KNAUER

# *Application Note*

## Determination of sugars, ethanol and glycerol in wine

AZURA

| | |
|---|---|
| **Category** | Food |
| **Matrix** | Wine |
| **Method** | HPLC |
| **Keywords** | Sugars, Alcohol, Glycerol, Wine, Eurokat Pb, AZURA Compact HPLC |
| **Analytes** | Saccharose, Glucose, Fructose, Ethanol, Glycerol |
| **ID** | VFD0121N_A_E |

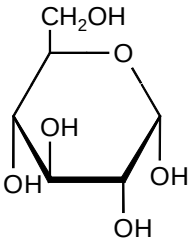

### Summary

This application note presents a simple and rapid method for the simultaneous determination of Saccharose, Glucose, Fructose, Ethanol and Glycerol. The method is designed for the quality and process control of wine. All five substances are baseline separated and no hydrolysis of Saccharose was observed. An extra sample preparation is not necessary, just a dilution. This method works with water as a low cost eluent.

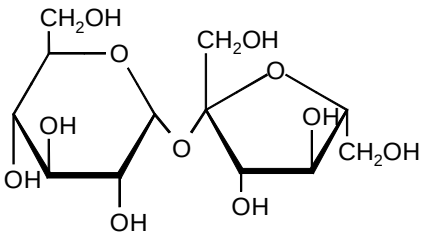

### Introduction

Rapid identification and quantitation of Saccharose, Glucose, Fructose, Ethanol and Glycerol in wines is important. During the fermentation the Saccharose is cleaved into Glucose and Fructose. Monosaccharides are metabolized into Ethanol and Glycerol. The determination of the sugar content is necessary to obtain the endpoint of the fermentation. Therefore these five compounds need to be checked to control the quality and process of wine fermentation. Furthermore Saccharose, Glucose, Fructose, Ethanol and Glycerol influence the taste of wine. The Glycerol concentration is also a marker for premium wines.

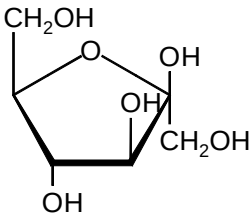
## Chemical Structures



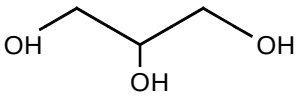

Glucose



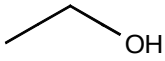

Saccharose




Fructose




Glycerol




Ethanol

## Experimental sample preparation

The wine sample can be injected directly after micro filtration and a 3:4 dilution with 2 mmol/l phosphate buffer pH 7.0 (750 µl wine + 250 µl phosphate buffer). All standard solutions were prepared with double-distilled water.

## Method parameters

| | |
|---|---|
| Column | Eurokat Pb, 300 x 8 mm and 30 x 8 mm as precolumn |
| Eluent A | water |
| Flow rate | 0.6 ml/min |
| Injection volume | 20 µl |
| Column temperature | 60 °C |
| System pressure | approx. 60 bar |
| Detection | Refractive Index |
| Run time | 25 min |

## Results

A calibration was made for Saccharose, Glucose, Fructose, Ethanol and Glycerol. All five substances are baseline separated (figure 1). The calibration concentration was in the range between 1.5 to 75 mg/ml for each substance (figure 2). The experimental data beyond show that the linearity range is higher. The linearity r2 for all substances was greater than 0.9999. Ethanol shows the smallest detector response and therefore the limit of detection is lower.

