UV method for approx. 3 x 8 assays

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

The method is contained in the German and Swiss food laws and in European regulation. Recommended e. g. by IDF, IFU, AIJN, MEBAK, OIV, VDLUFA. Standardized by DIN, EN, GOST, ISO, NEN, NF. Approved by AOAC.

Principle

(1) Citrate ——— CL ⟶ oxaloacetate + acetate

(2) Oxaloacetate + NADH + H^+ ——— L-MDH ⟶ L-malate + NAD^+
(Oxaloacetate ——— Ox-DC/metal ions ⟶ pyruvate + CO_2)

(3) Pyruvate + NADH + H^+ ——— L-LDH ⟶ L-lactate+ NAD^+

Ref.: Möllering, H. & Gruber, W. (1966) Determination of citrate with citrate lyase, Anal.Biochem. 17, 369-376

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 volume: 3.020 ml
Measurement: against air or against water
Sample solution: 1 to 80 µg citric acid in 0.200 to 2.000 ml sample solution.

Reagents

1: 3 bottles with lyophilizate with glycylglycine buffer, pH approx. 7.8, approx. 110 U L-MDH, approx. 220 U L-LDH, approx. 4 mg NADH (for stability see pack label). *Dissolve contents of one bottle # 1 with 9 ml redist. water*. The solution is stable for 2 weeks at +2 to +8°C, resp. for 4 weeks at -15 to -25°C. Bring to +20 to +25°C before use.

2: 3 bottles with lyophilizate with approx. 10 U citrate lyase (for stability see pack label*). Dissolve the content of one bottle # 2 with 0.2 ml redist. water, **and let the bottle stay for 30 min at room temperature** (swirl carefully).* The solution is stable for 5 days at +2°C to +8°C, respectively for 4 weeks at -15°C to -25°C.

In addition (not contained in the kit):

Standard solution citric acid, monohydrate, ultrapure, 0.4 g/l for test control only.

The reagents for the determination of citric 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 assay[1]**	**Sample assay[2]**	**Rerun assay[3]**	**Assay with internal standard[4]**	**High sensitive assay[5]**
Glycylglycine buffer, L-MDH, L-LDH, NADH solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
Sample solution[6] (e.g. 0.04 to 0.4 g citric acid/l)	-	-	**0.200 ml**	**0.100 ml**	**0.100 ml**	**2.000 ml**
Standard solution[6] (e.g. 0.4 g citric acid/l)	-	0.200 ml	-	-	0.100 ml	-
Redist. water	2.000 ml	1.800 ml	1.800 ml	1.900 ml	1.800 ml	-
Mix[7], after approx. 5 min read the absorbances A_1. Add:						
CL solution # 2	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix[7], after completion of the reaction (approx. 5 to 10 min) read the absorbances A_2.						

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. volume v.
4 Recovery = [(2 x $\Delta A_{sample+standard}$ - ΔA_{sample}) / $\Delta A_{standard}$] x 100 [%].
5 Assay recommended in the case of trace level compound analysis, with sample volume increased up to 2.000 ml (0.0005 to 0.04 g citric 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)

Calculation[8]

$\Delta A = (A_1 - A_2)_{\text{sample resp. standard}} - (A_1 - A_2)_{\text{blank}}$

In order to obtain safe and reproducible results, the absorbance difference Δ A should be between 0.100 and 1.000.

c = (V x MW x ΔA) / (ε x d x v x 1000) [g citric acid/l sample solution]

c = (3.020 x 192.1(resp. 210.1) x ΔA) / (6.3 x 1.00 x 0.200 x 1000) = **0.460 (resp. 0.504) x ΔA**
[g citric acid (monohydrate)/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:

$$\text{Content}_{\text{citric acid}} = \frac{C_{\text{citric acid}}\ [\text{g/l sample solution}]}{\text{weight}_{\text{sample}}\ [\text{in g/l sample solution}]} \times 100\ [\text{g/100 g}]$$

Sample preparation:

1. Dilute *clear, colorless and* almost *neutral liquid samples* to get a sample solution with 0.04 to 0.4 g citric 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 colored) solutions* with KOH or NaOH to approx. pH 8, incubate a few minutes, or dilute (see pt. 1) without pH adjustment in the case of colorless samples.
5. Treat *"strongly colored solutions"* used undiluted with 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.
8. Deproteinize samples with perchloric acid (choose one of the three methods below):
   - Homogenize a [g] sample (water content w in g/100 g) with b [ml] perchloric acid (1 M), filter. Neutralize [c] ml filtrate with [d] ml KOH (5M). Store at +4 °C for 15 min, filter.
   - Homogenize a [g] sample with b [ml] perchloric acid (1 M). Neutralize with KOH (5 M). Transfer into a 100 ml volumetric flask, fill up to mark with redist. water. (Fatty layer is above the mark.) Store at +4 °C for 15 min, filter.
   - Homogenize a [g] sample (water content w in g/100 g) with b [ml] perchloric acid (1 M). Neutralize with d [ml] KOH (5 M). Store at +4 °C for 15 min, filter.
9. Note: The Carrez clarification cannot be used in sample preparation because of too low results (adsorption of citric acid).

Assay characteristics

1. *Specificity*: *Specific* for citric acid. In the analysis of commercial citric acid monohydrate results of > 100 % have to be expected if the crystal water is lost during storage and the results are calculated with the molecular weight of citric acid monohydrate.

2. *Sensitivity:* 0.25 mg/l (ΔA = 0.005; v = 2.000 ml; V = 3.020 ml)

3. *Detection limit:* 0.5 mg/l (ΔA = 0.010; v = 2.000 ml; V = 3.020 ml)

4. *Linearity:* 1 µg/assay (v = 2.000 ml; V = 3.020 ml)
   to 80 µg/assay (v = 0.200 ml; V = 3.020 ml)

5. *Precision:* ΔA = +/- 0.005 to 0.010 absorbance units
   CV = approx. 1 to 3 %
   Fruit juice: $r = 0.095 + 0.025 \times c_{\text{citric acid in g/l}}$ [g/l]
   $R = 0.130 + 0.054 \times c_{\text{citric acid in g/l}}$ [g/l]

Wine:	r	R
< 400 mg/l:	14 mg/l	39 mg/l
> 400 mg/l:	28 mg/l	65 mg/l

6. *Interferences:* None known in the analysis of foodstuffs. (If necessary, remove phenolic compounds during sample preparation).

7. *Technical Information:* The results can be given as citric acid (recommended) or as citric acid monohydrate.

Notes

8 Results from acidimetric determination (protons are measured) calculated as citric acid cannot be compared with enzymatic analysis (citrate is measured)