Critical Assessment of Electrolyte Systems for the Capillary Electrophoresis Analysis of Phenolic Compounds in Herbal Extracts

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Abstract: This work presents a comparative evaluation of electrolyte systems for the capillary electrophoresis analysis of the phenolic compounds apigenin and luteolin, and their corresponding 7-*O*-glucosides naringenin, rutin, quercetin, umbelliferone, herniarin, chlorogenic, and caffeic acids, in methanolic, glycolic, and hydroalcoholic extracts of *Matricaria recutita* L. (Asteraceae). The electrolytes included tetraborate buffer at different pHs and concentrations, containing varied amounts of sodium dodecyl sulfate, β-cyclodextrin, and acetonitrile. The electrophoretic profile of the extracts changes considerably from electrolyte to electrolyte. However, in a single electrolyte, the profile of different extracts seems to be very similar (identical elution order), varying only in relative composition. The best condition for quantitative work was 20 mmol/L tetraborate buffer, pH 10, using direct detection at 337 nm. Baseline resolution of 9 among 11 selected standards was achieved readily in this electrolyte, showing distinct migration patterns for flavonoid aglycones (flavones, dihydroflavonols, and flavonols), coumarins, and acidic phenylpropanoids. To assure reproducibility, several capillary conditioning procedures were tested. Electrokinetic rinses with tetraborate buffer prior to sample injection gave the best results. Precision of migration times and peak areas were better than 4 and 2%, respectively, for 10 consecutive injections of the methanolic extract. A few method validation parameters also are reported, such as linearity ($r^2 < 0.999$, concentration range from 5.0 to 75.0 μg/mL), limit of detection (3.8 μg/mL), and limit of quantitation (11.5 μg/mL), referred to apigenin, a commonly used compound to standardize chamomile extracts for topical use. Flavonoid glucosides that have distinct sugar moieties were separated readily in free solution; however, those with similar sugar moieties were separated only in micellar medium, using β-cyclodextrin as the additive. The presence of reference compounds in the extracts was confirmed by spiking techniques and their identification was assisted by a UV-spectra library. The validity of the diode array detector as an identification tool for phenolic compounds also is discussed. J Micro Sep 13: 227–235, 2001

Key words: *Matricaria recutita; herbal extracts; capillary electrophoresis; micellar electrokinetic chromatography*

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INTRODUCTION

Chamomile is the name commonly used to designate two distinct species: the Roman or English chamomile (*Chamaemelun nobile* L. All; synonym *Anthemis nobilis* L.) and the German or Hungarian chamomile (*Matricaria recutita* L.; synonym *Chamomilla recutita* (L.) Rausch; synonym *Matricaria chamomila* L.). All these species belong to the Asteraceae family [1,2]. These plants are native to Europe and Western Asia, but they have been introduced into North America and Australia as well as in a few countries in South America, such as Argentina and Brazil [2,3].

Chamomile has been used abundantly throughout the history of humanity. The British Herbal Pharmacopoeia [3] suggests the use of German chamomile as an anti-inflammatory, spasmolytic, mild sedative, and carminative. Externally, chamomile is used for conditions that affect the skin and mucous membranes [4,5]. The anti-inflammatory activity of *Matricaria recutita* L. extracts is now well established. For a long period of time, this activity was attributed to essential oils, more specifically to chamazulene, bisabolol, and enyne-dicycloether [6]. However, Della Loggia et al. [7] observed that the plant predominantly is used as infusions and hydroalcoholic extracts, i.e., in extract forms that are poor in lipophilic compounds and rich in hydrophilic compounds, such as flavonoids, coumarins, and phenylpropanoids. Therefore, it was suggested that the flavonoids present in hydroalcoholic extracts are responsible for the anti-inflammatory action in topical applications [4,7–10].

In addition to the therapeutic use of herbs and their extracts, the demand for cosmetic products for skin care and hair treatment based on herbal extracts has increased considerably. Due to the complex nature of these extracts, it is a common practice to select sets of standards or marker compounds, characteristic of a particular plant, and to evaluate their concentration in the extract as an indicator of product quality during industrial processing and storage. Another benefit of the analytical evaluation of herbal extracts is to monitor adulterations in raw materials and final products.