**Figure 1**
Chromatogram at 75 mg/ml

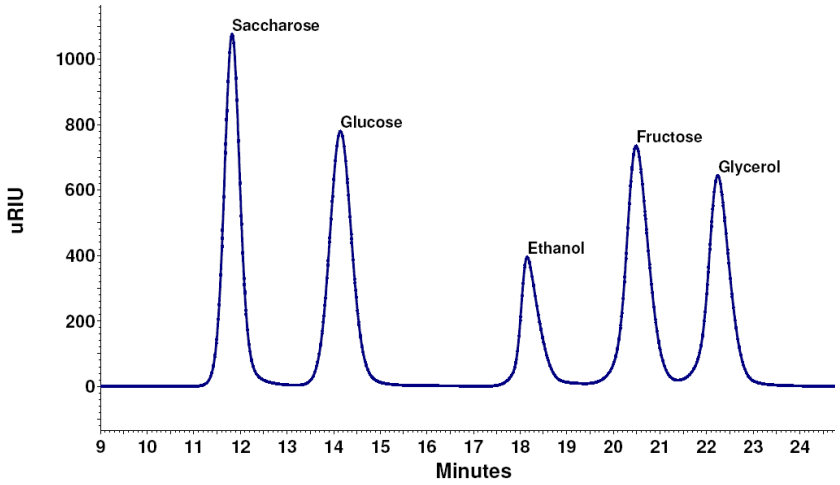
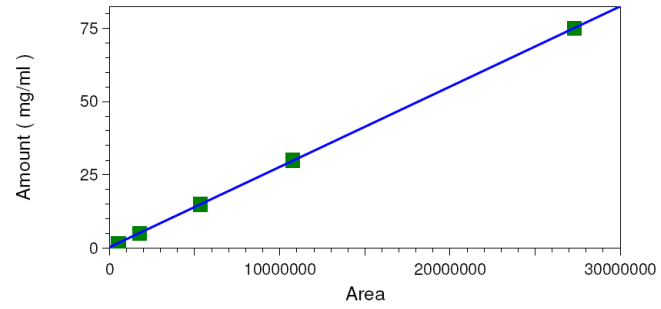



