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Short Communication

Analysis of flavonoids by MECC with ultraviolet diode array detection*

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Keywords: *Micellar electrokinetic capillary chromatography (MECC); diode array detection (DAD); flavonoids;* Helichrysum italicum.

Introduction

Capillary electrophoresis (CE) has developed into a high-efficiency separation technique [1]. CE in the micellar mode is complementary to reversed-phase high-performance liquid chromatography (RPLC), since the analytes separate under the influence of a high-voltage potential (10–20 kV) as a result of differences in partitioning into and out of the hydrophobic core of the micelles.

Although RPLC has been the conventional method for the analysis of flavonoids [2, 3], the use of micellar electrokinetic capillary chromatography (MECC) is assuming considerable importance [4–7]. In addition, recent technological improvement of the capillary electrophoresis apparatus for ultraviolet diode array detection (DAD) permits 'on-line' information to be obtained with wavelength spectral resolution and reproducibility better guaranteed than in the use of 'rapid scanning' detectors. This is very useful for flavonoids, whose spectra are different in relation to the structure. This approach has been applied to the analysis of most common flavonoid-glycosides and of *Helichrysum italicum* extracts.

Experimental

Materials

The flavonoid standards luteolin-7-glucoside (L), isoquercitrin (I), kaempferol-3-glucoside (K), apigenin-7-glucoside (A), hyperosid (H) and quercitrin (Q) were obtained from Extrasyntheses (Genay, France).

4,2′,4′,6′-tetrahydroxychalcone-2′-glucoside (C) and naringenin-4′-glucoside (N) were isolated from *Helichrysum italicum* according to the literature [8]. The structures of all these flavonoids are shown in Fig. 1.

The flowering tops of *H.italicum* were purchased from different commercial sources.

Apparatus

MECC analyses were performed using Eureka 2000 CE–DAD apparatus from Kontron Instruments (Milan, Italy), equipped with a 50 cm fused silica capillary column (75 μm i.d.). This apparatus is the first integrated system for capillary electrophoresis with diode array detection (512 diodes; spectral resolution, 1.3 nm). The analysis buffer was an aqueous solution containing 20 mM sodium borate (pH 8.2) and 40 mM sodium dodecyl sulphate (SDS). The analysis conditions were: voltage, +15 kV (current 54 μA); gravity injection, 15 mm × 30 s (10 nl); temperature, 25°C. The acquisition of UV spectra was automatic (220–430 nm, 2 nm steps). Buffer was filtered using 0.2 μm Spartan (Schleicher & Schuell, Dassel, Germany). Data were collected by means an IBM compatible computer (6 Mbyte RAM,

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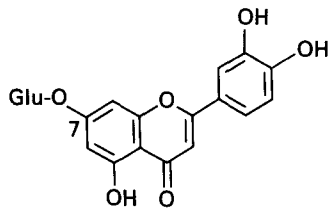
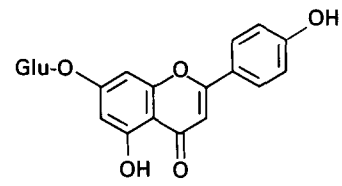
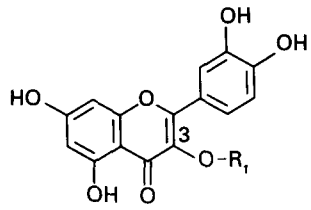


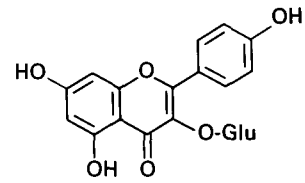
Luteolin-7-O-glucoside (L)



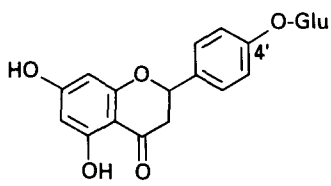
Apigenin-7-O-glucoside (A)



