OIV-MA-AS315-24 Determination of lysozyme in wine using high-performance capillary electrophoresis

Type IV method

1. Introduction

This method is used to detect the addition of lysozyme in wine but is not suitable for the assay or determination of lysozyme as an allergenic compound.

Determination of residual lysozyme in treated wines is performed using high-performance capillary electrophoresis (HPCE).

2. Scope

This method applies to lysozyme determination in white wines at concentrations ranging from 9 mg/L to 100 mg / L, and by dilution above this level.

3. Principle

The wine samples are directly injected into the capillary-electrophoresis instrument after filtration and dilution, as needed. The quantification of lysozyme is performed against an external standard.

4. Reagents

Lysozyme extract of chicken egg white [CAS No. 12650-88-3]

85% Phosphoric acid [CAS No. 7664-38-2]

Hydroxypropyl methylcellulose (HPMC) [CAS No 9004-65-3]

Purified water for laboratory use, for example to EN ISO 3696 grade (water for analytical laboratory use - specification and test methods [ISO 3696:1987]).

5. Apparatus

Standard laboratory apparatus

Capillary electrophoresis instrument with a UV spectrophotometric detector

6. Sample preparation

The wine to be analysed is diluted four-fold in distilled water for analysis by capillary electrophoresis, in order to fit within the linear dynamic range of the method (lysozyme content lower than 100 mg/l).

7. Analytical conditions

Capillary: fused silica (37 cm length, 75 µm diameter)

Buffer: phosphoric acid (75 mm) HPMC (0.1 %), pH 1.68
Injection time: 15 sec
Injection mode: hydrostatic procedure (3447.38 Pa)
Temperature: 25°C
Applied voltage: 7 kV
Detection: UV 214 nm

8. Calculation

A calibration curve is produced based on lysozyme solutions in water at 10, 20, 50, and 100 mg/l. Depending on the external calibration method, lysozyme quantification is performed by measuring the lysozyme peak area in the wine and comparing it with the corresponding concentration on the calibration curve.

9. Method characteristics

9.1. Linearity of response

As the maximum authorized dose of lysozyme which may be added to wines is 500 mg/l, a standard range containing 5 to 500 mg/l of lysozyme in aqueous solution was prepared. Each solution was analysed five times.

Above 100 mg/l, the response is no longer linear. The linear dynamic range of the method is from 5 to 100 mg/l, as shown on the calibration curve in Figure 1.

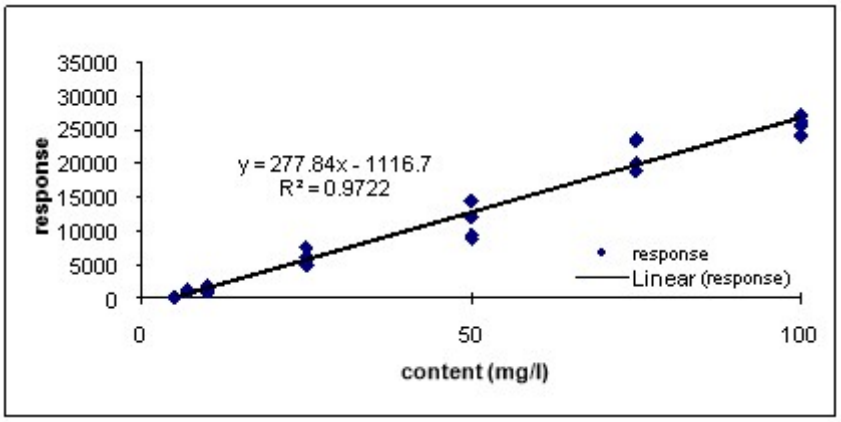


Figure 1: Linearity of lysozyme determination using HPCE

9.2. Repeatability

The repeatability of lysozyme determination in white wines has been determined from the results obtained across 20 wines with added lysozyme, analysed twice in

succession, in order to be tested under identical conditions. The results are given in table 1:

Table 1: Repeatability of lysozyme determination using HPCE

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	Calculated values
Repeatability	
standard deviation in mg/L	2.63
CV %	1.4%
r limit in mg/L	7.35
r limit %	4%

9.3. Reproducibility

The reproducibility of lysozyme determination in white wines has been determined by analysing the same white wine, with 200 mg/L of lysozyme added, 8 times on different dates. The results are given in Table 2.

Table 2 - Reproducibility of lysozyme determination using HPCE

Reproducibility	Calculated value
standard deviation in mg/L	11.75
CV %	5.8%
R limit in mg/L	32.90
R limit %	16%

9.4. Limits of detection and quantification

Limits of detection (LoD) and quantification (LoQ) are determined based on the background noise measured near the lysozyme peak corresponding to the first calibration point, i.e. 5 mg/L. The results obtained are as follows:

- LoD = 3 X background noise (mg/L) = 3 mg/L
- LoQ = 10 X background noise (mg/L) = 9 mg/L

9.5. Uncertainty

Uncertainty was determined using the intralaboratory reproducibility standard deviation, this is 12 %.

Bibliography

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