**Figure 2**
Calibration



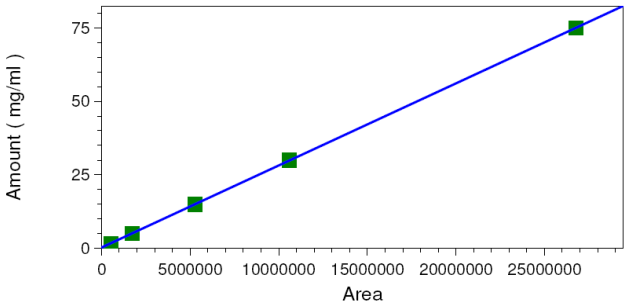

**Saccharose**

Range: 1.5-75 mg/ml

Linearity ($r^2$) = 0.999960

Linearity range = 0.75-150 mg/ml

Limit of detection = 0.02 mg/ml



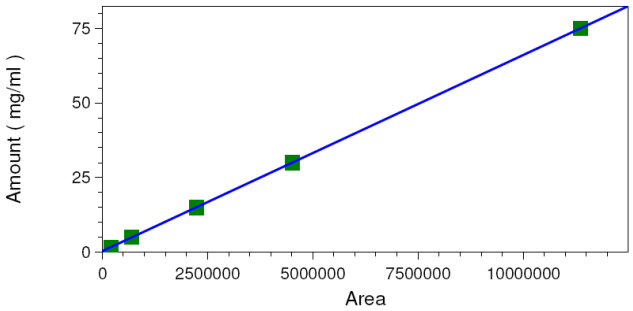

**Glucose**

Range: 1.5-75 mg/ml

Linearity ($r^2$) = 0.999985

Linearity range = 0.75-150 mg/ml

Limit of detection = 0.03 mg/ml



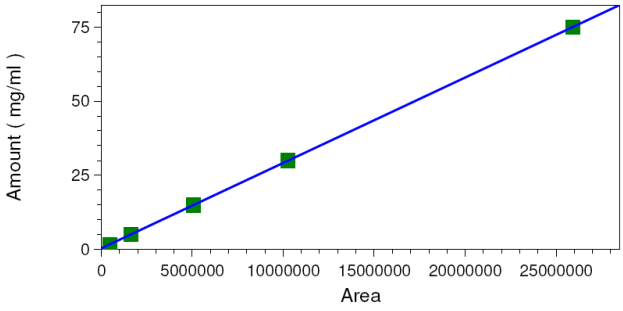

**Ethanol**

Range: 1.5-75 mg/ml

Linearity ($r^2$) = 0.999989

Linearity range = 1.0-150 mg/ml

Limit of detection = 0.06 mg/ml



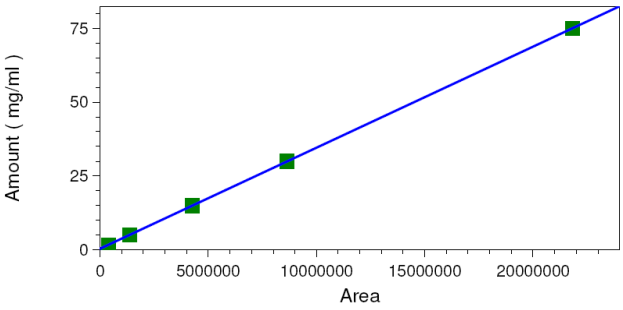

**Fructose**

Range: 1.5-75 mg/ml

Linearity ($r^2$) = 0.999984

Linearity range = 0.75-150 mg/ml

Limit of detection = 0.03 mg/ml




**Glycerol**

Range: 1.5-75 mg/ml

Linearity ($r^2$) = 0.999980

Linearity range = 0.75-150 mg/ml

Limit of detection = 0.04 mg/ml

As samples Federweißer (young wine) and red wine were used. Both samples contain no Saccharose. Federweißer contains a high concentration of Fructose and Glucose and a low concentration of Ethanol and Glycerol (Figure 3). The red wine shows a different pattern. The red wine has a high concentration of Ethanol and Glycerol and a low concentration of Fructose and Glucose (Figure 4). The red wine was spiked with Saccharose to show that Saccharose can be analyzed with wine as matrix (Figure 5). The recovery rate was 99 %. The dilution with buffer is necessary, because the wine acids hydrolyse the Saccharose at 60 °C. This results in a lower recovery rate.

