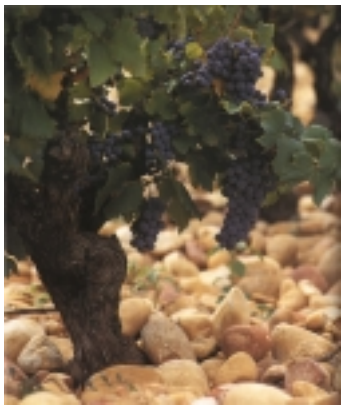
CE and HPLC-MS Methods for the Determination of Resveratrol and Glycosides in red Wine

G. Stecher[1], C.W. Huck[1], A. Premstaller[1], C.G. Huber[1], M. Popp[2], H. Scherz[3], G.K. Bonn[1]

[1] Institute of Analytical Chemistry and Radiochemistry, Leopold - Franzens University, Innrain 52a, A-6020 Innsbruck, Austria
[2] Bionorica/Plantamed GmbH, Kerschensteinerstraße 11-15, D-92318 Neumarkt/Oberpfalz, Germany
[3] Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergerstraße 4, D-85748 Garching, Germany

Introduction

Regular, but moderate consumption of red wine is correlated with many health aid effects: a decrease in coronary heart disease, lower formation of kidney stones, inhibition of cancer growth and a favourable HDL/LDL ratio in blood is documented. Many of these effects are attributed to resveratrol and its glycoside (piceid) [1,2]. Most research has been focused on the determination of trans-resveratrol since initial publication has reported the presence of trans-resveratrol in commercial wines [1].
The aim of this study was to develop new methods for the determination of saccharides, piceid and other glycosides in red wine.



Methods

Measurements were achieved by capillary electrophoresis. For this purpose the capillary was coated with quarternized trimethylamino-polystyrene nanoparticles [3]. Aldoses, ketoses and uronic acids were derivatized within 15 min at a temperature of 90°C by reductive amination with p-aminobenzonitrile [4]. Using 175 mM borate buffer, pH 10.5, as carrier, derivatized sugars could be separated as their borate complexes in the co-electroosmotic separation mode (fig.1).
For the simultaneous determination of cis- and trans-resveratrol, myricetin, quercetin and their glycosides in red wine extractives a HPLC-MS method was established.

References

[1] Siemann, E.H. and Creasy, L.L.; Am.J. Enol.Vitic.; Vol.43, No.1 (1992), 49-52
[2] Goldberg, D.M.; Ng, E.; Karumanchiri, A.; Yan, J.; Diamandis, E.P.; Soleas, G.J.; J. Of Chromatography A, 708 (1995), 89-98
[3] Kleindienst, G.; Huber, C.G.; Gjerde, D.T.; Yengoyan,L.; Bonn G.K.; Electrophoresis; 1998, 19, 262-269
[4]Schwaiger, H.; Oefner, P.J.; Huber C.; Grill, E.; Bonn, G.K.; Electrophoresis; 1994, 15, 941-952

Separation of Derivatized Carbohydrates by Co-electroosmotic CE

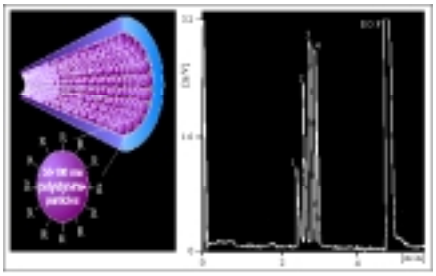


Figure 1
Capillary 50 cm x 50 µm x 375 µm (L_{eff}:30cm); buffer: 175 mM borate, pH= 10.5; voltage: -13kV; current: 71mA; detection: direct UV at 285 nm. Peak assignments: 1 galacturonic acid; 2 galactose; 3 arabinose; 4 xylose

HPLC-ESI-MS - Blauburgunder 1997 spiked with Resveratrol standard

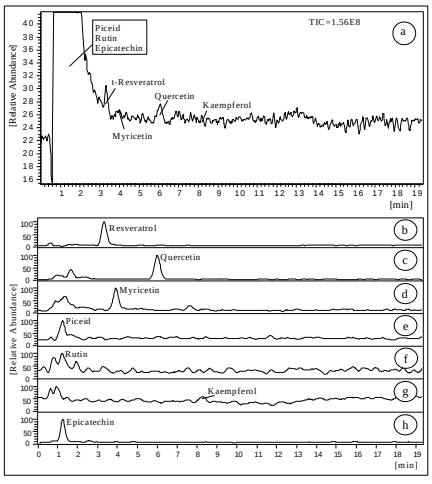


Figure 2
Column: Phenomenex Luna C18(2) (50 x 2mm I.D., 3µm) plus a Phenomenex precolumn C18 (4 x 4mm I.D., 5µm); **mobile phase:** (A) 225 g H_2O, 19.8 g methanol, 2.5 ml formic acid; (B) 150 g H_2O, 66.8 g THF, 19.8 g methanol, 2.5 ml formic acid; **gradient:** 0 min 50%A, 10 min 16,7%A, 11 min 50%A, 15 min 50%A; **flow rate:** 0,2 ml/min, **temp.:** 60 °C; **MS**: Capillary Temp.: 270 °C, capillary voltage: 3.5 kV
(a) TIC=Total Ion Current; (b) resveratrol; (c) quercetin, (d) myricetin, (e) piceid, (f) rutin, (g) kämpferol, (h) epicatechin

MS/MS of Piceid (Resveratrolglycoside, m/z=390) in Blauburgunder 1997

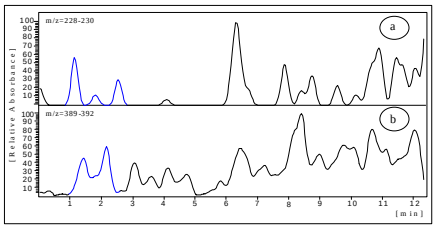


Figure 3
Separation conditions see Figure 2. MS/MS collision energy: 25%. (a) m/z=228-230 (=Resveratrol), (b) m/z=389-392 (=Piceid).

Results and Discussion

The developed CE separation technique allows very fast analysis of wine ingredients with high efficiency (Fig.1). A mixture of galacturonic acid, galactose, arabinose and xylose could be separated in approximately 3 minutes.
After optimizing the separation of cis- and trans-resveratrol, myricetin and quercetin by HPLC, further investigations with HPLC-MS were carried out (fig.2). The applied H_2O-THF-MeOH-HCO_2H gradient allowed the separation of the compounds of interest on a C-18 column in approximately 8 min (fig.2). The analysis of a wine-sample (Blauburgunder 1997) showed, that the detection of quercetin, myricetin, piceid, rutin, kaempferol and epicatechin is possible. Trans-resveratrol could only be detected, when the sample was spiked with standard solution (fig.2). Detection limits are shown in Table 1. For the identification of Piceid MS/MS of m/z=390 (Piceid) was done. Figure 3 shows the increase of resveratrol and the simultaneous decrease of piceid at 1.5 min.
Finally the HPLC-MS method gives the possibility of a rapid identification and quantification of polyphenols and glycosides even in complex sample matrices.

Table 1:
Limits of detection using HPLC-ESI-MS

	LOD [ng]	LOD [pmol]
Kaempferol	18.0	62.7
Rutin	40.5	66.4
Myricetin	21.9	69.0
Quercetin	40.5	133.7
Epicatechin	149.5	515.5
t-Resveratrol	720	3160.0