The quantitative assessment of herbal extracts, especially those used in cosmetology, demands well established techniques for separation and analysis of a variety of naturally occurring classes of compounds in the presence of product additives. High-performance liquid chromatography (HPLC) has replaced thin layer chromatography as the technique of choice for the analysis of herbal extracts. The literature that describes methodologies for a variety of herbal extracts in hydroalcoholic media is vast [11–18]. For herbal extracts for topical use, however, where large amounts of propyleneglycol, water, alcohol, and glycerine, in addition to preservatives, are incorporated during the extraction process, the analysis is a bit of a challenge. Due to the complexity of such matrices, or perhaps due to industrial confidentiality, the literature regarding analytical methodologies for herbal extracts for topical use is far from comprehensive.

In the past 10 years, capillary electrophoresis (CE) has been introduced for analysis of secondary plant metabolites. The technique is very versatile and several modes can be implemented to analyze cationic and anionic compounds as well as neutral and hydrophobic molecules selectively. Phenolic compounds, such as flavonoids, coumarins, phenylpropanoids, and phenolic acids, as well as alkaloids and terpenes all have been characterized by this technique [19,20]. The presence of ionic moieties in conjunction with neutral organic groups in phenolic compounds makes them eligible for analysis in both free solution and micellar medium capillary electrophoresis. Due to their distinct separation mechanisms, both techniques usually offer complementary information.

Pieta et al. [21] published the first work that describes the CE separation of flavonoids. Since then, a great number of publications have appeared, reporting the analysis of phenolic compounds by both free solution capillary electrophoresis (FSCE) and micellar electrokinetic chromatography (MEKC) [19]. Several herbal extracts have been characterized, but to our knowledge, the composition of *Matricaria recutita* L. has never been investigated by capillary electrophoresis.

This work describes the CE analysis, in both free solution and micellar medium modes, of apigenin and luteolin, and their corresponding 7-*O*-glucosides, naringenin, rutin, quercetin, umbelliferone, herniarin, chlorogenic, and caffeic acids in methanolic, glycolic, and hydroalcoholic extracts of *Matricaria recutita* L. (Asteraceae). These compounds, whose structures are depicted in Figure 1, were chosen by taking into consideration their pharmacological activity as well as their widespread use in cosmetic applications [22–41].

EXPERIMENTAL

Reagents and solutions: Reference compounds. All reagents were of analytical grade; solvents were of chromatographic purity. β-Cyclodextrin was obtained from Aldrich (St. Louis, MO) and sodium dodecylsulfate (SDS) was obtained from Riedel-Haën (Seelze, Germany). Buffer electrolyte solutions (compositions specified in the figure captions) were prepared fresh daily and filtered through a 0.22

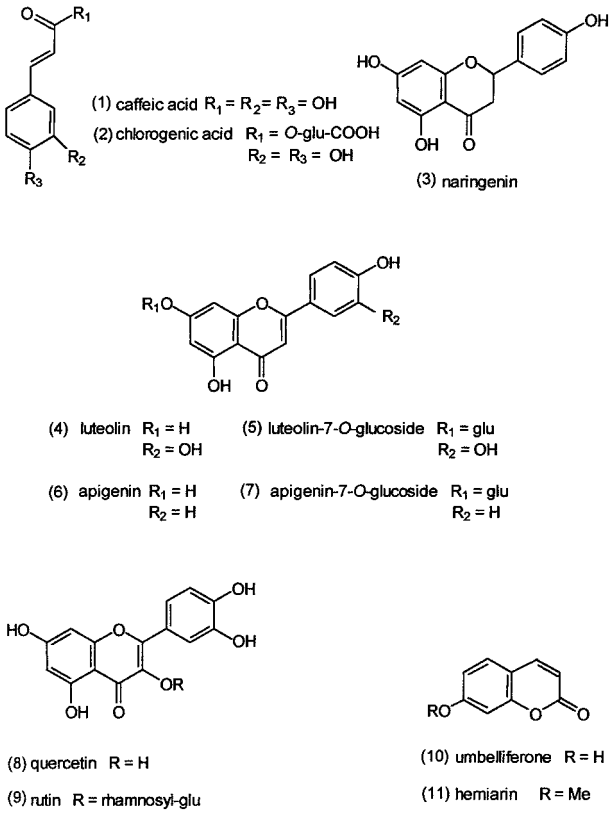

Figure 1. *Structures of the phenolic compounds studied in this work.*

μm membrane filter (Millipore, Pittsburgh, PA) just prior to use. Standard compounds 1–4 and 8–10 (Figure 1) were obtained from Sigma (St. Louis, MO), compounds 5–7 were purchased from Extrasynthese (Genay, France), and compound 11 was purchased from Fluka (St. Louis, MO). Stock solutions of the standards in 10:90 Me_2SO:MeOH at a concentration of 1 mg/mL were prepared every other week and stored in a freezer. A mixture containing 100 μL of each standard was prepared, resulting in a final concentration of 0.091 mg/L of each individual standard.