**Figure 3**
**Federweißer (young wine)**

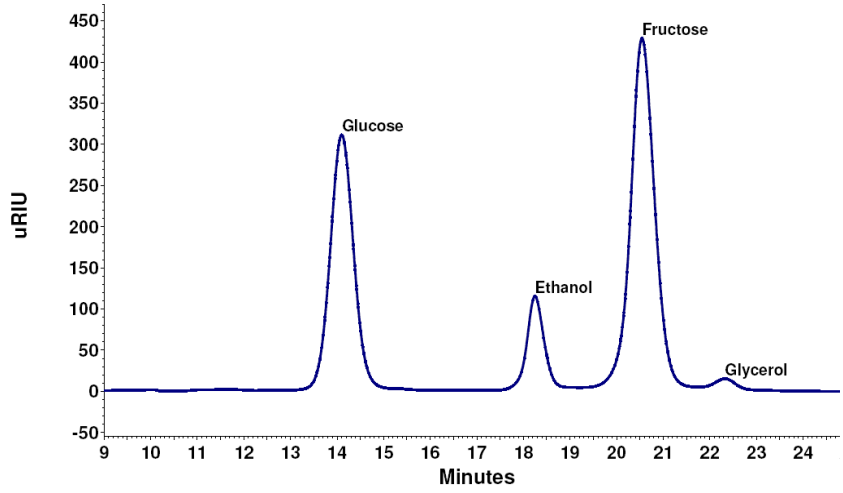


**Figure 4**
**Red Wine**

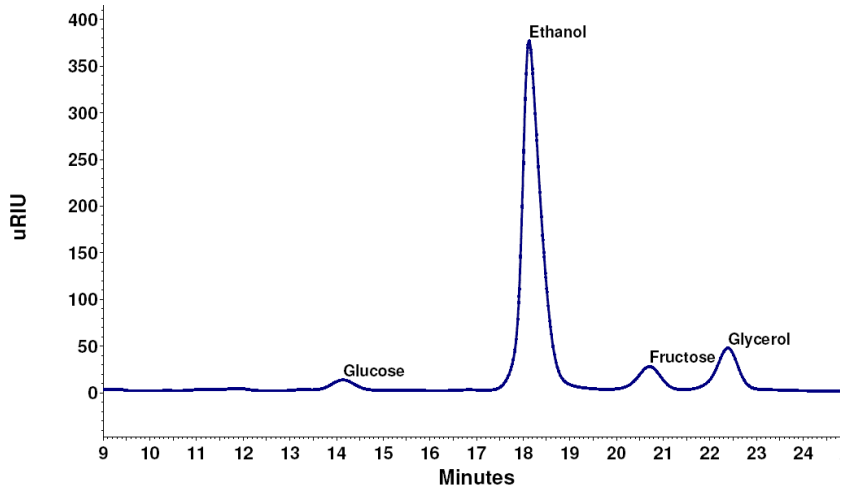



**Figure 5**
**Red Wine spiked with saccharose (blue line) compared to unspiked sample (green line)**

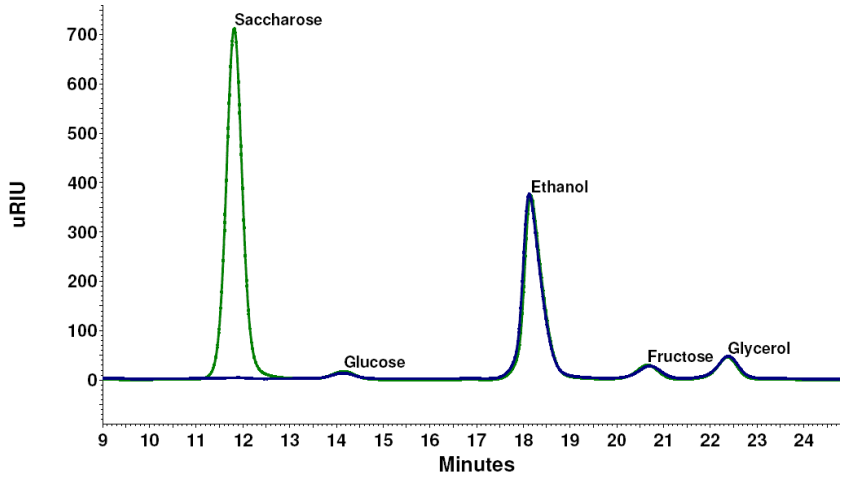



**Figure 6**
**Results of samples**

| | Federweißer (young wine) | Red Wine |
|---|---|---|
| Saccharose | 0.00 mg/ml | 0.00 mg/ml |
| Glucose | 29.9 mg/ml | 1.4 mg/ml |
| Ethanol | 21.2 mg/ml (2.7 %Vol) | 93.6 mg/ml (11.9 %Vol) |
| Fructose | 43.6 mg/ml | 3.4 mg/ml |
| Glycerol | 1.5 mg/ml | 7.0 mg/ml |

**Method performance**

| | |
|---|---|
| Limit of detection | 0.02 – 0.06 mg/ml (S/N = 3) |
| Linearity ($r^2$) | 0.999960-0.999989 |
| Linearity range | 0.75-150 mg/ml |
| Retention time precision* | < 1 % RSD |
| Peak area precision* | < 2 % RSD |

## Conclusion

The use of this method facilitates to determine Saccharose, Glucose, Fructose, Ethanol and Glycerol in young and red wine without the loss of Saccharose. Saccharose hydrolyse very fast under acidic conditions. Therefore the sample is diluted with buffer.

If you use a sulfonated cross-linked styrene-divinylbenzene copolymer in H-form, Saccharose will hydrolyze over 25 C. Using the H-form under this temperature leads to an incomplete baseline separation of acids and monosaccharides. Due to the high concentration range of the wine compounds, it is possible that a high concentrated substance overlaps a low concentrated one. The newly described method here circumvents this problem.

Another method to determine the sugar is the use of a HILIC (hydrophilic interaction chromatography) method. HILIC works very fine with sugar, but it is impossible to analyze Ethanol. Furthermore you need a high concentration of acetonitrile. The presented method only needs water as an eluent, which keeps the cost low.

The fact that Methanol and organic acids in wine cannot be assigned is avoidable by using KNAUER Eurokat H columns.

## Authors

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## Physical properties of recommended column

Packing materials in the Eurokat family are sulfonated cross-linked polystyrene copolymers. The lead forms are the phase of choice for the separation of sugars and sugar alcohols. The analytes are separated by ion exclusion and ligand exchange chromatography

| | |
|---|---|
| Stationary phase | sulfonated cross-linked styrene-divinylbenzene copolymer in lead form |
| USP code | L34 |
| Cross Linkage | 6 % |
| Particle size | 10 µm |
| Form | spherical |
| Dimensions | 300 x 8 mm and 30 x 8 mm |
| Order number | 30GX350EKN and 03GX350EKN |

## Recommended instrumentation

This application requires an isocratic HPLC system equipped with degasser, autosampler, column oven, and refractive index detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

| Description | Order No. |
|---|---|
| AZURA Compact HPLC isocratic | AYIABACA |
| Autosampler KNAUER Optimas | A5007 |
| Column Thermostat | A0585 |
| RI Detector 2300 | A5160 |
| AZURA Eluent tray E 2.1L | AZC00 |

## Contact information

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