Simultaneous Determination of Resveratrol- and Piceid - Isomeres in Wines by Capillary Electrochromatography (CEC)

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Abstract

The stilbene resveratrol is a polyphenolic compound found in grapes and wines [1]. It shows promising effects as preventive therapeutic agent against arterosclerosis, inflammation and against several types of cancer [2]. Basing on the French paradox [3] and the first publication [4], which documented the presence of resveratrol in red wine, further pharmacological and analytical investigations were carried out and therefore a high demand for new analytical techniques still consists. The use of HPLC and CE as analytical tools and UV-, FLD- and MS- detection [5] of the substances of interest are documented. In this work, a capillary electrochromatography (CEC) method for the determination of stilbene-isomers (resveratrol and piceid) and flavonoids (kaempferol and epicatechin) is presented. Silica C18 (L_{eff} = 40 cm, 100 µm I.D., 3 µm) was used as stationary phase and different mobile phases e. g. KH_2PO_4 and NH_4OAc were investigated. Also the influence of the temperature and different amounts of organic additives such as acetonitrile (ACN) were tested in order to develope a CEC-separation system also for CEC-MS coupling.

References

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Separation of polyphenolic compounds using a KH_2PO_4-buffer

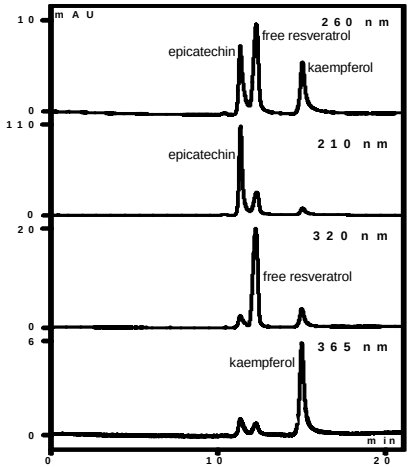

Fig.1: Capillary: HP-CEC-C18, 3 µm, 40 cm L_{eff}, 100 µm I.D.; buffer: 10 mM KH_2PO_4, 60% ACN, pH 7; Temp: 25°C; U = 25 kV, p = 10 bar, I = 12,5 µA

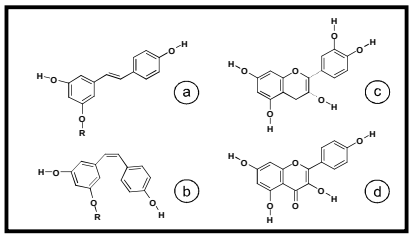

Fig.2: Chemical structures. a: *trans*-resveratrol (R = H), *trans*-piceid (R = glucose), b: *cis*-resveratrol (R = H), *cis*-piceid (R = glucose), c: epicatechin, d: kaempferol

Separation of stilbene-isomeres using a NH_4OAc-buffer

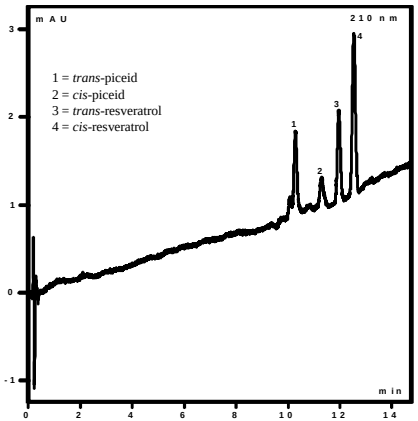

Fig. 3: Capillary: HP-CEC-C18, 3 µm, 40 cm L_{eff}, 100 µm I.D.; buffer: 10 mM NH_4OAC, 50% ACN, pH 6,7; Temp: 25°C; U = 25 kV, p = 10 bar, I = 10,6 µA

Table 1: Calibration curves, correlation factors, limits of detection from *trans*- and *cis*-resveratrol and *trans*- and *cis*-piceid.

SUBSTANCE	CALIBRATION CURVE	r^2	LOD [µg/mL]
***Trans*-resveratrol (320nm)**	**y=0,0022x+0,0011**	**0,9989**	**5,5**
***Cis*-resveratrol (210nm)**	**y=0,0036x-0,0053**	**0,9993**	**2,3**
***Trans*-piceid (320nm)**	**y=0,0034x+0,0008**	**0,9984**	**6,5**
***Cis*-piceid (210 nm)**	**y=0,0034x+0,0006**	**0,9973**	**5,6**

Results and discussion

Figure 1 shows the separation of a standard-mixture containing *trans*- and *cis*- resveratrol, epicatechin and kaempferol. As the free resveratrols could not be separated the influence of temperature and amount of acetonitrile were investigated. First temperature was changed from 25 to 30 and 40°C working with 60% acetonitrile, after that the amount of acetonitrile was varied from 40 to 50 and 60% at a temperature of 25°C. As expected with higher temperature retention times decreased, but the free resveratrols could not be separated. Changing the acetonitrile amount from 60 to 40% same results were yielded, retention times increased, but the stilbeneisomeres could not be separated.

As we were interested in the determination of different stilbeneisomeres we tested NH_4OAc as running buffer and after optimizing the system, 10 mM NH_4OAc, pH 6,7 with 50% ACN showed best conditions for the separation of *trans*- and *cis*- piceid, and *trans*- and *cis*- resveratrol (see figure 3). Using UV-absorbance detection at 210 and 320 nm the plots of peak area versus concentration for all standard solutions showed good linearity (r^2 > 0,997). Standards demonstrated limits of detection between 2 and 6 µg/mL calculating them at a signal to noise ration of 2 to 1 (see table 1).

The used method for the determination of stilbeneisomeres showed good stability and reproducibility. Further investigation in the quantification of polyphenols by fluorescence- and mass spectrometric detection, and finally by chip is a seminal development for fast analysis of wine ingredients with high efficiency.