Application Note 71838

Evaluation of a Fully Automated Method for the Measurement of Glycerol in Wine

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Introduction

During fermentation, glycerol is synthesized from the glucose within yeast cells. Glucose is converted to glyceraldehyde-3-phosphate and dehydroxyacetone phosphate. Most of the dehydroxyacetone phosphate produced converts to glyceraldehyde-3-phosphate eventually producing ethanol. The remainder produces glycerol.

Glycerol is the third most common chemical compound in wines and an important by-product of alcoholic fermentation. Usually the glycerol concentration in wines is around 5 g/L, but concentrations can be as high as 15-20 g/L and depend upon fermentation conditions, especially the level of sulfur dioxide. The influence of glycerol in finished wine is usually at or below the level of sensory perception. Wines with elevated levels of alcohol tend to have more body and viscosity and a sweet taste which has often been attributed to the presence of glycerol.

The purpose of this study is to evaluate the performance of the Thermo Scientific™ system reagent kit for determination of glycerol in wine using a Thermo Scientific™ Arena™ discrete automated analyzer to complete the photometric measurements. Results are compared to those analyzed with the WineScan™ FT120 (FOSS). Five proficiency test samples, analyzed by the accredited enzymatic reference method of ALKO, Inc., are also analyzed with the Arena analyzer.

The method used in this study is an enzymatic assay with glycerokinase (GK), ADP dependent hexokinase (ADP-HK) and glucose-6-phosphate-dehydrogenase (G6P-DH). The principle of the method is shown in equations 1-3.

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{L} - \text{Glycerol} - 3 - \text{phosphate} + \text{ADP} \quad [1]$$

$$\text{ADP} + \text{D} - \text{Glucose} \xrightarrow{\text{ADP-HK}} \text{D} - \text{Glucose} - 6 - \text{phosphate} + \text{AMP} \quad [2]$$

$$\text{D} - \text{Glucose} - 6 - \text{phosphate} + \text{NAD}^{+} \xrightarrow{\text{G6P-DH}} \text{Gluconate} - \text{d} - \text{lactone} - 6 - \text{phosphate} + \text{NADH} + \text{H}^{+} \quad [3]$$

Materials and Method

Instruments

A Thermo Scientific™ Arena™ 20XT analyzer (Thermo Fisher Scientific, Vantaa, Finland) and a WineScan FT120 (FOSS, Hillerod, Denmark) were used. The Glycerol method has also been adapted for use with the fully automated discrete Thermo Scientific Gallery™ and Gallery™ Plus analyzers.

Application

The Glycerol method designed for use with the automated discrete analyzer consists of two reagents, an end-point measurement with sample blank, and a linear calibration curve used for result calculation. The measuring range is from 0.07 to 30.00 g/L.

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First, a 5 µL sample was incubated at 37 °C with 100 µL of buffer containing D-glucose, GK, ADP-HK and G6P-DH for 120 seconds followed by a blank reading. Then, 25 µL of reagent with NAD^+ and ATP was added and incubated for 420 seconds and the absorbance was measured at 340 nm.

Reagents and Calibrator

The system reagent Glycerol kit was used along with the Glycerol standard as a calibrator.

Table 1. Sample types and number.

Sample Type	Number
White wine	17
Rosé	3
Red wine	33

Samples and Controls

Samples of white wine, rosé wine, and red wine were used. The total number of samples was 53. Controls were self-made from glycerol (AnalaR, BDH, CAS: 56-81-5). Controls had two different concentration levels and were water-based.

All the samples were analyzed without pretreatment.

Result and Discussion

Calibration

The method calibration was performed as a two point calibration with two replicates. The responses and concentrations of the calibrators as well as the coefficient of regression are shown in Table 2.

Table 2. An example of the calibration results.

Standard	Response (Abs)	Calculated conc. (mg/L)	Concentration (mg/L)
Water	0.177	-0.007	0.000
Water	0.178	0.007	0.000
Glycerol STD	0.687	12.610	12.600
Glycerol STD	0.686	12.590	12.600
Factor	0.412		
Bias	0.177		

Comparison Study

For the comparison, each sample was measured three times. Results obtained from the Arena analyzer are correlate to those obtained by WineScan as shown in Figure 1.

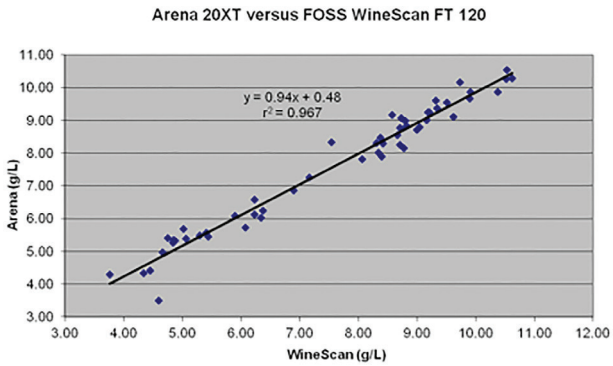

Figure 1. A method comparison study.

Five proficiency test samples were also analyzed using the Arena analyzer. Results are shown in Table 3 and compared to those measured with the ALKO, Inc. accredited enzymatic method.

Table 3. Results from the five proficiency test samples.

Sample Type	Tolerance (g/L)	Arena results (g/L)
White wine	5.4 ± 0.8	5.22
Red wine	7.1 ± 1.1	6.82
Sparkling wine	6.0 ± 0.9	5.61
Aromatized wine	8.7 ± 1.3	8.33

Conclusions

The Thermo Scientific Glycerol test used with the Arena discrete analyzer is accurate and repeatable both at high and low levels. The results correlate well with those measured by the WineScan and very well with those analyzed according to the accredited ALKO, Inc. enzymatic method. The correlation coefficient was 0.964 when compared to the WineScan and 1.000 when compared to the accredited enzymatic method. The repeatability of the method was tested with a water based standard as well as wine samples in the range of 4.25 g/L to 10.39 g/L. The observed within-run repeatability (SD) was between 0.036 and 0.099 g/L.

Acknowledgements

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References

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