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Identification of antioxidative compounds in plant beverages by capillary electrophoresis with the marker index technique

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Abstract

Two capillary zone electrophoretic methods were developed for the routine separation of flavonoids and phenolic antioxidative compounds. The analyses were optimized for reference compounds at pH values of 7.00 and 8.85, using a 30 m*M* mixture of sodium dihydrogen phosphate and/or disodium hydrogen phosphate as the electrolyte solution. Analytes were detected on-column with UV absorption at 220 nm. The methods were optimized using reference compounds, with the aim of obtaining good resolution of flavonoids and phenolic compounds in real samples. The repeatability of the analyses was improved by using triphenyl acetic acid and benzoic acid as marker compounds for calculating the indices of the analytes. The separation method optimized at pH 7.00 was applied to the analysis of extracts from *Eucommia ulmoides* olive leaves and the method optimized at 8.85 was applied to an untreated Bulgarian red wine. Plant leaves were pretreated in four different ways, i.e., by conventional digestion both with boiling water and a water–methanol mixture, by Soxhlet extraction with an acetone–dichloromethane mixture and by supercritical fluid extraction with carbon dioxide by collection into acetone. The marker index technique, using organic carboxylic acids, was used to give indices for the flavonoids and phenolic compounds and for identification of the analytes. It was noticed that the reliability of peak identification was generally enhanced if the analytes migrated between the marker compounds. High relative standard deviations [R.S.D.s (%)] between the electrophoretic mobilities calculated using the absolute migration times of the analytes in samples obtained using different sample pretreatment techniques were due to the difference in ionic strengths of the analysed samples. The selectivity of the method (pH 7.00) was calculated for the plant extracts. The detection limit for all of the antioxidative compounds was 3 pmol.

Keywords: *Eucommia ulmoides*; Wine; Sample handling; Flavonoids; Phenolic compounds; Antioxidants

1. Introduction

Flavonoids were first identified in the pigments responsible for the yellow, orange and red colours of flowers, but lately also from fruits, green tea, red wine, green vegetables, seeds, flowers and berries. In plants, they are secondary metabolites with important pharmacological, physiological and ecological effects [1–5]. As antioxidants, they inhibit oxidation in fats and, accordingly, the pharmaceutical, food, polymer and rubber industries employ them as additives. Flavonoids seem to protect tissues against the damage caused by free radicals and possess anti-allergenic, anti-inflammatory, anti-viral (quercetin, rutin) and anti-carcinogenic activities.

Phenolic compounds also inhibit the oxidative degradation of food products and are used for this purpose by the food industry. They also have been shown to inhibit the mediators of inflammation. Typically, the reactive chemical moiety in both

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natural and synthetic phenolic antioxidants is an aromatic ring with at least one hydroxyl group [6].

The most widely used methods for the separation of flavonoids and phenolic compounds are thin-layer, liquid and gas chromatography [6–8]. More recently, capillary electrophoresis (CE) has been applied [9,10]. Its selectivity, short analysis time, small sample volumes and on-line concentration possibilities makes CE an extremely promising method [11–14]. Usually, the migration order of the analytes depends on the ratio of their molecular masses and charges. However, the separation efficiency is very much dependent on the concentration of the individual analytes and on the total concentration of all compounds in the sample. For this reason, analysis of real samples requires either an internal standard or marker compounds to identify them. Marker compounds are used to measure the real electrophoretic mobilities of the analytes and to identify them through the assigning of indices.

In this work, we optimized capillary zone electrophoresis (CZE) methods for the separation of flavone, rutin, quercitrin, chlorogenic acid, ferulic acid, caffeic acid and protocatechuic acid at pH 7.00 and for the separation of rutin, D-catechin, quercetin, epicatechin and myricetin at pH 8.85. The method at pH 7.00 was applied to the separation of antioxidants extracted from leaves of the tree *Eucommia ulmoides* olive, the source of a traditional Chinese medicine that has been famous since the Ming dynasty [15,16]. Its usefulness in treating hypertension is well known. Previously, only the bark of *Eucommia ulmoides* olive was thought to contain the medicinally effective compounds, but lately, interest has focused on the leaves, as the bark of the *Eucommia ulmoides* olive tree can only be peeled after twenty years and after that the tree will probably die. Chlorogenic acid in the leaves has strong antibiotic properties and the antibiotic and anti-inflammatory effects are indirectly associated with the effect on hypertension. The leaves of *Eucommia ulmoides* olive were pretreated by digestion in boiling water or water–methanol or by extraction with Soxhlet equipment or under supercritical conditions. In a second application, the method at pH 8.85 was used to separate the antioxidative compounds in red wine. In this case, no pretreatment step was employed.