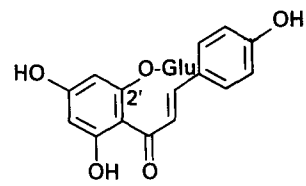
Isoquercitrin (I) R_1 = Glucose
Hyperosid (H) R_1 = Galactose
Quercitrin (Q) R_1 = Rhamnose



Kaempferol-3-O-glucoside (K)



Naringenin-4'-O-glucoside (N)



4,2',4',6'-Tetrahydroxychalcone-
-2'-O-glucoside (C)

Figure 1
Structures of the investigated flavonoid-glucosides.

hard disk 50 Mbyte, MS-DOS 3.3) equipped with mouse and Eureka 2000 software.

To maintain the capillary conditions, fresh buffer was introduced into the capillary between each run (aspiration for 2 min).

Sample preparation

Standard stock solutions (0.4 mg ml^{-1}) of L, I, K, A, H, Q, N and C were prepared in methanol. Different aliquots of these solutions were diluted with 30% methanol (concentration range 0.001–0.2 mg ml^{-1}).

The extracts of *H.italicum* were obtained according to the literature [9].

Results and Discussion

Results of MECC–DAD experiments are shown in Fig. 2. The flavonoid-glucosides migrated in the following order in less than 10 min: luteolin-, quercetin- kaempferol-, naringenin- and apigenin-glucoside. The relative standard deviations of the migration times were 0.6% (n = 30) and 0.9% (n = 15) for intra-day and inter-day assays, respectively.

It should be noted that the separation of these compounds by HPLC is problematic even by a gradient mode. In the case of quercetin-3-*O*-glucoside (I) and quercetin-3-*O*-galactoside (H), the separation by HPLC is impossible. On the other hand, the described MECC conditions allow the resolution of these quercetin-derivatives (Fig. 3).

Ultraviolet photo-diode array detection has been found very useful in the differentiation of separated flavonoids. The 'on-line' spectra of the flavonoid glycosides standards are in good agreement with those obtained by HPLC–DAD, although there was a wavelength shift (about 10 nm) due to the different solvents

