DEVELOPMENT AND VALIDATION OF A REAGENT BASED ON ENZYMATIC REACTIONS FOR DETERMINING HISTAMINE IN WINES

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Abstract
The presence of histamine in wine is becoming increasingly important to consumers and producers alike, due to the potential threats of toxicity to humans and consequently health implications. In the scientific field, histamine has the potential to be applied as indicator of unsanitary conditions during wine production procedures.

Current methods for analysis of histamine are HPLC, ELISA, and fluorimetry which sometimes require expensive and sophisticated instrumentation and as consequence skilled technicians. BioSystems presents a new, simple and rapid enzymatic method for determination of histamine in red and white wine.

The method is based on the specific reaction of histamine with recombinant histamine dehydrogenase (from E. Coli) causing the reduction of a soluble tetrazolium salt to form a formazan salt that absorbs at 420 nm. Thus can be measured by visible spectrophotometry and correlate it, through a calibration, with histamine concentration. A simple method to remove interfering substances (reducing agents like polyphenols and anthocyanins) from wine sample is also presented.

This new reagent has a long stability, is liquid and ready-to-use, avoiding end-user influence. It has been formulated to be used in any photometer or automated analyzer.

Linearity, limit of detection (LoD), limit of quantification (LoQ), repeatability, within-laboratory reproducibility, trueness and recovery were characterized using a BioSystems Y15® automated analyzer and HPLC method OIV-MA-AS315-18 as the reference.

Measurement procedure
Histamine in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry:

\[ \text{Histamine} + \text{Reagent} \rightarrow \text{Formazan} \]

Sample preparation: Dilute wine ½ with distilled water.
Add approximately 10 mg of PVPP and shake.
Centrifuge sample to obtain a firm sediment and use the supernatant.

Samples are assayed in a Y15® which automatically are dispensed with the reagents in the reaction rotor. Absorbance at 420 nm is read and concentration is calculated through a 5 points calibration.

Reagents and calibrators are ready to use and stable until expiry date.

Conclusions
Results confirms that FoodQuality® Histamine Enzymatic (BioSystems) can quantitatively measure histamine in wine samples with similar accuracy and precision than the UHPLC method.

Furthermore, this test can be automated in a analyzer (the kit is adapted for Y15® or Y25® analyzers from BioSystems) or can be used in a photometer with a simple sample treatment making histamine analysis more accurate, fast and easy.

The small volumes used also allows an affordable analysis and better waste management.

Bibliography

Product performance

Precision

Samples: control materials with low and high histamine concentration.

Results are expressed as coefficient of variation (%CV).

<table>
<thead>
<tr>
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<th>8 mg/L</th>
<th>13 mg/L</th>
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<tbody>
<tr>
<td><strong>Repeatability</strong></td>
<td>1,3 %</td>
<td>0,8 %</td>
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<tr>
<td><strong>Between-run</strong></td>
<td>1,7 %</td>
<td>1,7 %</td>
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<tr>
<td><strong>Within-laboratory</strong></td>
<td>2,2 %</td>
<td>1,9 %</td>
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Limit of detection (LoD)/quantification (LoQ)
Establish the LoD and/or LoQ of a measurement procedure; Approved guideline - Second edition. CLSI EP17-A2.

<table>
<thead>
<tr>
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<th>1 mg/L</th>
<th>3 mg/L</th>
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<tbody>
<tr>
<td><strong>Limit of Detection (LoD)</strong></td>
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<tr>
<td><strong>Limit of Quantification (LoQ)</strong></td>
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Measurement range

| Measurement range | 3 - 80 mg/L |

Trueness
Red and white commercial wines from Spain where spiked with histamine to reach different concentration levels. Samples were tested using Biosystems FoodQuality® Histamine Enzymatic according to the instructions with Y15® automated analyzer from BioSystems. The same samples were assayed with UHPLC according to the Latorre et al. protocol, an optimization of OIV-MA-AS315-183. All samples where assayed (extraction and test) in duplicate.

Data is analyzed using Passing-Bablok linear regression and Bland-Altman difference analysis with Analyse-it® software.

Recovery
The average recovery for all the wines is 97% (86 – 109%). There are no difference between the type or origin of wine (red or white).