Samples. *Matricaria recutita* L. (Asteraceae) was obtained from an experimental field (SEARA, Jacupiranga, SP, Brazil). Planting was conducted in April 1998 and harvesting occurred in the following months of July and August. Floral heads of *Matricaria recutita* L. were collected and dried in an air-forced oven (40°C), until constant weight was achieved. About 16 g of the dried material were placed into 400 mL of 1:1 MeOH:H_2O and submitted to ultrasonic bath for 1 h. This procedure was repeated three times and all recovered fractions were filtered and evaporated completely under vacuum. The residue was suspended in 24 mL of MeOH, diluting 10 mL of this solution with 20 mL of H_2O to give a stock solution. Exactly 2.5 g of the stock solution was diluted in 10 mL 1:1 MeOH:H_2O. Hydroalcoholic and glycolic extracts were obtained from Farma Service Ind. Farmacêutica Ltda (São Paulo, SP, Brazil) and diluted appropriately in 1:1 MeOH:H_2O (2.5 g extract to 10 mL solvent). Approximately 10% (w/w) of floral heads was used in the preparation of these industrial extracts. In the glycolic extract, the solvent is constituted of propylene glycol (92.7% w/w), glycerine (2% w/w), and ethanol (5% w/w); in the hydroalcoholic extract, a 70% ethanolic–aqueous solution was employed. In both cases, a mixture of methylparaben (0.2% w/w) and propylparaben (0.1% w/w) was used as a preservant.

Instrumentation. All experiments were conducted in a capillary electrophoresis system (model HP^{3D} CE, Agilent Technologies, Palo Alto, CA) equipped with a diode array detector set at 337 nm, a temperature control device maintained at 25°C, and data acquisition and treatment software (HP ChemStation, rev. A.06.01). Samples were injected hydrodynamically (50 mbar; 0.2 s for standards and 3 s for extracts) and the electrophoresis system was operated under normal polarity and constant voltage conditions of +25 kV. A fused-silica capillary (Polymicro Technologies, Phoenix, AZ) with dimensions 63 cm total length (54 cm effective length), 75 μm i.d., and 375 μm o.d. was used.

Analytical procedures. At the beginning of the day, the capillary was conditioned by a pressure flush (930 mbar) of 1 mol/L NaOH solution (20 min), deionized water (10 min), and electrolyte solution (30 min), followed by an electrokinetic flush of electrolyte (+25 kV during 15 min). Between runs, the capillary was replenished with fresh electrolyte solution (1 min, pressure flush), followed by an electrokinetic flush (+25 kV, 4 min).

RESULTS AND DISCUSSION

Free solution CE. The separation of phenolic compounds is possible by free solution capillary electrophoresis. If the pH is raised above 9 (typical pK_a value), dissociation of the phenolic OH groups occurs. Consequently, the compounds become negatively charged and can be separated advantageously under counterelectroosmotic flow, a condition in which larger singly charged species migrate first. Figure 2 presents electropherograms of phenolic standards in tetraborate buffer at various conditions of pH and concentration. An increase in buffer concentration promotes separation of the critical pair (glucoside forms of luteolin and apigenin, compounds 5 and 7), however, at the expense of analysis

time (Figure 2a and c), which increases considerably. Raising the buffer pH from 10 to 11 (Figure 2a and b) also causes an overall increase in analysis time. The solute migration times at higher pH change markedly after 10 min elution, despite the fact that the electroosmotic flow does not vary much at that pH level. A possible explanation for this behavior is that the solutes that elute after 10 min may have suffered further dissociation, exhibiting a larger amount of negative effective charge. Whereas the solute migration occurs in counterflow, an increase in its electrophoretic mobility will result in a longer elution profile.

Ultraviolet photodiode array detection (DAD) has been found very useful in the differentiation of a variety of compounds including flavonoid glucosides [42]. Figure 3 shows the association of on-line UV spectra, with peaks in the electropherogram recorded at 337 nm, for the phenolic compounds under investigation in this work. This wavelength of 337 nm is particularly useful for trace analysis of phenolic compounds, considering that at lower wavelengths, the absorbance of the background electrolyte tends to interfere. The UV spectra of compounds 5 and 7, which comigrated under optimum conditions (20 mmol/L tetraborate buffer at pH 10), were recorded separately during migration of the compounds. All records were used to create a spectrum library. Even though UV spectral differences might not be very distinct among compounds that belong to the same chemical class, the spectra showed in Figure 3 suggest that a DAD may be useful in assigning peaks in more complex electropherograms.

