

High Performance Thin-layer Chromatography with Automated Multiple Development (AMD-HPTLC) of Anthocyanins in Red Wine

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Introduction: Colour is an important factor in the acceptability of food products. In red wine, the colour is due to the presence of anthocyanins, which belong to the group of the flavonoids. The wine of *Vitis vinifera* contains mixtures of the monoglucosides of the anthocyanins Malvidin, Delphinidin, Cyanidin, Petunidin and Paeonidin. Hybrids of European and American varieties contain also diglucosides of anthocyanins, mainly Malvidin-3,5-diglucoside (Mv-3,5-Gl₂).

The aim of this work was to determine in a rapid and cost-effective manner the qualitative and quantitative pattern of the anthocyanins in hybrid red wines, especially with regard to the Mv-3,5-Gl₂ content. We suspected this compound to be responsible for a sometimes bluish-red colour of the hybrid wines. Thus, this method could detect hybrids with high Mv-3,5-Gl₂ content in order to avoid their propagation during the process of grape wine breeding.

Material and Methods: Up to 17 wine samples as well as standard solutions of anthocyanins were applied on a 20x10 cm HPTLC normal phase silica plate. The chromatographic system consisted of an equipment for the Automated Multiple Development (AMD) technique. Six runs with mixtures of isopropyl acetate - formic acid – acetic acid – water with descent polarity and crescent running distances were performed. The chromatograms were submitted to densitometric evaluation with a TLC-scanner at 520 nm in reflectance mode. Peak areas were integrated and the percentile distribution of the different anthocyanin peaks was calculated.

Results and discussion: The densitometric evaluation of the chromatograms revealed up to 14 peaks. In non-hybrid wines, we found a relative uniform distribution of the anthocyanins with Malvidin-3-monoglucoside (Mv-3-Gl) as the dominant one, in accordance with the literature [1]. In non-hybrid wines, we detected a much higher variability of anthocyanins pattern. An example

chromatogram of the anthocyanins of a hybrid red wine is given in Fig.1: The major anthocyanin in this wine is Mv-3-Gl with 48,2% of total anthocyanins. Furthermore, one can detect Mv-3,5-Gl₂ (6,4% of total anthocyanins). In Fig. 2 a hybrid wine with extremely high Mv-3,5-Gl₂ content (50,3%) is represented. In this case Mv-3-Gl amounts to only 8,3%

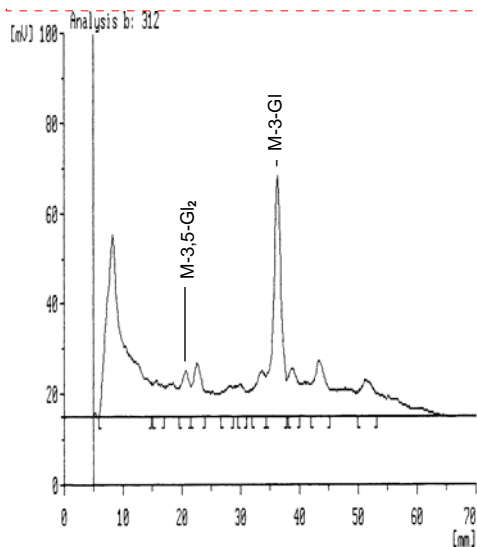


Fig. 1

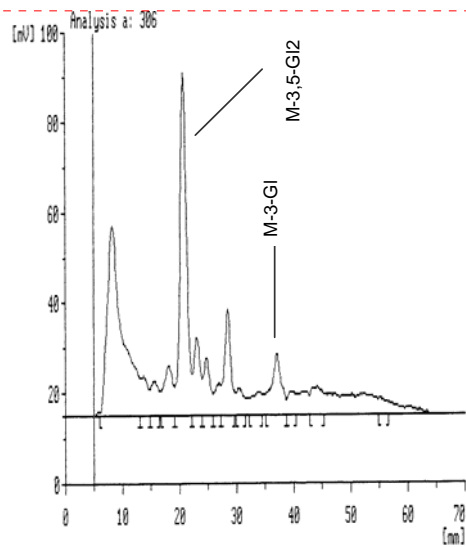


Fig. 2

Comment [HH1]: noch mal Boehm fragen, welche Weine hybrid / nichthybrid (siehe e-mail 14.10.05)

of total anthocyanins. We also found hybrid wines without any traces of Mv-3,5-Gl₂ (not shown). In our working hypothesis, we thought, that a high M-3,5-Gl₂ content could be responsible for the bluish-red colour of the hybrid wines. However, preliminary results indicate, that there is no correlation between these two parameters.

Conclusions:

The method presented here is the first one published for the separation of red wine anthocyanins with the AMD-technique. This new method is suitable for the monitoring of the red wine anthocyanins pattern, without the need for sample clean-up, with short analysis time and low solvent consumption.

References:

[1] L. W. Wulf, and C. W. Nagel. High-pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. Am. J. Enol. Vitic. 29 : 42-49 (1978)