

Application Note

► Determination of ingredients in wine



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|----------|--|
| Category | Food |
| Matrix | Wine |
| Method | HPLC |
| Keywords | Sugars, sugar alcohols, organic acids, wine, Eurokat H polymer column |
| Analytes | Citric acid, Tartaric acid, Malic acid, Succinic acid, Lactic acid, Acetic acid, Glucose, Fructose, Glycerol, Butandiol, Ethanol |
| ID | VFD6, December 2007 |

Summary

All major wine compounds can be analyzed by HPLC from a single injection. Simple and rapid method enables simultaneous determination of sugars, organic acids and alcohols.

Introduction

The amount of sugar present in wines determines its classification as a dry or sweet wine. Being a fruit product, wine can be expected to contain fructose and glucose and other additive sugars. Sorbic acid and citric acid are commonly used as acidulants and/or preservatives [1]. Citric acids add a fresh taste. Whereas succinic acid gives a more salty bitter taste. The malic acid content, for example, gives wine its distinctive flavor and is also an indicator of the quality of the fermentation process which has been undertaken by the yeast in the wine. If the fermentation process is not properly controlled however, any malic acid remaining can lead to spoilage from bacterial fermentation after bottling. Such wine is said to suffer from an imbalance of acid or to have spoiled. Objective values related to sweetness, acidity, maturity and alcohol content can be obtained, simultaneously with indication of deterioration or adulteration. All listed compounds can be quickly analyzed by way of HPLC in combination with Eurokat H column.

Experimental Sample Preparation

Due to the simple matrix influence all wine samples can be directly injected into the HPLC system. The wine sample can be injected directly after micro filtration and dilution with ultra pure water if necessary.

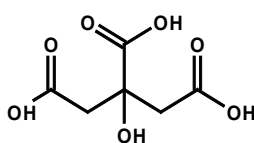
Experimental Preparation of Standard Solution

All standard solutions were prepared with double-distilled water. The following concentrations of the standard solution mixture is used:

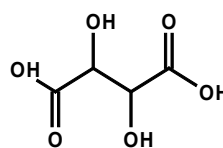
| | |
|---------------|------------|
| Citric acid | 10 µg/µl |
| Tartaric acid | 10 µg/µl |
| Glucose | 10 µg/µl |
| Malic acid | 10 µg/µl |
| Fructose | 10 µg/µl |
| Succinic acid | 10 µg/µl |
| Lactic acid | 10 µg/µl |
| Glycerol | 160 µg/µl |
| Acetic acid | 10 µg/µl |
| Butandiol | 10 µg/µl |
| Ethanol | 160 µg/µl. |

From the stock solution mixture the standard solutions for calibration are prepared with different dilution steps. The calibration range is 0.2 g/L up to 10 g/L. For glycerol and ethanol a larger standard range from 2 g/L up to 160 g/L is analyzed.

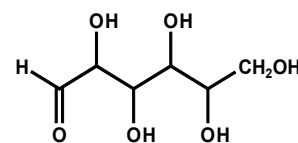
Chemical Structures



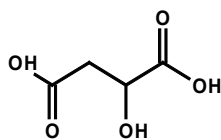
Citric acid



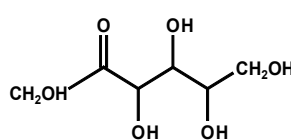
Tartaric acid



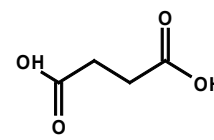
Glucose



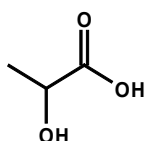
Malic acid



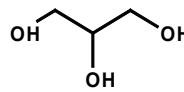
Fructose



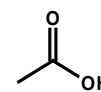
Succinic acid



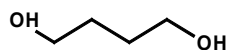
Lactic acid



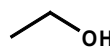
Glycerol



Acetic acid



1,4 Butandiol



Ethanol

Method Parameters

A simple and fast analysis of the components in wine can be made by using a Smartline HPLC from KNAUER with a Eurokat H column. The chromatographic conditions (eluent concentration, flow rate, temperature) have been optimized so that the components mentioned above are separated with baseline resolution.

