Validation of a method based on inmunoturbidimetry for determining gluten in wines with BioSystems Y15 autoanalyser

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Abstract

Gluten is a protein found in wheat, barley, and rye, which can cause damage to the small intestine in individuals with celiac disease. It can also lead to symptoms like abdominal pain, bloating, and diarrhea in those with gluten intolerance. Both conditions can result in long-term health issues.

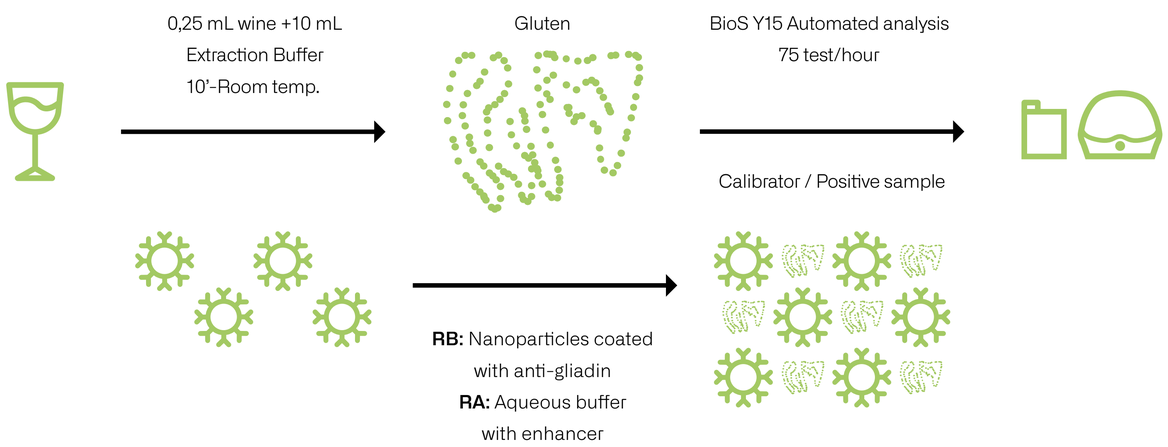
Most wines contain less than 20 ppm of gluten, meeting the EU and US definition of “gluten-free.” While there are rare instances of gluten contact during wine production, such as through the use of wheat flour paste or gluten as a clarifying agent, these practices are not commonly employed. However, certain countries like Brazil and Canada have specific regulations requiring gluten-free certification or testing for gluten presence in wine labeling. The EU Regulation 1169/2011 and the OIV state that from 2023, the presence of gluten exceeding the legal limit must be indicated on wine labels.

Different methods exist for gluten analysis, with rapid immuno-chromatographic tests and ELISA kits being the most popular. Rapid tests are user-friendly but lack quantification capabilities, while ELISA offers quantitative results but is prone to errors due to its less automated process.

This study introduces a novel method for quantifying gluten in wine, using a turbidimetric immunoassay on a BioSystems Y15 spectrophotometric analyser.

Measurement procedure

In this method, the antibody is immobilized on the surface of latex nanoparticles, causing them to aggregate when exposed to gluten. The extent of agglutination is directly proportional to the amount of gluten in the sample. To achieve this, a highly specific monoclonal antibody has been developed, specifically designed to recognize the 33-mer fragment of gluten. This fragment is well-known for its significant immunogenicity and toxicity in individuals with celiac disease. Additionally, an innovative and environmentally friendly extraction buffer, free from hazardous substances, has been developed for this method. Coupled with a fast and straightforward protocol, it enables a one-step extraction process completed in just 10 minutes.



Conclusions

The validation data for the BioSystems Y15 analyser shows that the method has a linear range of 0 to 40 mg/L, with a high correlation coefficient (r^2) of over 0.99. The limit of quantification (LOQ) is below 2.5 mg/L, and the relative standard precision values (RSD%) range from 3 to 10% under intermediate conditions. Recoveries for purified gliadin fall within the 90-110% range. Furthermore, comparisons with the reference method (AOAC-2012.01) demonstrate good correlation.

The Gluten Y15 BioSystems method has been validated specifically for wines and proves to be a valuable tool for automated, rapid, accurate, and straightforward gluten determination. Automating the measurement process in the BioSystems Y15 analyser enhances precision and eliminates potential user errors, allowing for the analysis of a wide range of sample sizes, from a few to a high throughput of 75 samples per hour.

Additionally, the BioSystems Y15 automation capability provides the opportunity to combine gluten analysis with other routine enological control parameters like sulfite levels, sugars, or organic acids. This advantage improves efficiency and speed in decision-making during the wine production process.

Product performance

The method validation has been conducted according to the criteria established by the AOAC (Appendix F: Guidelines for Standard Method Performance Requirements).
Finished red wine samples from various varieties and origins have been used for the study.
Recovery studies were performed by adding purified gliadin using the reference material from the Prolamin Working Group (PWG).

Measurement range - Linearity

	Measured value, mg/L					Results			
Calibrator value mg/L	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Mean value mg/L	Sr	RSDr (%)	Bias mg/L
0	0.0	0.1	0.1	0.1	0.0	0.1	0.03	-	0.1
4	3.9	4.0	3.9	3.9	4.0	3.9	0.07	1.8	-0.1
8	7.8	8.0	8.1	8.0	8.1	8.0	0.12	1.4	0.0
12	11.5	11.9	12.1	12.1	12.1	11.9	0.26	2.2	-0.1
16	15.2	16.0	16.0	16.0	16.0	15.8	0.36	2.3	-0.2
20	18.4	19.2	19.8	19.2	19.6	19.2	0.54	2.8	-0.8
24	24.0	23.2	24.0	24.5	24.1	24.0	0.48	2.0	0.0
32	32.1	31.9	31.8	32.1	32.1	32.0	0.13	0.4	0.0
40	37.6	37,6	40.8	39.0	42.1	39.4	1.99	5.1	-0.6

Limit of detection (LoD) / quantification (LoQ)

Lowest analyte that can be determined with acceptable precision and accuracy in a sample under the conditions of the method used.

Limit of Detection (LoD)	0.50 mg/L
Limit of Quantification (LoQ)	1.50 mg/L

Precision and recovery

		BioSystems Gluten Y15 results						
Matrix	ELISA r-Biopharm mg/L	Spiking PWG mg/L	Total gluten mg/L	Mean (n=5) mg/L	Sr	RSDr (%)	Recovery (%)	Bias mg/L
Red wine 1	<LoQ	0	0.1	-	-	-	-	-
Cabernet		5	5.1	5.0	0.26	5.24	99%	0.0
Sauvignon		10	9.6	9.5	0.32	3.35	95%	-0.5
		20	20.4	20.3	1.12	5.52	102%	0.3
Red wine 2	<LoQ	0	0.0	-				
		5	4.60	4.60	0.41	4.50	92%	-0.4
Merlot		10	9.84	9.84	0.76	4.30	98%	-0.2
		20	21.2	21.2	0.95	2.24	106%	1.2
Red wine 3	<LoQ	0	0.2	-	-			
		5	5.0	4.9	0.13	4.73	97%	-0.1
Pinot noir		10	10.9	10.7	0.29	4.87	107%	0.7
		20	21.5	21.3	0.71	6.03	107%	1.3

Bibliography

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Official Methods of Analysis (2016) 20th Edition, AOAC INTERNATIONAL, Rockville, MD, Appendix F/K.