2. Experimental

2.1. Chemicals

The flavonoids and phenolic compounds (Fig. 1) were purchased from the Division of Pharmacognosy (University of Helsinki, Department of Pharmacy, Helsinki, Finland) and the Alcohol Testing Laboratory (Alko, Helsinki, Finland). Chlorogenic acid, ethacrynic acid and the two carboxylic acids used as marker compounds, triphenyl acetic acid (TFA) and benzoic acid (BA), were from Sigma (St. Louis, MO, USA). Methanol, 2-propanol, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium tetraborate decahydrate, tris(hydroxymethyl)-aminomethane (Tris), acetone and dichloromethane were of HPLC grade and were from Merck (Darmstadt, Germany). Mandelic acid was from EGA-Chemie (Steinheim, Germany) and *meso*-2,3-diphenylsuccinic acid was from TCI (Japan). 3-[N-Morpholino]propanesulfonic acid (MOPS), 1,3-diamine, lauryl sulfate (SDS) and β-cyclodextrin were from Sigma. 3-(Cyclohexylamino)-1-propanesulfonic acid (CAPS) was from Sigma (Poole, UK). Sodium hydroxide (0.1 *M*) was prepared from Titrisol (Merck). Powdered leaves from the *Eucommia ulmoides* olive tree were from Northwestern College of Forestry (Yangling, Shaanxi Province, China). Distilled water was further purified with a Water-I instrument using 0.2 μm filters (Gelman Sciences, Ann Abor, MI, USA). All chemicals were of analytical grade.

2.2. Apparatus

Two capillary electrophoresis systems were used: A Beckman 2050 P/ACE System 2000 (Beckman Instruments, Fullerton, CA, USA) for the leaf extracts and a Hewlett-Packard 3D system for the wine. In the Beckman instrument, the total length of the capillary (Composite Metal Services, The Chase, Worchester, UK) was 87 cm (80 cm to the detector)×50 μm I.D., 360 μm O.D.). The applied voltage was +30 kV and the current was 16.6 μA. Injection was made under pressure (5 s, 35 mbar). During separations, the temperature was kept at +25°C. The analytes were detected at 220 nm.