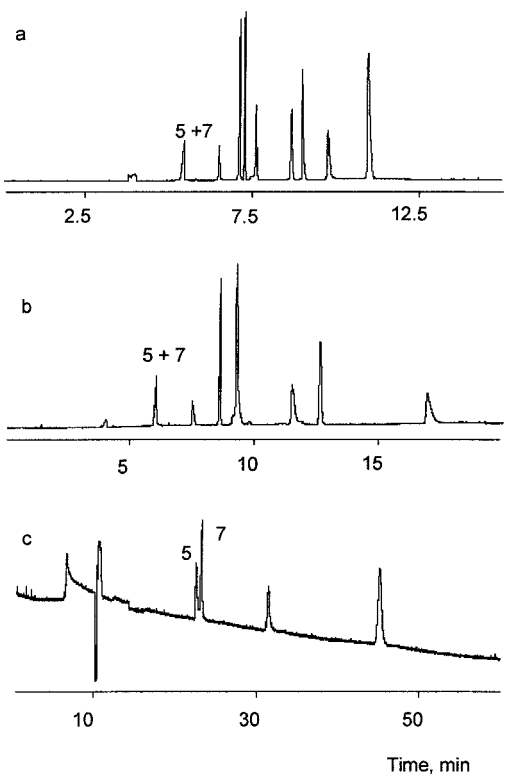

Figure 2. *Analysis of phenolic compounds by free solution capillary electrophoresis. Electrolytes: (a) 20 mmol/L tetraborate buffer, pH 10; (b) 20 mmol/L tetraborate buffer, pH 11; (c) 100 mmol/L tetraborate buffer, pH 10. Other conditions: standard concentration, 0.091 mg/mL; +25 kV; 0.2 s pressure injection (50 mbar); 337 nm detection. Peak identification as in Figure 1.*

To test the validity of the DAD as an identification tool, methanolic, hydroalcoholic, and glycolic extracts of *Matricaria recutita* L. were analyzed by free solution capillary electrophoresis under optimum conditions. The resulting electropherograms are organized in Figure 4. The hydroalcoholic and glycolic extracts are industrialized and, therefore, they contain a preservative mixture of alkyl parabens; the peaks are labeled with an asterisk in Figure 4b and c. Even though differences in migration time occurred, possibly due to day-to-day electroosmotic flow variability, small pH changes during buffer storage, and matrix effects, the profiles of the three extracts are very similar and the elution order of the selected standards is identical. It is worth noting that the migration of the structurally related compounds, luteolin, apigenin, and quercetin, increases considerably from the methanolic to the glycolic and hydroalcoholic extracts, in that order, which might be indicative of adduct formation.

The effect of borate complexation on the electrophoretic behavior of carbohydrates in capillary electrophoresis is well known [43]. Flavonoids with ortho-dihydroxy groups, luteolin and quercetin, and those with sugar moieties that present the same feature as the *cis*-1,2 diol system of rhamnosyl in rutin should complex more strongly with the borate molecules of the electrolyte and, therefore, are expected to migrate further on in relation to compounds without vicinal hydroxyl groups. That was the case among the glucoside compounds (5) and (7), which migrated earlier than rutin. Luteolin and quercetin are the last eluting compounds among the flavonoids studied.

Considering that the source of the chamomile plant used to produce the extracts was distinct and the extract preparation process (maceration, time of drying, temperature, etc.) was not standardized, comparison of the extractive power of each solvent system might be speculative. However, certain differences are notable when the electropherograms of Figure 4 are inspected. The plant used in the prepa-