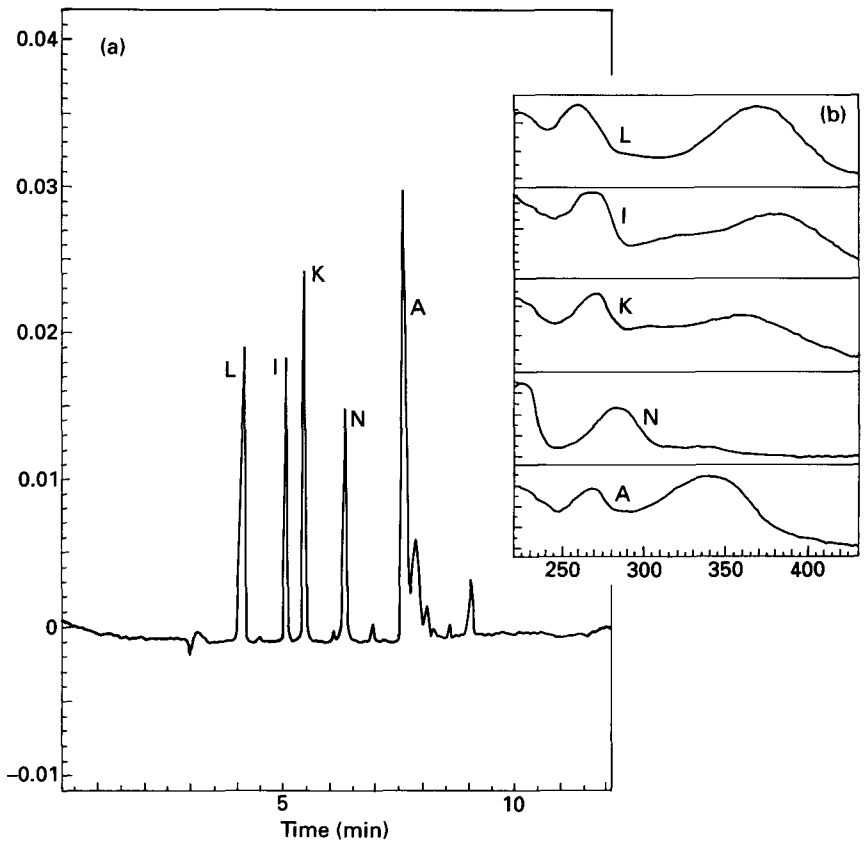


Figure 2
Separation of a standard solution of (a) flavonoid-glucosides and (b) relative DAD spectra. Capillary: fused silica (50 cm × 75 μm i.d.). Electrolyte: 20 mM borate buffer (pH 8.2) with 40 mM sodium dodecyl sulphate. Voltage: +15 kV; current: 54 μA. Temperature: 25°C. Injection: gravity, 15 mm × 30 s. For peaks see Fig. 1.

[borate–SDS (pH 8.2) buffer in MECC, and propanol–water in HPLC] used. Figure 4 shows the comparison between the UV spectra of quercitrin (Q) in HPLC eluent (line a) and MECC buffer (line b).

Sets of these standards were run and the detector response was linear over the range 0.002–0.2 mg ml^{-1} (n = 20; RSD 1.5%). The minimum detectable amount was 0.001 mg ml^{-1}.

To confirm the validity, MECC–DAD was applied to the analysis of *Helichrysum italicum* purified extracts, which represent a valid model, since they contain flavonoids from three different classes, i.e. flavonol (kaempferol-3-*O*-glucoside, K), flavanone (naringenin-4′-glucoside, N) and chalcone (4,2′,4′,6′-tetrahydroxychalcone-2′-glucoside, C).

Figure 5 shows a typical electropherogram [5(a)] and 'on-line' UV spectra [5(b)] of a *Helichrysum italicum* extract, where the three components of interest are readily identified by comparing migration times and UV spectra with those from relative standards. Peaks 1 and 2 showed 'on-line' UV spectra typical of chalcone derivatives, whereas the spectra of peaks 3 and 4 presented an UV profile characteristic of kaempferol and apigenin derivatives, respectively.

A peak purity check ('purity index' >990) confirmed the homogeneity of each peak.

From these results it may be concluded that the new integrated system for CE–DAD combines high separation capacity with a reproducible spectral information and represents a complementary technique to HPLC–DAD.

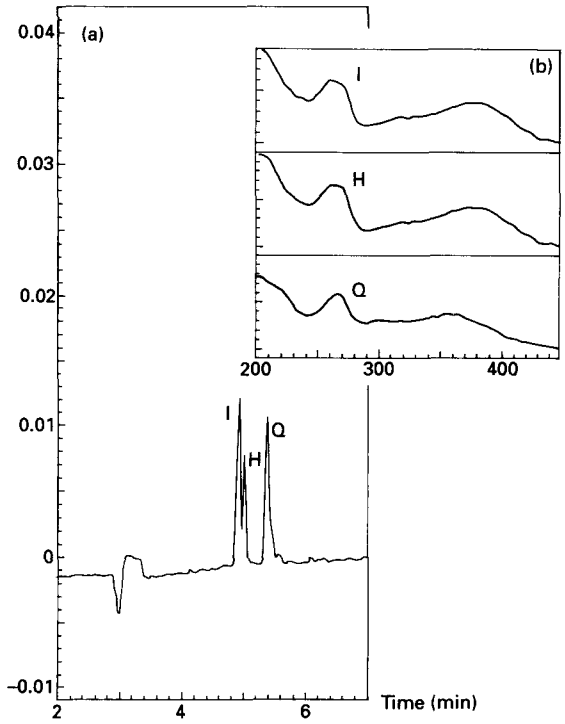


Figure 3
Separation (a) of quercetin-3-glucoside (isoquercitrin, I), quercetin-3-galactoside (hyperoside, H) and quercetin-3-rhamnoside (quercitrin, Q) and relative DAD spectra (b). For experimental conditions see Fig. 2.