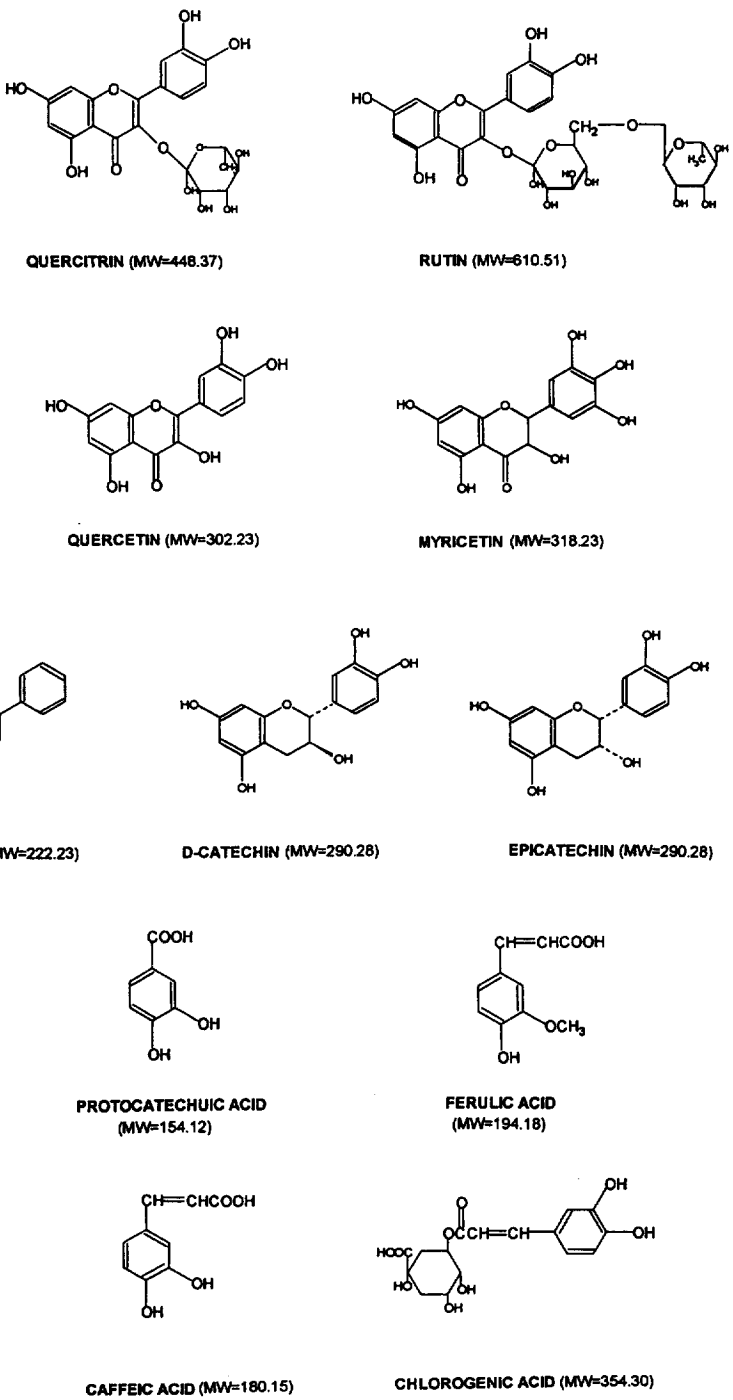

Fig. 1. Structures of flavonoids and phenolic compounds.

The Hewlett-Packard 3D instrument was used for the separation of flavonoids in red wine. A diode array detector (DAD) was employed for identification of the analytes at 220 and 380 nm. The total length of the capillary (Composite Metal Services) was 67 cm (60 cm to the detector)×50 μm I.D.×360 μm O.D. The applied voltage was +20 kV. Injection was made under pressure (5 s, 50 mbar). During separations, the temperature was kept at +22°C.

Before use, all capillaries were washed for 15 min with 0.1 *M* NaOH (for the Hewlett-Packard set-up) and with 0.1 *M* KOH (for the Beckman instrument set-up) and finally for 15 min with water.

Supercritical fluid extractions (SFE) were performed with a Suprex Prep Master (Suprex, Pittsburgh, PA, USA) using a Suprex 5 ml extraction vessel. A 10-cm linear fused-silica capillary with a 30-μm I.D. (Composite Metal Services) was used as a restrictor and was connected to the outlet capillary with a 1/16 in. Swagelok (1 in.=2.54 cm). Carbon dioxide pressurized with helium (Air Products and Chemicals, Allentown, PA, USA) was used as the supercritical fluid. The pressure during extractions was 40.5 MPa and the temperature was 120°C.

2.3. Preparation of electrolytes for CZE

The electrolyte solutions for preliminary CZE work were prepared using disodium hydrogen phosphate and sodium dihydrogen phosphate, borate, MOPS, CAPS or Tris, which were of different concentrations and pH values, depending on the buffer capacity in the neutral and basic regions. Borate, phosphate and MOPS buffers were also modified with β-cyclodextrin, SDS, 1,3-diamine or 2-propanol, or with some mixtures of these.

Phosphate buffers were selected for optimization experiments. The final electrolyte solutions for the optimized conditions were a mixture of 30 m*M* sodium dihydrogen phosphate and 30 m*M* disodium hydrogen phosphate for work at pH 7.00 and 30 m*M* disodium hydrogen phosphate for work at pH 8.85.

The pH of the solutions was determined using a Jenway 3030 pH meter by using a combination electrode filled with 3 *M* KCl. Before use, the buffer solutions were filtered and degassed in an ultrasonic bath.

2.4. Preparation of samples

Flavonoids and phenolic compounds were isolated from the dried and ground leaves of *Eucommia ulmoides* by four methods: (**1**) Analytes were extracted with a mixture of boiling water–methanol (30:70, v/v) for 30 min and thereafter at ambient temperature for 12 h. (**2**) Analytes were extracted with boiling water alone, as in step 1. (**3**) Analytes were isolated from 3.5 g of leaves by Soxhlet extraction with acetone–dichloromethane for 20 h. (**4**) Supercritical fluid extraction (SFE) was applied to 1.0 g of the powder that had been modified with methanol–water (2:1, v/v). SFE was applied at 40.5 MPa and 120°C, with CO_2 being used as the supercritical fluid at a flow-rate of 1 ml/min. The extraction was done for 15 min in static mode and then for 25 min in dynamic mode. Extracts were collected into 3.5 ml of acetone, placed in a 7.5 ml glass screw-top vial. All extracts were filtered and marker compounds were added to the filtrate, which was used as the final sample.

The red wine samples (Oriachovitza Cabernet Sauvignon Reserve, 1990, Bulgaria) were directly injected into the instrument, with no pretreatment.

