2-A_0) for blank and sample. The ΔA calculation is different from the calculations in other enzymatic tests, because with the equilibrium of the preceding indicator reaction, there is no linear (direct) proportionality between the measured absorbance difference and the acetic acid concentration. The following formula, which should generally be used for preceding indicator reactions, serves to calculate the $\Delta A_{acetic\ acid}$:

$$\Delta A_{acetic\ \ acid} = \left[(A_2 - A_0)_{sample} - \frac{(A_1 - A_0)^2_{sample}}{(A_2 - A_0)_{sample}} \right] - \left[(A_2 - A_0)_{blank} - \frac{(A_1 - A_0)^2_{blank}}{(A_2 - A_0)_{blank}} \right]$$

The measured absorbance difference, as a general rule in all enzymatic tests, should be at least between 0.100 and 1.000 absorbance units to achieve sufficient precise results.

Concentration is calculated according to the general Lambert-Beer formula:
c = (V x MW x Δ A) / (ε x d x v x 1000) [g acetic acid/l sample solution]
c = (3.230 x 60.05 x Δ A) / (6.3 x 1.00 x 0.100 x 1000) = **0.3079 x Δ A [g acetic acid/l sample solution]**

If the sample has been diluted during preparation, multiply the result with dilution factor F.

When analyzing samples which are weighed out for sample preparation, calculate the content from the amount weighed:

$$Content_{acetic\ acid} = \frac{c_{acetic\ acid}\ [g/l\ sample\ solution]}{weight_{sample}\ [in\ g/l\ sample\ solution]} \times 100\ [g/100\ g]$$

Sample preparation

1. Dilute *clear, colourless and almost neutral* liquid *samples* to get a sample solution with 0.015 to 0.15 g acetic acid/l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide*, e.g. by filtration, or add $NaHCO_3$ till the solution is slightly alkaline, dilute (see pt. 1).
4. Adjust *acid* (esp. *slightly coloured*) solutions with KOH or NaOH to approx. pH 8 to 9, incubate a few minutes, or dilute (see pt. 1) without pH adjustment in the case of colourless samples.
5. Treat *strongly coloured solutions* used undiluted with activated charcoal, PVPP or polyamide, e.g. 1 g/100 ml, mix, incubate a few minutes, filter.
6. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples*, extract with water, or dissolve in water, filter and dilute (see pt. 1) if necessary.
7. Extract *fat containing samples* with hot water at a temperature above the melting point of fat, e.g. in a 100 ml volumetric flask. Adjust to +20°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter. Alternatively, clarify with Carrez reagents (which can be recommended).
8. Clarify *samples containing protein* with Carrez reagents:
   Weigh sufficient quantity of solid or pasty sample into 100 ml volumetric flask, add approx. 60 ml water. Or pipette liquid sample into 100 ml volumetric flask containing approx. 60 ml water. Add, and mix after each addition, 5 ml Carrez-I-solution (3.60 g $K_4[Fe(CN)_6]$ x $3H_2O$ = potassium hexacyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g $ZnSO_4$ x 7 H_2O = zinc sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g. 10 ml NaOH (0.1 M). Fill the flask to the mark, mix and filter.
9. Deproteinize *samples containing protein* with perchloric acid.

Assay characteristics

1. *Specificity:* Specific for acetic acid. In the analysis of commercial glacial acetic acid results of 100 % have to be expected, in the analysis of water-free sodium acetate results of < 100 % because the material absorbs moisture.
2. *Sensitivity:* 0.1 mg acetic acid/l (Δ A = 0.005; v = 2.000 ml; V = 3.230 ml)
3. *Detection limit:* 0.15 mg acetic acid/l (Δ A = 0.010; v = 2.000 ml; V = 3.230 ml)
4. *Linearity:* 0.3 µg acetic acid (v = 2.000 ml; V = 3.230 ml)
   to 30 µg acetic acid/assay (v = 0.100 ml; V = 3.230 ml)
5. Precision: Δ A = +/- 0.005 to 0.010 absorbance units
   CV = approx. 1 to 3 %

Pork sausage: x = 0.3 g/100 g	r = 0.017 g/100 g	s(r) = +/- 0.006 g/100 g
	R = 0.023 g/100 g	s(R) = +/- 0.008 g/100 g
Tomato ketchup:	r = 0.05 g/100 g	s(r) = +/- 0.02 g/100 g
	R = 0.07 g/100 g	s(R) = +/- 0.02 g/100 g
Bread: x = 131.89 mg/100 g	r = 7.53 mg/100 g	s(r) = +/- 2.66 mg/100 g
	R = 21.12 mg/100 g	s(R) = +/- 7.46 mg/100 g
Bread: x = 204.55 mg/100 g	r = 7.41 mg/100 g	s(r) = +/- 2.62 mg/100 g
	R = 19.35 mg/100 g	s(R) = +/- 6.84 mg/100 g

6. *Interferences:* Esters of acetic acid (e.g. in wine) are slowly saponified under assay conditions which is responsible for a slow creep reaction that has to be considered by extrapolation of the absorbance back to the time of the addition of the sample.
7. *Technical Information:* Acetic acid is volatile. This has to be taken into consideration e.g. when preparing sample solutions.