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| Column | Eurokat H, 300 x 8 mm |
| Eluent | 0.00125 M sulfuric acid |
| Flow rate | 0.6 ml/min |
| Injection volume | 20 µl |
| Column temperature | 95 °C |
| System pressure | approx. 37 bar |
| Detection | Refractive Index |
| Run time | 40 min |

Results

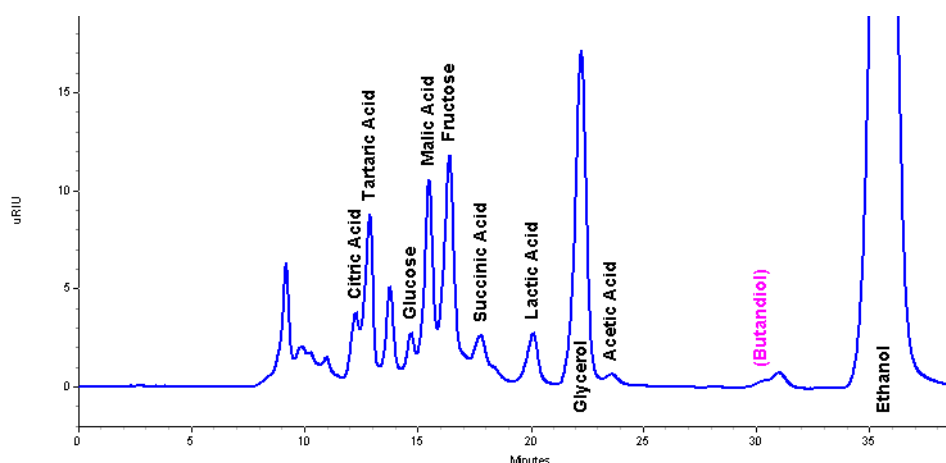


Fig. 1

Major wine components in a typical red Wine sample

The above-named substances were identified in different wines as being 12 – 13 % of the sample weight. Interestingly, white wine # 2 contained a markedly higher amount of malic acid (11.585 g/L) and fructose (12.919 g/L) and less alcohol (87.202 g/L) in comparison to all of the other wines analyzed. This result causes us to conclude that the fermentation process proceeded quickly and then was prematurely stopped. Consequently, a wine is produced with high residual sugar content, and higher fructose and lower glucose concentrations. The flavor of the wine is dependent on the tartaric acid and malic acid concentrations, as well as the total acid content comprised mainly of lactic acid, citric acid, and succinic acid. The wines analyzed gave an average total acid content of 7 – 8 g/L, with the exception of white wine # 2. The concentration of volatile acetic acid was nearly 0.36 g/L in the white wine sample and, as expected, 0.65 g/L in the red wine, twice the amount of the white wine.

Table 1

Assay results for red wine

| Substance | t _r (min) | Area | Conc. [g/l] |
|---------------|----------------------|---------|-------------|
| Citric acid | 12.283 | 98127 | 1.28 |
| Tartaric acid | 12.900 | 236473 | 4.28 |
| Glucose | 14,714 | 73412 | 1.07 |
| Malic acid | 15.500 | 305124 | 3.55 |
| Fructose | 16.417 | 425668 | 5.73 |
| Succinic acid | 17.800 | 140279 | 2.07 |
| Lactic acid | 20.100 | 104133 | 1.64 |
| Glycerol | 22.283 | 614282 | 19.40 |
| Acetic acid | 23.600 | 23710 | 0.65 |
| Butandiol | 30.345 | - | - |
| Ethanol | 35.650 | 4159017 | 111.59 |

Method Performance

| | |
|----------------------------------|----------------------------------|
| Limit of detection | g/l range (S/N = 3) |
| Linearity (r²) | 0.99980-0.99993 |
| Linearity range | 0.2 to 10 g/l (2 g/l to 160 g/l) |
| Retention time precision* | < 2 % RSD |
| Peak area precision* | < 3 % RSD |

*repeatability calculated over 5 replicate runs

Conclusion

A rapid and simple method for the determination of all major compounds in wine is easy to realise with the Eurokat H column in combination with an isocratic HPLC system.

References

- [1] Official Methods of Analysis, food Compositions; Additives, Natural Contaminants, 15th ed; AOAC: Arlington, VA, 1990, Vol. 2.; Official Method AOAC 986.13: quinic, malic, citric acid in cranberry cocktail and apple juice.

Physical Properties of recommended Column

Eurokat H is a sulfonated cross-linked styrene-divinylbenzene copolymer. This particular cation exchanger is characterized by a very high density of functional groups. Thus it is not the appropriate polymer for applications in ion exchange but in ion exclusion and ligand exchange chromatography.



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| Stationary phase | sulfonated cross-linked styrene-divinylbenzene copolymer (Eurokat H) |
| USP code | L17 |
| Particle size | 10 µm |
| Form | spherical |
| Cross Linkage | 8 % |
| Dimensions | 300 x 8 mm |
| Order number | 30GX340EKN |

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. |
|--|-----------|
| Smartline Pump 1000, incl. 10 ml pump head | A50303 |
| Smartline Manager 5000 degasser | A5316 |
| Autosampler 3900 | A1508 |
| Smartline Column Oven 4000 | A5300 |
| Smartline RI Detector 2300 | A5200 |
| ChromGate Software | A1493 |

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