2.5. Marker index technique

The marker technique was developed for high-accuracy identification by CZE. The markers, carboxylic acids of known electrophoretic mobilities, are used under neutral and basic conditions to determine the effective field strength and electroosmotic flow velocity of the system, which then allow calculation of the electrophoretic mobilities of the analytes [17]. However, the marker technique cannot be used in micellar electrophoretic systems because of the partition of the markers into the micelles. To overcome this problem, a migration index technique was developed that replaces the electrophoretic mobilities of the markers with indices [18]. The marker index technique is also more reliable in CZE where complex multicomponent matrices of high ionic strength are to be analyzed.

We used TFA and BA as marker compounds in the test mixture and real samples, to improve the reliability of the CZE method. The migration times of the analytes, electroosmotic velocities and migra-

tion times of the markers and the effective length of the capillary were used to determine the migration indices of the analytes under the separation conditions. The results were calculated with laboratory-designed programs operating in MATLAB (Mathworks, USA) [18].

The migration index of TFA is set at 1000 (Ind1) and the migration index of BA (Ind2) is calculated from Eq. (1), for each CZE analysis, separately:

$$\mathrm{Ind2} = \mathrm{Ind1}(t_{eo}/t_2 - 1)/(t_{eo}/t_1 - 1) \quad (1)$$

The indices for the analytes (Ind_x) are calculated from Eq. (2):

$$\mathrm{Ind}_x = [t_1 t_2(\mathrm{Ind1} - \mathrm{Ind2}) - t_x(\mathrm{Ind1}\,t_1 - \mathrm{Ind2}\,t_2)]/t_x(t_2 - t_1) \quad (2)$$

where Ind1 and Ind2 are the indices of the marker compounds TFA and BA, respectively, t_1 and t_2 are their respective migration times and t_{eo} is the electroosmotic migration time.

The reliability of the identification was evaluated for the analytes in the standard mixture using Eq. (3).

$$Q_{id} = (x_2 - x_1)/(\sigma_1 + \sigma_2) \quad (3)$$

where values x_1 and x_2 are the responses of interest, and σ_1 and σ_2 are their standard deviations. The identification is reliable when Q_{id} is greater than two.

3. Results and discussion

Several inorganic and organic buffers were tested in the search to find an electrolyte solution that was optimal for the CZE separation of flavone, rutin, quercitrin, chlorogenic acid, ferulic acid, caffeic acid, protocatechuic acid, D-catechin, epicatechin, quercetin and myricetin. The pH values of the electrolyte solutions were in the neutral or basic region. Because of the identical migration times of some analytes, two different methods were needed to achieve baseline separation for all peaks in the electropherogram. Different phosphate buffers were chosen for the final electrolyte solutions. The optimization was completed by testing the instrumental parameters and sample injection.

Analysis of the compounds by CZE was validated for the separation of the antioxidants at both pH 7.00 and pH 8.85. The neutral and moderately basic pH range was chosen to minimize the dissociation of the glucose-conjugated analytes, rutin and quercitrin. The method at pH 8.85 was developed especially for the red wine sample, since selectivity for its numerous compounds was better at a higher pH value. In general, the migration times at pH 7.00 (Fig. 2) increased with polarity, molecular mass and the degree of dissociation of the analytes, and the migration order was flavone>rutin>quercitrin>chlorogenic acid>ferulic acid>caffeic acid>protocatechuic acid. The migration order at pH 8.85 was D-catechin > epicatechin > rutin > quercetin > myricetin.

The plant samples were pretreated for isolation of the flavonoids and phenolic compounds. The solvents used for the conventional techniques (refluxing,

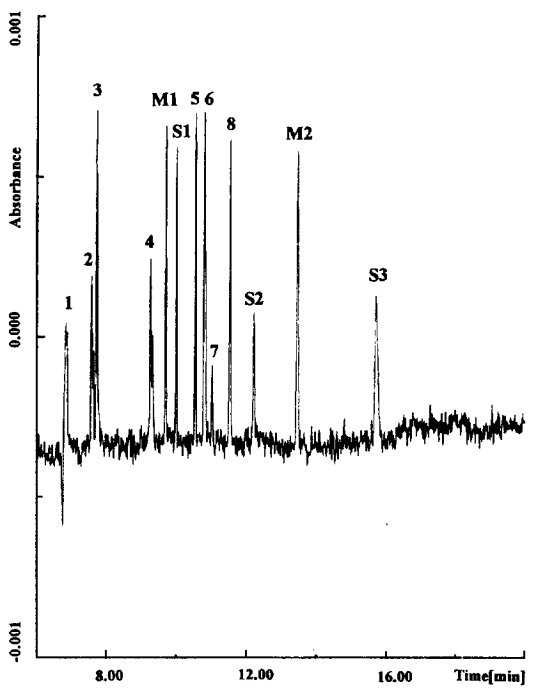

Fig. 2. Electropherogram obtained from a standard mixture (pH 7.00). Peaks: (1) flavone, (2) rutin, (3) quercitrin, (4) chlorogenic acid, (5) ferulic acid, (6) caffeic acid-1, (7) caffeic acid-2, (8) protocatechuic acid, (S1) ethacrynic acid, (S2) mandelic acid, (S3) *meso*-2,3-diphenylsuccinic acid, (M1) triphenyl acetic acid and (M2) benzoic acid.

