BIO-RAD

Capillary Electrophoresis
Application Note 57

Analysis of Flavonoids by Micellar Electrokinetic Chromatography with Scanning Detection

Flavonoids constitute a large family of plant phenolic pigments. Many plant species contain complex mixtures of up to 50 compounds and each plant tends to have a distinctive profile.

Flavonoids are widely used as remedies because of their spasmolytic, antiphlogistic, antiallergic, and diuretic properties. The role of flavonoids is related to their chemical structures. Their structure is based on 2-phenylbenzopyrone, with flavonoids differing one from another in their degree of saturation, type of conjugation (glycosylation, malonylation, sulphonation), and position of hydroxyl, methoxy, and sugar residues.

Flavonoid analysis has been performed by a variety of techniques, including thin-layer chromatography, gas chromatography, and HPLC.[1] More recently, capillary electrophoresis was demonstrated to be a powerful alternative to HPLC in the determination of honey flavonoids for characterization of the botanical origin of honeys[2] and in the analysis of flavonoid drugs from plant extracts.[3,4]

The present study demonstrates the successful use of micellar electrokinetic chromatography (MEKC) with scanning detection for the rapid and sensitive analysis of two flavonoids, naringenin and quercetin (Figure 1).

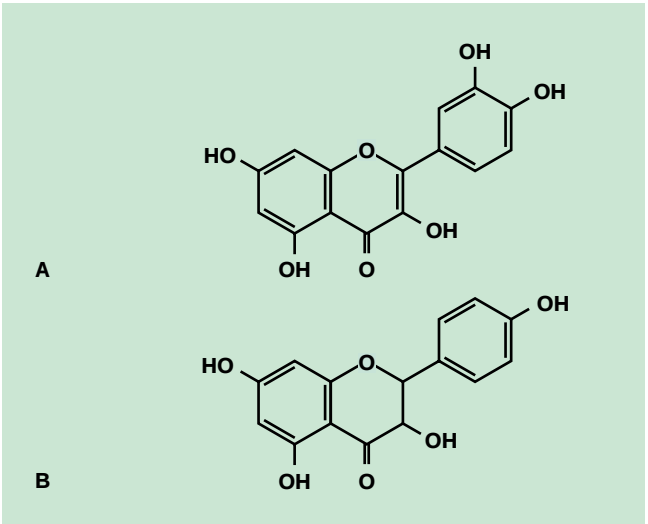

Fig. 1. Structure of quercetin (A) and naringenin (B).

Standards

Flavonoid standards, quercitin, and naringenin, were kindly provided by Dr. Nancy L. Paiva from the The Nobel Foundation, Ardmore, Oklahoma. Stock solutions were prepared in methanol at a concentration of 150–200 µg/ml. All dilutions were made in 100% methanol.

Analysis Conditions

Instrument	BioFocus® 3000 system
Capillary	50 cm x 50 µm, uncoated
Run buffer	200 mM sodium borate (pH 8.5) + 50 mM SDS
Capillary purge	60 seconds with run buffer
Injection	pressure at 5 psi*second
Polarity	positive to negative
Run voltage	18 kV (observed current 34 µA)
Cartridge temperature	20 °C
Autosampler temperature	20 °C
Detection	scanning, 200–360 nm

Results

In chromatography, flavonoids are best separated using reversed-phase HPLC (RP-HPLC) in which the separation mechanism is based on flavonoid hydrophobicity. In MEKC, ionic surfactants (*e.g.* SDS) are added directly to the analysis buffer to generate micelles with a charged surface and a hydrophobic interior. In such micellar systems, flavonoids are separated by a combination of factors: charge, hydrophobicity, and conformational state. The charges are obtained by ionization of the hydroxyl functions. Depending on the presence and position of hydroxyl functions, flavonoids can also acquire additional negative charges by complexing with the