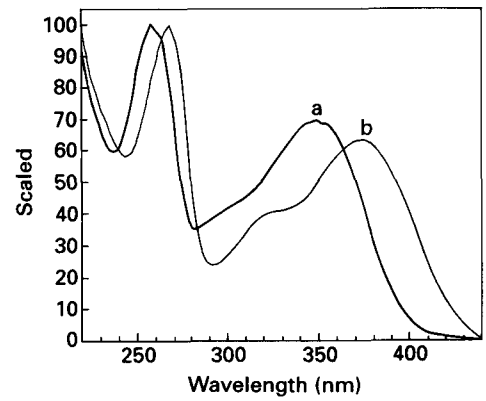


Figure 4
UV spectra of quercitrin (Q) (a) in HPLC–DAD eluent [2-propanol–tetrahydrofuran–water (10:5:85, v/v/v)] and (b) in MECC–DAD buffer [20 mM borate (pH 8.2) with 40 mM SDS].

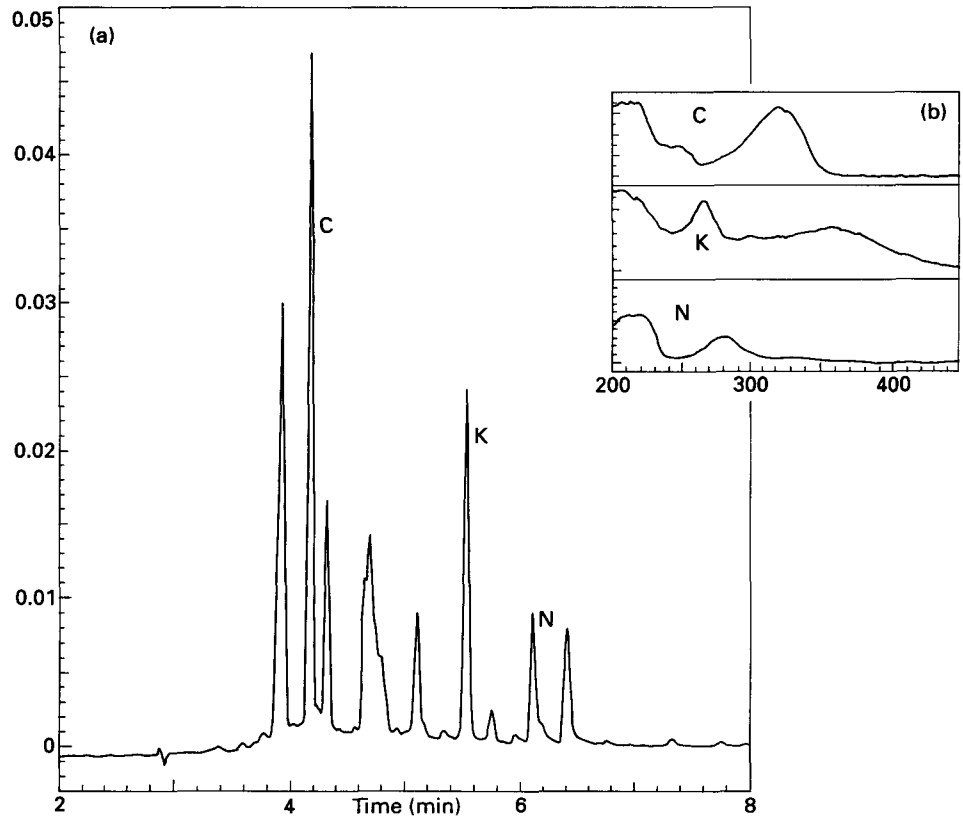


Figure 5
Typical electropherogram of a *Helichrysum italicum* purified extract (a) and MECC–DAD spectra (b). For peaks see Fig. 1 and for experimental conditions see Fig. 2.

Acknowledgements — Kontron Instruments (Milan, Italy) is thanked for providing Eureka 2000 CE-DAD apparatus.

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