Table 1
Plate numbers for the analytes studied in a phosphate electrolyte solution at pH 7.00

Compounds	Plate numbers/80 cm
Flavone	77 700
Rutin	51 100
Quercitrin	53 100
Chlorogenic acid	141 700
Ferulic acid	221 930
Caffeic acid	494 200
Protocatechuic acid	264 900

digestions) were chosen on the basis of the solubility of the analytes in the solvent. Bioflavonoids are usually extracted with hot, aqueous alkaline solvents or with water-miscible organic solvents, such as isopropanol [19]. Since *Eucommia ulmoides* olive leaves are used as tea material, we designed the digestion steps to simulate this use. We also took note of the fact that caffeic acid occurs in plants only in conjugated forms, such as chlorogenic acid, and chlorogenic acid forms caffeic acid in alkaline solutions. Soxhlet extraction with acetone–dichloromethane (90:10, v/v) was tested as a means of extraction recovery from the matrix, and SFE was tested as a new method for isolating the flavonoids and phenolic compounds.

The separation methods were optimized for flavone, rutin, quercitrin, chlorogenic acid, ferulic acid, caffeic acid and protocatechuic acid standards at pH 7.00 (Table 1). The repeatability of the separation, based on absolute migration times of the analytes in the electrolyte solutions, was improved by adding markers to the sample and calculating the results with MATLAB (Table 2). The marker index technique, which is a particularly valuable tool for complex matrices like our plant materials, with high concentrations of organic, hydrophilic and hydrophobic compounds, provided highly accurate identifications in CZE. The marker compounds, TFA and BA, were used to calculate the indices for the analytes in extracts. The analytes found in both water and water–methanol extracts were rutin, chlorogenic acid, ferulic acid and caffeic acid (Fig. 3); rutin, chlorogenic acid, caffeic acid and protocatechuic acid, in the SFE extract and rutin, chlorogenic acid and caffeic acid in the Soxhlet extract (Fig. 4). Benzoic, mandelic and *meso*-2,3-diphenylsuccinic acids were used as internal standards to calculate the relative migration times of the analytes. The supercritical fluid extract also contained chlorophylline. The analytes found in red wine samples were D-catechin, epicatechin, rutin, quercetin and myricetin (Fig. 5). Tables 3–5 show how the marker indices improved the repeatability of the analyses. The standard deviations for the migration indices of the reference compounds were less than 1% for compounds from quercitrin to protocatechuic acid, and were only higher for flavone and rutin. Evidently, a third marker compound would be in order, since it is preferable that the analytes should migrate between the marker compounds, or very near them. Our marker compounds arrived at the detector in the region of phenolic compounds, allowing excellent repeatabilities. The results also showed that the more the marker compounds resemble the analytes, the more repeatable is the separation.

When the standard mixtures were screened for analytes through the use of the absolute migration times, the relative standard deviations (R.S.D.s)

Table 2
Repeatability of the analysis in terms of absolute migration times and migration indices for the flavonoids and phenolic compounds. Intra-day results with nine replicates for the reference mixture

Compound	t_{abs} (av.), min	SD	R.S.D. (%)	Ind (av.)	SD	R.S.D. (%)
Flavone	6.89	0.053	0.77	47.5	6.52	13.73
Rutin	7.61	0.077	1.02	358.2	8.77	2.449
Quercitrin	7.56	0.069	0.89	413.0	3.62	0.877
Chlorogenic acid	9.30	0.094	1.01	892.9	1.41	0.158
Ferulic acid	10.58	0.118	1.11	1184.2	0.609	0.051
Caffeic acid-1	10.83	0.126	1.17	1231.6	0.766	0.062
Caffeic acid-2	11.06	0.132	1.20	1274.7	1.253	0.098
Protocatechuic acid	11.57	0.143	1.23	1364.5	0.847	0.062