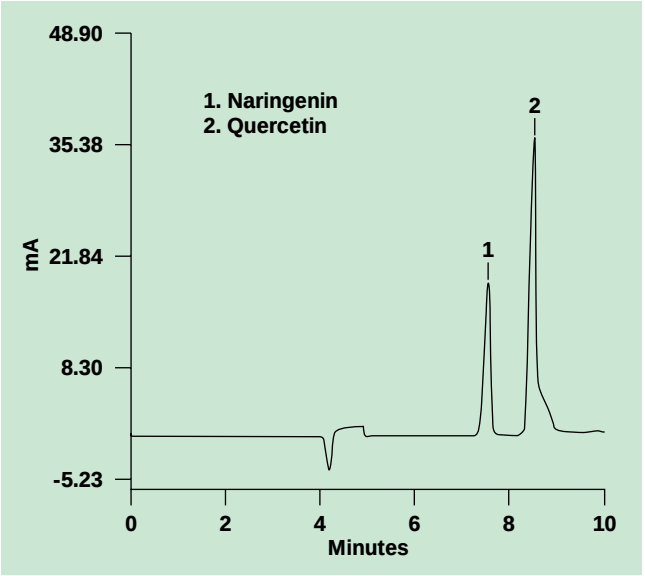

Fig. 2. MEKC separation of flavonoids. Detection at 205 nm.

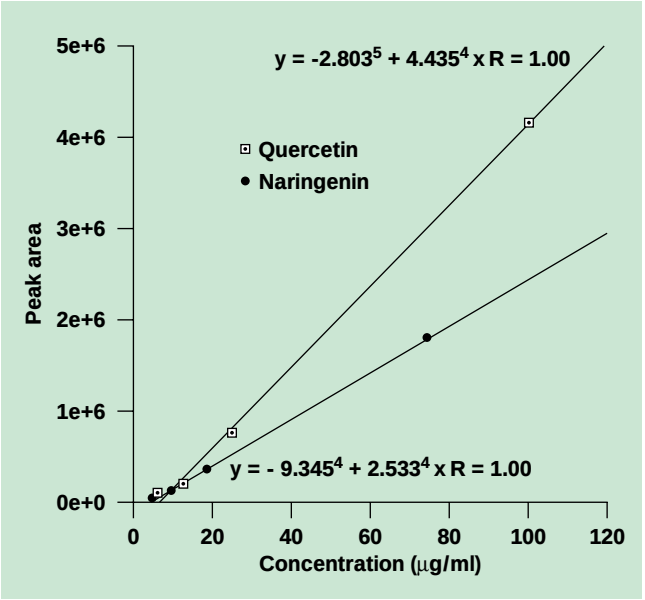

Fig. 3. Flavonoid response at 205 nm.

borate present in the MEKC buffer. Flavonoids migrate according to the number of negative charges; the more negative a molecule the longer its migration time. Due to this additional selectivity, flavonoids such as naringenin and quercetin, which often co-migrate when analyzed by RP-HPLC, can be easily resolved to baseline by MEKC, as shown in Figure 2.

Flavonoid response at 205 nm is shown in Figure 3. The detector response was linear from the minimum detectable concentrations (MDC) to at least 100 µg/ml for quercetin and 75 µg/ml for naringenin. Using single wavelength detection at 205 nm, MDCs of 0.45 µg/ml (naringenin) and 0.31 µg/ml (quercetin) were calculated from a peak-to-peak noise value of 39 µAU at a signal-to-noise of 3.

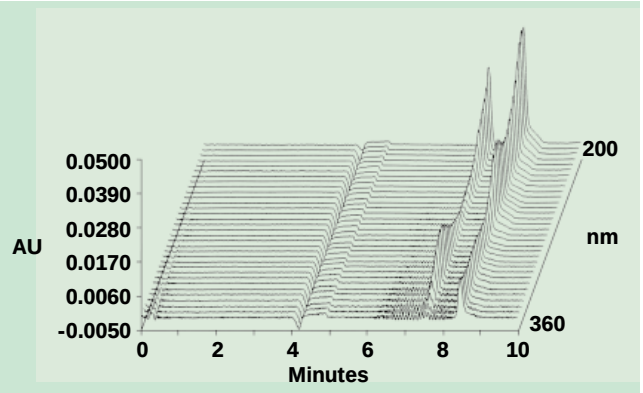

Fig. 4. 3-dimensional plot of a flavonoid standard mixture.