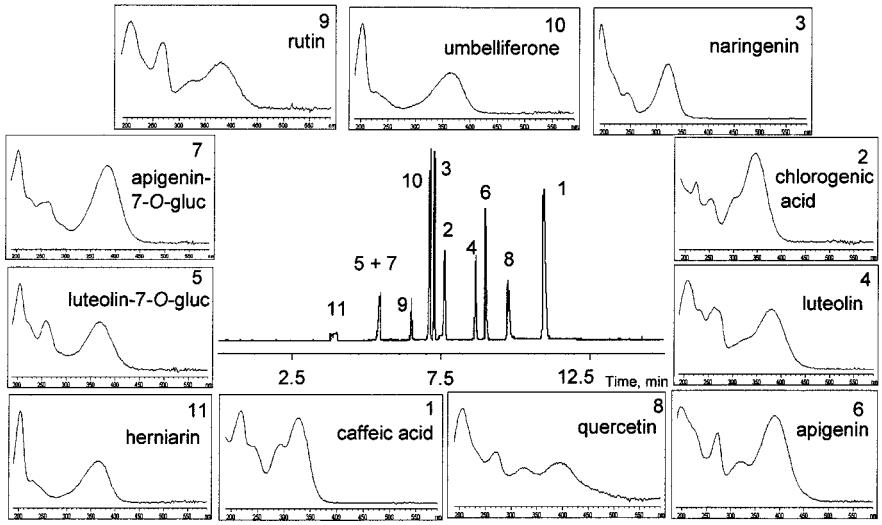

Figure 3. *Analysis of phenolic compounds by free solution capillary electrophoresis under optimum conditions: 20 mmol/L tetraborate buffer, pH 10; standard concentration, 0.091 mg/mL; +25 kV; 0.2 s pressure injection (50 mbar); 337 nm. The insets show on-line UV spectra of the standards.*

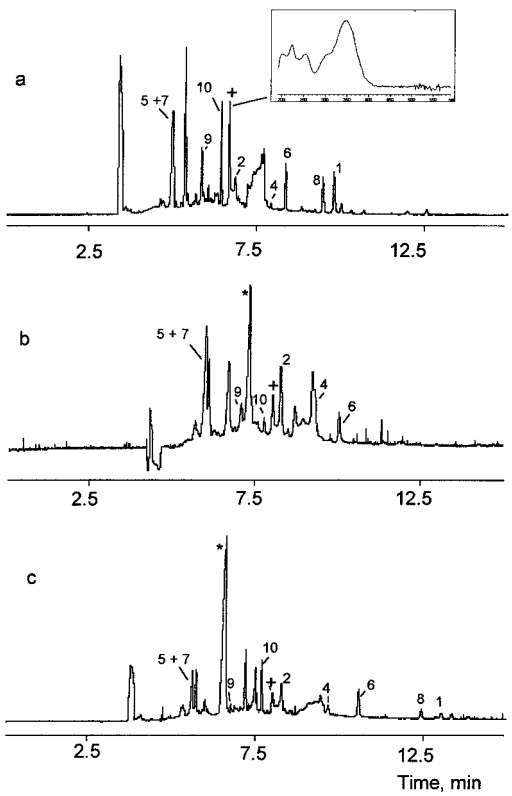

Figure 4. *Analysis of (a) methanolic, (b) glycolic, and (c) hydroalcoholic extracts of Matricaria recutita L. by free solution capillary electrophoresis under optimum conditions. Conditions as in Figure 3, except for injection (3 s at 50 mbar). Peak identification as in Figure 1. The peaks labeled with * correspond to the extract preservative. For comments on the peaks labeled +, see text. The inset shows the on-line UV spectrum of peak +.*

ration of the methanolic extract is rich in umbelliferone followed in descending order by rutin, apigenin, quercetin, caffeic acid, and the glucoside forms of luteolin and apigenin. Chlorogenic acid and free luteolin appear in much smaller concentrations. Comparatively, the plant used in the glycolic extract is richer in chlorogenic acid and luteolin, but lacks quercetin and caffeic acid. The plant used in the hydroalcoholic extract seems to be intermediate between the methanolic and glycolic extracts, resembling more closely the composition of the methanolic extract. About 9 of the 11 compounds under investigation were identified by comparison of the on-line spectrum and the UV spectra library. Naringenin and herniarin were not identified in any of the extracts under the conditions studied.

For the major components of the extracts, peak identification by spectral comparison is straightforward. Checks of peak purity resulted in an index greater than 998 for solutes 9, 10, 2, 6, 8, and 1 in the methanolic extract, for instance, confirming the peak homogeneity, whereas solutes 5 and 7, which comigrate in all electropherograms, present a peak index of about 818. When the component abundance is low, spectral identification may not be conclusive. In this case, spiking techniques are in order. For all electropherograms depicted in Figure 4, peak identity was confirmed by spiking with pure standards.