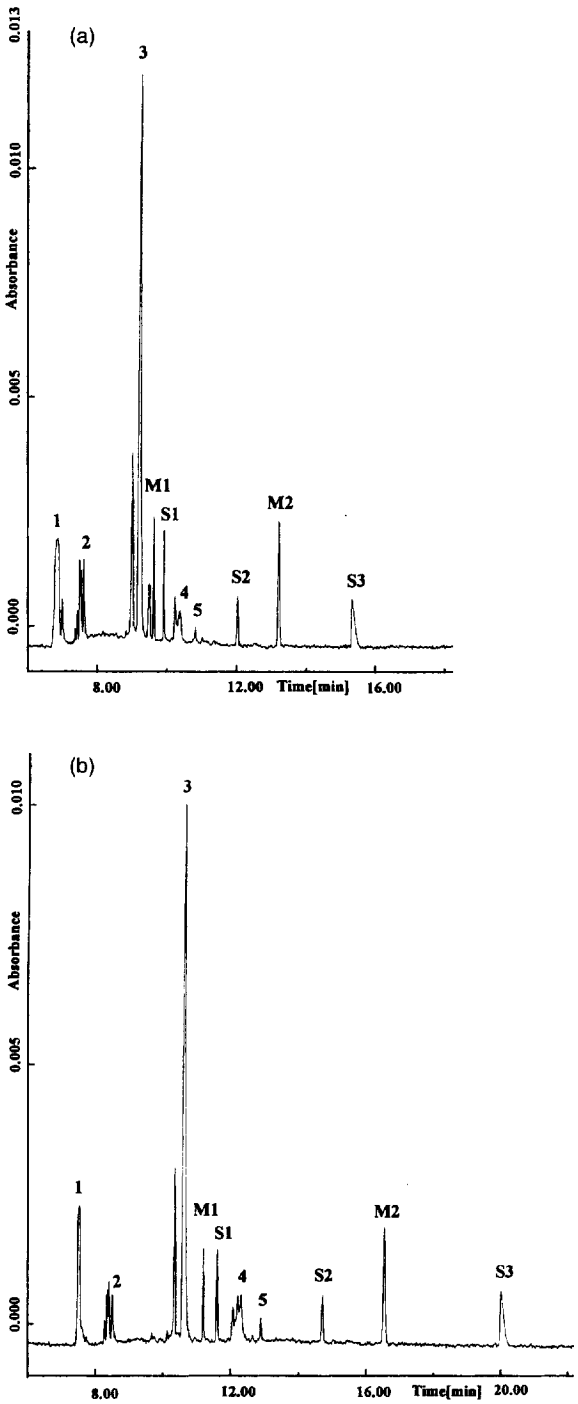

Fig. 3. Electropherograms of (a) methanol–water and (b) water extracts of the leaves of *Eucommia ulmoides* olive. Compounds: (1) flavone, (2) rutin, (3) chlorogenic acid, (4) ferulic acid and (5) caffeic acid-2. Marker compounds and internal standards as in Fig. 2.