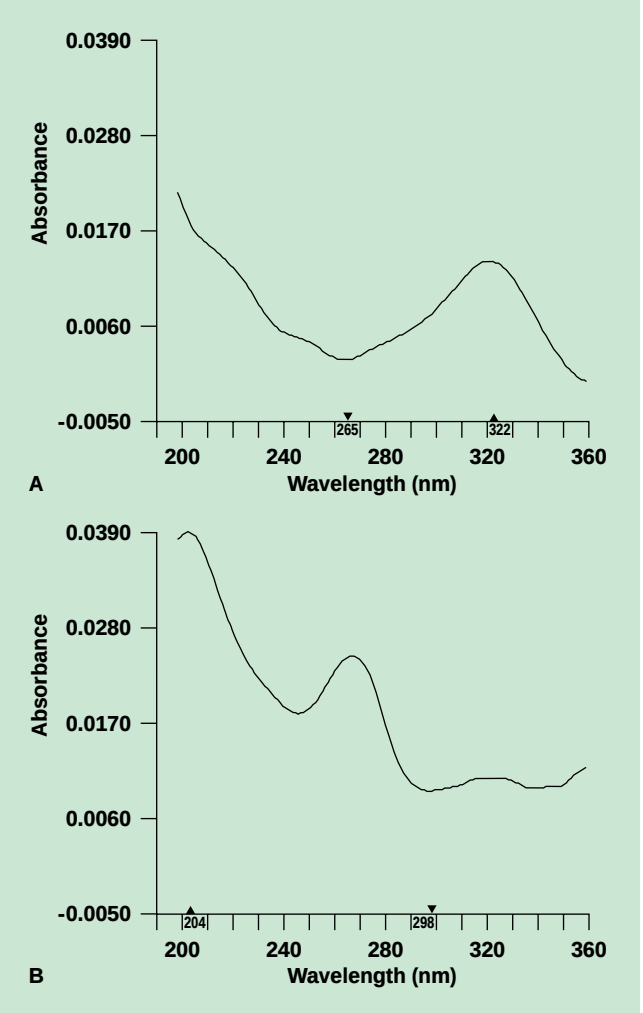

Fig. 5. UV spectra of naringenin (A) and quercetin (B) in MEKC buffer.

The 3-dimensional plot of time vs spectra as shown in Figure 4 is useful in the identification of unknown peaks in the analyzed sample.

Under MEKC conditions, the flavonoids give different UV spectra from those obtained in HPLC using methanol.[5] The UV spectra for naringenin and quercetin in the MEKC buffer are shown in Figure 5. There is a clear difference between the spectrum of quercetin (a flavonol) and that of naringenin (a flavanone). This allows flavonols and flavanones to be easily distinguished by their very characteristic UV spectra.

This study demonstrates that MEKC with scanning detection is a promising tool for rapid and sensitive analysis of flavonoids. It offers automation, high selectivity and resolution, and it is faster than HPLC. In addition, it has the advantage of very low solvent and sample consumption.

References

1. Franke, A. A. and Custer, L. J., *J. Chromatogr.,* **662**, 47 (1994).
2. Delgado, C., Tomas-Barberan, F. A., Talou, T. and Gaset, A., *Chromatographia,* **38**, 71,(1994).
3. Seitz, U., Oefner, P. J., Nathakarnkitkool, S., Popp, M. and Bonn, G. K., *Electrophoresis,* **13**, 35, (1992).
4. Pietta, PO. G. and Mauri, P. L., *J. Chromatogr*., **549**, 367 (1991).
5. Mabry, T. J., Markham, K. R., and Thomas, M. B., *"The Systematic Identification of Flavonoids,"* Springer Verlag, Berlin, 1970.

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