An interesting illustration of the concept of peak identity assisted by DAD detection relates to the peak labeled as + in Figure 4. A comparison of its migration time with the migration times of the standards (Figure 3) suggests that peak (+) might be

naringenin. However, its on-line spectrum (inset of Figure 4a) differs significantly from that of naringenin (in Figure 3). As a matter of fact, it closely resembles the spectrum of its adjacent peak, chlorogenic acid (spectrum also in Figure 3). Peak 2 was spiked with standard chlorogenic acid to confirm its identity unequivocally. This procedure indicates that peak + is not naringenin and suggests that it might be a compound that is structurally similar to chlorogenic acid, such as a positional isomer or other closely related compound.

Method validation parameters. Before a method is routinely used, it must be validated. Validation is the process of proving that the method is acceptable for its intended purpose. To assure reproducibility of migration time for peak identification (mobility data) and reproducibility of peak area for quantitation purposes, a few capillary conditioning procedures were tested: the capillary was just replenished with the electrolyte (procedure A); the capillary first was rinsed with sodium hydroxide solution and water prior to the electrolyte (procedure B); the capillary was rinsed with organic solvent mixtures, followed by the electrolyte (procedure C); and, finally, the capillary was rinsed with the electrolyte buffer using pressure and voltage flushes (procedure D). The results are compiled in Table I for apigenin, a compound commonly used to standardize chamomile extracts of cosmetic interest. The precision of each procedure was given in terms of coefficient of variation (% CV). Reproducibility of the migration time of apigenin for 10 consecutive injections of a methanolic extract was comparable for procedures A, C, and D, but reproducibility of peak area was poor. A simple electrokinetic flush of the electrolyte seemed to improve both migration time and peak area reproducibility greatly, and it was adopted throughout this work.

A calibration curve for apigenin was linear over the concentration range of 5.0–75.0 μg/mL, as shown by the statistical data organized in Table II. The results showed a good linearity between peak area and concentration ($r^2 > 0.999$). The limits of detection (LOD) and quantitation (LOQ) were 3.8 and 11.5 μg/mL, respectively. The criteria used to determine the LOD and LOQ were based on the standard deviation of the response (SD) and the calibration curve slope (S) according to the expressions LOD = 3.3(SD/S) and LOQ = 10(SD/S) [44]. The standard deviation of response was determined from the y intercept standard deviation of the regression line. The calibration curves consisted of five points, and three replicate injections of standards at each concentration level were performed.

MEKC. The separation of phenolic compounds is also possible by micellar electrokinetic chromatography [19]. In a common version of this separation mode, anionic surfactants such as sodium dodecylsulfate are introduced in the electrolyte buffer to create a pseudo-stationary phase that migrates slowly

Table I. *Evaluation of reproducibility of migration time and peak area of apigenin during capillary conditioning procedures.*

Capillary conditioning	Procedure							
	A		B		C		D	
NaOH 1 M	No		1 min		No		No	
H_2O	No		1 min		1 min		No	
H_2O:ACN 1:1	No		No		1 min		No	
ACN	No		No		5 min		No	
H_2O	No		No		1 min		No	
Electrolyte[a]								
200 mbar	5 min		5 min		5 min		1 min	
+25 kV	No		No		No		4 min	
	Migration time (min)	Peak area	Migration time (min)	Peak area	Migration time (min)	Peak area	Migration time (min)	Peak area
Average[b]	9.46	52.72	9.69	52.39	9.18	47.41	8.66	53.87
Std.[c]	0.54	5.43	1.05	5.45	0.37	3.11	0.32	0.88
CV[d] (%)	5.4	10.3	10.8	10.4	4.0	6.6	3.7	1.6

[a] Electrolyte: 20 mmol/L tetraborate buffer pH 10. Other conditions: +25 kV, 0.2 s pressure injection (50 mbar), 337 nm.
[b] 10 consecutive injections of methanolic extract.
[c] Standard deviation.
[d] Coefficient of variation.

Table II. *Method validation regarding linearity, limit of detection and limit of quantitation for apigenin in methanolic extracts.*[a]

Parameter	Statistical data
Concentration range (μg/mL)	5.0–75.0
Intercept	1.0468
Slope	1.0078
Correlation coefficient, r^2	0.9988
Standard deviation of intercept	1.16
Limit of detection (μg/mL)	3.8
Limit of quantitation (μg/mL)	11.5

[a] Electrolyte: 20 mmol/L tetraborate buffer pH 10. Other conditions: +25 kV, 0.2 s pressure injection (50 mbar), 337 nm.

in the direction of the cathode by imposition of a strong electroosmotic flow (high pH solutions). Several additives can be considered, aiming to increase solute solubility or to provide an auxiliary phase to improve selectivity. Whereas separation of the glucoside forms of luteolin (5) and apigenin (7) was not possible in free solution CE (Figure 3) unless extreme conditions were held (Figure 2c), MEKC seemed to be an interesting alternative, because it explores two distinct separation mechanisms simultaneously, migration and partition.