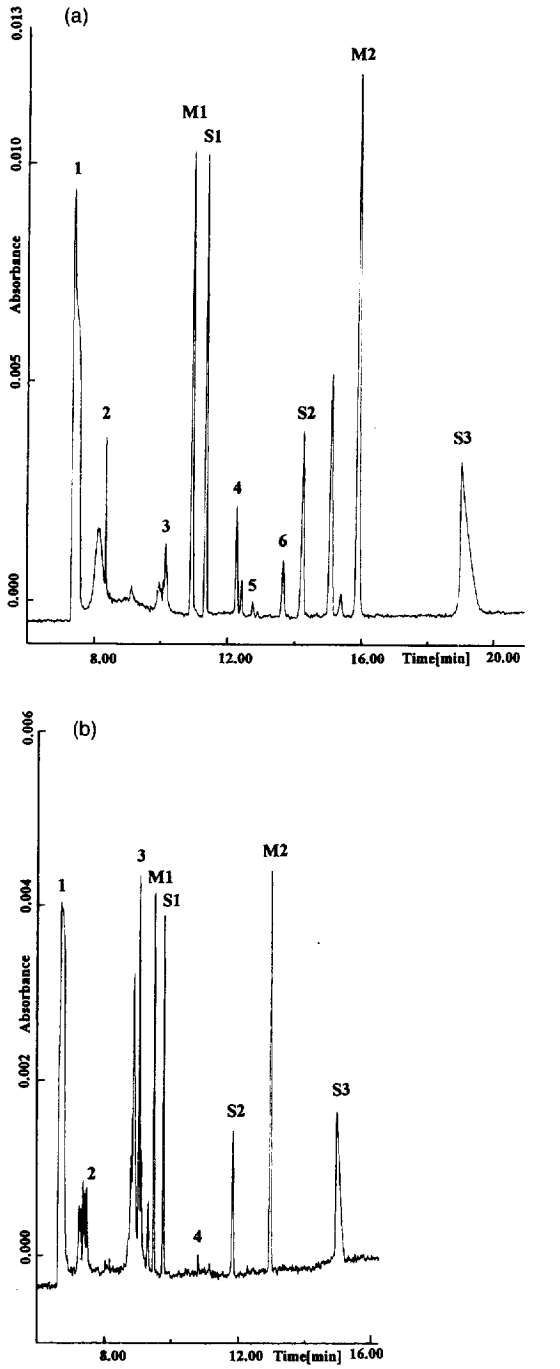

Fig. 4. Electropherograms of (a) SFE and (b) Soxhlet extracts of the leaves of *Eucommia ulmoides* olive trees. Compounds: (a) (1) flavone, (2) rutin, (3) chlorogenic acid, (4) caffeic acid-1, (5) caffeic acid-2 and (6) protocatechuic acid, (b) (1) flavone, (2) rutin, (3) chlorogenic acid and (4) caffeic acid-1. Marker compounds and internal standards as in Fig. 2.

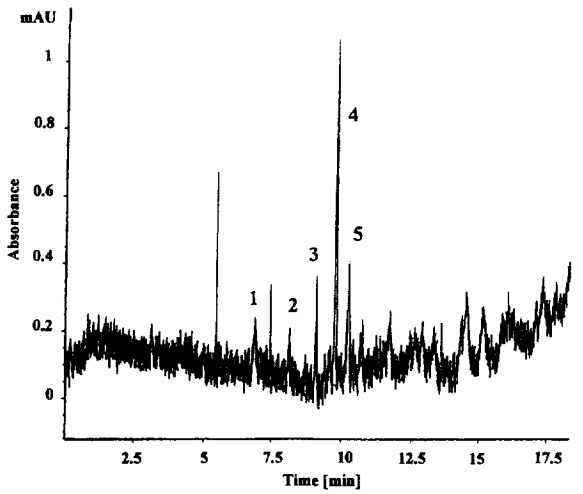

Fig. 5. Electropherogram of red wine. Compounds: (1) D-catechin, (2) epicatechin, (3) rutin, (4) quercetin and (5) myricetin.

varied from 1.5 to 2.6%. However, the values increased with real samples because of the increase in ionic strength (total concentration of all compounds in the sample). For incompletely dissociated flavonoids, the R.S.D. values were between 5 and 7% at pH 7.00, and for D-catechin and epicathechin, they were even higher, due to partial dissociation of the compounds or the immiscibility of the analyte with the buffer. The values of deprotonation (pK_a) of the 3-hydroxy group are 4.6 and 4.3 for catechin and rutin, respectively. In addition, the dissociation constants (pK_1) for quercetin and rutin are 6.74 and 7.1, respectively [20]. The pK_a values of the flavonoids differed by less than two units from the pH value of the electrolyte solution, which has been suggested to be the ideal condition for baseline separation and for use of the marker technique.

Our calculations confirmed the existence of flavone, chlorogenic acid and caffeic acid in all extracts and of protocatechuic acid in the SFE extract. However, the concentrations of flavone and the other neutral compounds comigrated with EOF were very high. Therefore, the identification of flavone in the extracts was excluded. *meso*-2,3-Diphenylsuccinic acid was used as a standard to estimate the concentration level of the sample, since its migration time increased with increasing total concentration. Table 6 shows the electrophoretic mobilities of the analytes isolated from the matrices. The R.S.D. values of the μ_{ep} from sample-to-sample are very low for rutin and *meso*-2,3-diphenylsuccinic acid (Table 7).

Table 3
Repeatability of the analysis in terms of migration indices for the flavonoids and phenolic compounds extracted from *Eucommia ulmoides* olive leaves (water–methanol and water extracts)

Peak in	Water–methanol			Water		
electropherogram	Ind (av.)[a]	SD	R.S.D.(%)	Ind (av.)	SD	R.S.D. (%)
Rutin	346.1	4.63	1.337	351.8	4.91	1.395
Chlorogenic acid	897.0	2.27	0.253	893.8	0.63	0.071
Ferulic acid	1183.0	2.54	0.214	1185.5	0.68	0.058
Caffeic acid-2	1278.9	2.83	0.221	1268.7	0.60	0.047

[a]Ind (av.) = average migration index from nine replicates, see Eqs. (1) and (2).

