Colorimetric assay for wine, food and beverages
1 x 20 ml (10 assays)

Only for laboratory use
Store between +2 and +8°C

Principle

Oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. Hydrogen peroxide reacts in presence of peroxidase (POD) with MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3-dimethylamino benzoic acid) forming a blue quinone compound. The intensity of color is proportional to the concentration of oxalate in the sample and it is read at 590 nm:

$$\text{Oxalate} + O_2 \xrightarrow{\text{Oxalate oxidase}} 2\,CO_2 + H_2O_2$$

$$H_2O_2 + \text{MTBH} + \text{DMAB} \xrightarrow{\text{POD}} \text{Blue quinone} + H_2O$$

Assay specifications

Wavelength:	590 nm (570-620 nm)
Light path:	1.00 cm (glass; plastic)
Temperature:	37°C
Method:	end-point
Reaction:	15 minutes
Measurement:	against air or against water
Linearity:	up to 90 mg/L
Sample/reagent:	1/20/2

Reagents

1: Reagent 1, buffer **1 x 20 ml** (buffer 20 mmol/L, pH 3.1 ± 0.1, MBTH 0.2 mmol/L, DMAB 0.9 mmol/L, activators, stabilizers). Reagent 1 is liquid and ready-to-use

2: Reagent 2, **one vial lyophilized** reagent (Oxalate oxidase from Barley 2 KU/L, POD 1000 U/L). Add 2 ml of distilled water, mix gently until dissolution. Stability: 30 days at 2-8°C.

3: Standard, **1 x 5 ml** (Oxalic acid 45 mg/L = 0.5 mmol/L). The standard is liquid and ready-to-use.

Reagents 1 and 3 are ready for use. They are stable at 2-8 °C up to the expiry date shown on the package, if not contaminated during handling.

Let the reagents reach the room temperature before use. Mix kindly before pipeting. Close immediately after handling. The reagents have to be used properly, to avoid contamination.

The reagents 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.

Sample preparation

- For samples containing **reducing or oxidating substances**, please use the Enzytec Sample purifier kit (ref. E2250)
- Use colorless and clear samples (< pH 7.0), undiluted if oxalate concentration is up to 90 mg/L; otherwise, dilute with water to reduce it in this range.
- Oxalic acid is stable at low pH (<3.3). If the pH is above 7.5, oxalate will be transformed into CO_2 and will be lost. But increasing the pH allows increasing solubility of Ca-Oxalate. So there are two possibilities:
  - For direct testing, adjust sample pH to 2.9 – 3.1 by using HCl or NaOH/KOH.
  - If Ca-oxalate is present and should be solubilized, adjust the pH to 5.0 – 7.0.
- In some cases, oxalic acid and its salts have to be released: adjust the sample to pH = 3.0 with HCl (1 M), then boil the sample at 100°C for 15-30 minutes.
- Turbid solutions have to be filtered or centrifuged.
- Samples containing carbon dioxide have to be degassed.
- Spinach and rhubarb juices have to be diluted 1:10 with distilled water. Adjust to pH 2.9 – 3.1 as above.
- Strongly colored samples have to be treated with PVPP (polyvinylpolypyrrolidone e.g. 1 g/100 mL sample).
- Deproteinize samples containing proteins with trichloro-acetic acid (the Carrez clarification and the perchloric acid method are not applicable to the oxalic acid test)
- For fat-containing samples, de-fatting is performed on ice

Procedure

Pipette into cuvettes	Reagent blank (RB)	Standard	Samples
Reagent 1 (buffer)	2000 µl	2000 µl	2000 µl
Distilled water	100 µl	-	-
Standard (vial 3)	-	100 µl	-
Sample	-	-	100 µl
Mix carefully, incubate 5 minutes at 37°C. Read the absorbance A_1, then add:			
Reagent 2 (enzyme)	200 µl	200 µl	200 µl
Mix carefully and incubate at 37°C until end of the reaction (approx. 15 min). Read the absorbance A_2. The color is stable for 60 min.			

Calculation

$$\Delta A = (A_2 - df \times A_1)_{\text{sample or standard}} - (A_2 - df \times A_1)_{RB}$$

with df = dilution factor of the optical densities by reagent volumes:
df = (sample volume + R1) / (sample volume + R1 + R2) = 0.913

$$\text{and } C_{sample}\,[mg/L] = \frac{c_{standard}\,[mg/L]}{\Delta A_{standard}} \times \Delta A_{sample}$$

Since the concentration of the standard is fixed at 45 mg/L, this gives the following calculation formula:

$$C_{sample}\,[mg/L] = (\Delta A_{sample} / \Delta A_{standard}) \times 45$$

Notes

1. A proportional variation of the reaction volumes does not change the results.
2. We suggest do not mix reagents from different production lots.
3. For concentrations higher than 90 mg/L, dilute the sample with distilled water in the mentioned ranges; repeat the determination and multiply the result by the dilution factor.
4. Sensitivity: in the manual procedure, the lowest detection limit is around 1.5 mg/l (Δ A = 0.020 and v = 100 µl)
5. Specificity: this test is specific for Oxalic Acid.
6. Ascorbic acid and other reducing substances may interfere at high concentrations

Literature

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