Figure 5 presents the separation of the 11 phenolic standard compounds by micellar electrokinetic chromatography in the presence of additives. The addition of SDS to the electrolyte medium (Figure 5A) was not successful to promote separation of all solutes; early eluting peaks were broadened, suggesting that some peak overlap might be occurring. The situation is even worse in the electrolyte with acetonitrile (Figure 5C). In this case, clear coelution occurred, the flow decreased, and many bands are distorted and broadened. The addition of β-cyclodextrin (Figure 5B) was quite satisfactory and the separation of compounds (5) and (7) was then achieved in a reasonable time.

Figure 6 shows the association of on-line UV spectra with peaks in the electropherogram obtained under optimum electrolyte conditions (20 mmol/L tetraborate buffer, 20 mmol/L SDS, 15 mmol/L β-cyclodextrin at pH 10) for the phenolic compounds under investigation in this work. In this medium, baseline resolution of all solutes was achieved even though compounds 3, 10, 6, 2, and 4 migrated closer together when compared to the profile in free solution (Figure 3). The order of elution is also altered as a result of the discriminatory interaction of the solutes within the micelle and

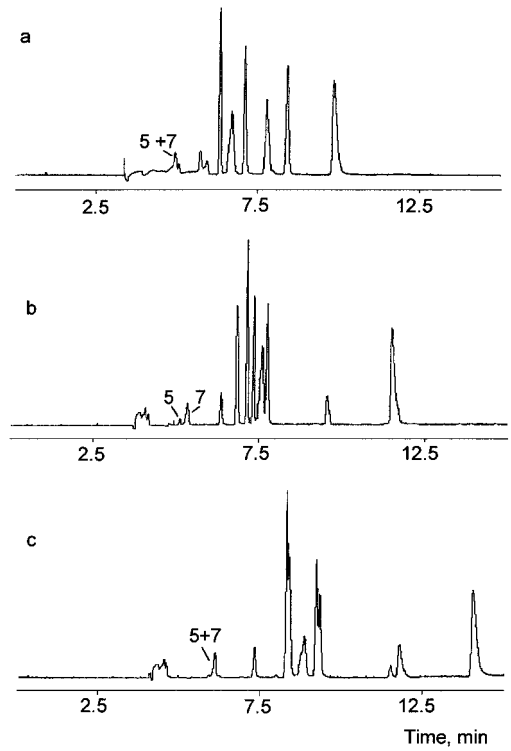

Figure 5. *Analysis of phenolic compounds by micellar electrokinetic chromatography. Electrolytes: (a) 20 mmol/L tetraborate buffer, 20 mmol/L SDS, pH 10; (b) 20 mmol/L tetraborate buffer, 20 mmol/L SDS, 15 mmol/L β-cyclodextrin, pH 10; and (c) 20 mmol/L tetraborate buffer, 20 mmol/L SDS, 5% ACN, pH 10. Other conditions: standard concentration, 0.091 mg/mL; +25 kV; 0.2 s pressure injection (50 mbar); 337 nm detection. Peak identification as in Figure 1.*

cyclodextrin cavity. Another interesting feature when Figures 3 and 6 are contrasted is that the on-line UV spectra of all solutes are remarkably similar regardless of the differences in the medium due to the addition of micelles and β-cyclodextrin. This is very convenient when complex matrices are examined, because peak identification can be performed readily without resorting to labor-intensive spiking techniques.

Figure 7 presents MEKC electropherograms of methanolic, hydroalcoholic, and glycolic extracts of *Matricaria recutita* L. under optimum conditions. All peaks were identified by comparison of the corresponding on-line UV spectrum and the spectra library, and later by spiking techniques. The preliminary spectral identification was correct, endorsing the use of DAD as an identification tool. The information obtained with respect to composition of the different extracts was quite similar to that obtained