Table 4
Repeatability of the analysis in terms of migration indices for the flavonoids and phenolic compounds extracted from *Eucommia ulmoides* olive leaves (SFE and Soxhlet extractions)

Peak in	SFE			Soxhlet extraction		
electropherogram	Ind (av.)[a]	SD	R.S.D. (%)	Ind (av.)[a]	SD	R.S.D. (%)
Rutin	345.8	51.76	14.96	352.4	6.37	1.806
Chlorogenic acid	837.0	0.50	0.060	883.9	0.93	0.105
Caffeic acid-1	1222.2	1.85	0.152	–	–	–
Caffeic acid-2	1243.8	2.12	0.171	1298.7	2.65	0.204
Protocatechuic acid	1410.4	3.38	0.240	–	–	–

Table 5
Repeatability of the analysis in terms of migration indices for the flavonoids and phenolic compounds in a red wine sample

Compound	t_{abs} (av.), min	SD	R.S.D. (%)	Ind (av.)[a]	SD	R.S.D. (%)
D-catechin	8.78	0.093	1.06	130.9	9.84	7.510
Epicatechin	9.14	0.555	6.06	206.1	5.89	2.860
Rutin	10.62	0.393	3.70	668.4	2.93	0.439
Quercetin	11.71	0.129	1.11	941.2	2.27	0.241
Myricetin	12.17	0.126	1.04	1033.1	0.65	0.063

[a]Ind(av.)=average migration index, see Eqs. (1) and (2).

Table 6
Electrophoretic mobilities of the analytes in four *Eucommia ulmoides* olive leave extracts

Compound	Standard mixture μ_{ep} (10^{-8} m^2 V^{-1} s^{-1})	Methanol–water μ_{ep} (10^{-8} m^2 V^{-1} s^{-1})	Water μ_{ep} (10^{-8} m^2 V^{-1} s^{-1})	SFE μ_{ep} (10^{-8} m^2 V^{-1} s^{-1})	Soxhlet μ_{ep} (10^{-8} m^2 V^{-1} s^{-1})
Rutin	−0.6144	−0.5484	−0.5953	−0.6084	−0.5217
Quercitrin	−0.7115	–	–	–	–
Chlorogenic acid	−1.5447	−1.4434	−1.5199	−0.8237	−1.4958
Ferulic acid	−2.0507	−1.9069	−2.0157	–	–
Caffeic acid-1	−2.1324	–	–	−1.4831	–
Caffeic acid-2	−2.2074	−2.0421	−2.1579	−1.6015	−2.1971
Protocatechuic acid	−2.3652	–	–	−1.8041	–

Table 7
Repeatability of electrophoretic mobilities in inter-day assays of *Eucommia ulmoides* olive samples ($n=9$)

Compounds	μ_{ep} (10^{-8} m^2 V^{-1} s^{-1})	SD	R.S.D. (%)
Rutin	−5.7762	0.3675	6.36
meso-2,3-Diphenylsuccinic acid	−3.2026	0.0850	2.65

CZE provided good separation of antioxidative compounds in extracts from the medicinal plant, *Eucommia ulmoides* olive, and from the red wine sample. On addition of the two marker compounds, the identification (Q_{id}) was accurate (Table 8). The reliability was increased when the analytes migrated between the marker compounds. However, the standard deviations of the indices were high when the analytes migrated before the first and after the last marker compound. The standard deviations were

Table 8
Values of the coefficients for identification (Q_{id}) obtained for successive peak pairs. The responses are absolute migration times and migration indices

Pair of compounds	Absolute migration times Q_{id}(Ind)	Migration indices Q_{id}(Ind)	Ratio Q_{id}(Ind)/Q_{id}(abs)
Flavone–rutin	0.408	19.2	47
Rutin–quercitrin	0.079	16.4	209
Quercitrin–chlorogenic acid	0.811	464	572
Chlorogenic acid–ferulic acid	0.604	1394	2308
Ferulic acid–caffeic acid-1	0.110	419	3826
Caffeic acid-1–caffeic acid-2	0.097	369	2776
Caffeic acid-2–protocatechuic acid	0.210	561	2674

especially large when the ionic strength of the sample after pretreatment was high.

4. Conclusions

Flavonoids and phenolic compounds were separated by CZE at pH values of 7.00 and 8.85 using a 30-m*M* phosphate solution, which provided the electrolyte. Marker techniques were developed for high-accuracy identification. One marker compound of known index value and another with a calculated value were used to determine indices for the analytes. Use of the two markers improved the repeatability of the analysis, especially for phenolic compounds. The method was excellent for screening flavonoids and phenolic compounds from *Eucommia ulmoides* olive leaves after refluxing and digestion, Soxhlet extraction or supercritical fluid extraction. The red wine samples were analysed without clean-up steps.

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