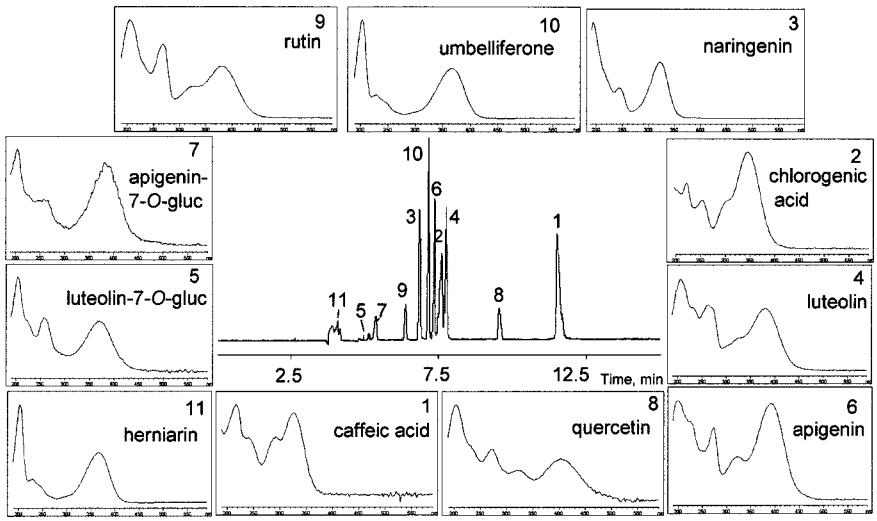

Figure 6. *Analysis of phenolic compounds by micellar electrokinetic chromatography under optimum conditions: 20 mmol/L tetraborate buffer, 20 mmol/L SDS, 15 mmol/L β-cyclodextrin, pH 10; standard concentration, 0.091 mg/mL; +25 kV; 0.2 s pressure injection (50 mbar); 337 nm. The insets show on-line UV spectra of the standards.*

during analysis by free solution CE (Figure 4). One exception is the discrimination between the glucosides luteolin (5) and apigenin (7). Free apigenin seems to be more abundant in methanolic extracts, whereas apigenin-7-*O*-glucoside prevails in the glycolic and hydroalcoholic extracts. The opposite is true for luteolin and its glucoside.

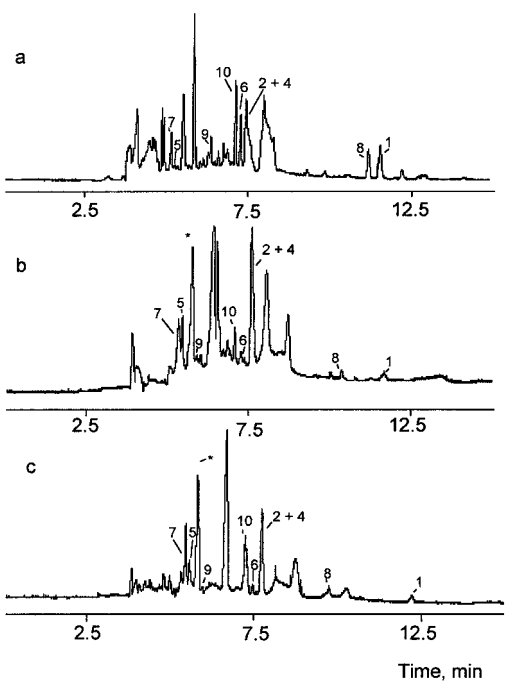

Figure 7. *Analysis of (a) methanolic, (b) glycolic, and (c) hydroalcoholic extracts of Matricaria recutita L. by micellar electrokinetic chromatography under optimum conditions. Conditions as in Figure 6, except for injection (3 s at 50 mbar). Peak identification as in Figure 1. The peaks labeled as * correspond to the extract preservative.*

CONCLUSION

This work introduced capillary electrophoresis in free solution and micellar medium coupled to diode array detection as a reliable and sensitive technique for the evaluation of herbal extracts, especially methanolic, glycolic, and hydroalcoholic extracts of *Matricaria recutita* L. The composition of the different extracts in terms of 11 phenolic compounds was assessed and contrasted readily. Both techniques presented complementary information.

A few advantages of the CE methodologies over other well established liquid chromatographic techniques, specifically for herbal extract composition evaluation, can be enumerated. In CE, multiple injections can be performed sequentially without compromising column performance. CE capillaries are low cost and durable. There is no need for sample clean-up procedures to remove either ionic components (carbohydrates, etc.) or high molecular mass compounds (e.g., triterpenes, waxes, etc.), which often are detrimental to column packings. Moreover, CE provides adequate performance characteristics, fast separations, good sensitivity, and spectral selectivity for the determination of flavonoids in real matrices.

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