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Use of molybdate as novel complex-forming selector in the analysis of polyhydric phenols by capillary zone electrophoresis

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Abstract

Molybdate was examined as a complex-forming additive to the CE background electrolytes (BGE) to affect the selectivity of separation of polyhydric phenols such as flavonoids (apigenin, hyperoside, luteolin, quercetin and rutin) and hydroxyphenylcarboxylic acids (ferulic, caffeic, *p*-coumaric and chlorogenic acid). Effects of the buffer concentrations and pH and the influence of molybdate concentration on the migration times of the analytes were investigated. In contrast to borate (which is a buffering and complex-forming agent generally used in CE at pH ≥9) molybdate forms more stable complexes with aromatic *o*-dihydroxy compounds and hence the complex-formation effect is observed at considerably lower pH. Model mixtures of cinnamic acid, ferulic acid, caffeic acid and 3-hydroxycinnamic acid were separated with 25 mM morpholinoethanesulfonic acid of pH 5.4 (adjusted with Tris) containing 0.15 mM sodium molybdate as the BGE (25 kV, silica capillary effective length 45 cm × 0.1 mm I.D., UV–vis detection at 280 nm). With 25 mM 2-hydroxy-3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulphonic acid/Tris of pH* 7.4 containing 2 mM sodium molybdate in aqueous 25% (v/v) methanol as the BGE mixtures of all the above mentioned flavonoids, *p*-coumaric acid and chlorogenic acid could be separated (the same capillary as above, UV–vis detection at 263 nm). The calibration curves (analyte peak area versus concentration) were rectilinear ($r > 0.998$) for ≈8–35 μg/ml of an analyte (with 1-nitroso-2-naphthol as internal standard). The limit of quantification values ranged between 1.1 mg l^{-1} for *p*-coumaric acid and 2.8 mg l^{-1} for quercetin. The CE method was employed for the assay of flavonoids in medicinal plant extracts. The R.S.D. values ranged between 0.9 and 4.7% ($n = 3$) when determining luteolin (0.08%) and apigenin (0.92%) in dry *Matricaria recutita* flowers and rutin (1.03%) and hyperoside (0.82%) in dry *Hypericum perforatum* haulm. The recoveries were >96%.

Keywords: Molybdate selector; Complex formation; Hydroxyphenylcarboxylic acids; Flavonoids; Plant extracts; *Matricaria recutita*; *Hypericum perforatum*

1. Introduction

Valuable features of some electro-migration analytical techniques (such as CE, isotachophoresis, ITP, ITP-CE, isoelectric focusing, IEF, capillary gel electrophoresis, CGE, micellar electrokinetic chromatography, MEKC, and capillary electrochromatography, CEC), i.e. their high separation efficiency and short time of analysis make them appropriate for the assays of pharmaceutically important compounds in plant materials and similar natural products of complex composition [1–3]. In the recent decade increased attention has been paid to the development of CE methods suitable for rapid determination of potential antioxidants such as flavonoids and similar polyhydroxy compounds of phenolic character in herbal samples (for relevant review see, e.g., [3]). To achieve ionisation of hydroxy compounds (as relatively weak acids) enabling their separation by CE, BGEs based on borate buffer of pH 9–11 are widely used and in many cases simultaneously the complex-formation ability of B(III) is utilized for manipulating or enhancing the selectivity of CE separation [4–12]. It is well known that polyhydric phenols are readily oxidised by oxygen in alkaline aqueous media but it seems that this fact is not much considered when using alkaline borate buffers as BGE in the CE assay of such antioxidants. Possible risk of spontaneous analyte oxidation with oxygen during the sample processing could be reduced by performing the CE separation under anaerobic conditions (which is not always effortlessly feasible) or in suitable BGE of pH close to 7 containing a complex-forming selector that can eventually convert the appropriate analytes to anionic complexes of different stability. Capability of Mo(VI) to form anionic complex species

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with hydroxy compounds that are generally more stable than corresponding B(III) complexes has been known for a long time [13]. In 1971 Soni and Bartusek [14] proved by spectrophotometry and by paper electrophoresis that MoO_4^{2-} reacts with 1,2-diphenols (such as catechol and pyrogallol, H_2L) in neutral aqueous solutions to form $[MoO_2L_2]^{2-}$ species characterised by overall formation constants $\log K_2 \cong 5$–6.5. Complex-formation between Mo(VI) and various organic ligands involving −OH moieties has been studied extensively in recent years; these studies were often promoted by the interest in the investigation of the role of molybdenum as a component of the cofactors of various redox enzymes [15–17]. The formation of a number of stable anionic Mo(VI)–malate [17,18], Mo(VI)–citrate [19,20], Mo(VI)–fructose and sorbose [21], Mo(VI)–galacturonic and glucuronic acid [22] and Mo(VI)–gluconic acid [23] complexes was reported. In spite of these facts it seems that potential use of Mo(VI) as a selector in CE separation of polyhydroxy compounds has not yet been recognized.

In the present paper complex-formation effect of molybdate as an additive to CE BGEs of different pH on the separation of polyhydroxy compounds of pharmaceutical importance occurring in medicinal herbs such as flavonoids (apigenin, hyperoside, luteolin, quercetin and rutin) and cinnamic acid derivates (ferulic acid and caffeic acid) is examined. Possible use of a CE method employing neutral BGE with molybdate as an additive for the assay of flavonoids in medicinal plant extracts of *Matricaria recutita* flowers and *Hypericum perforatum* haulm is demonstrated.

2. Experimental

2.1. Apparatus

A computer-controlled PrinCE 650 capillary electrophoresis system with integrated temperature control unit (Prince Technologies B.V., The Netherlands) equipped with LAMBDA (Leonberg, Germany) UV-Vis HPLC detector and WinPrinCE software (as an integrated module of DAX data acquisition and analysis package) was used. The PHM 220 (Radiometer, France) pH-meter with PHC2401-8 combined glass electrode calibrated with standard aqueous Radiometer buffers was employed for the pH measurements.

2.2. Capillary conditioning and electrophoretic separation conditions

The separations were performed at 25 kV with TSP0753100 (Composite Metal Services, Ltd., The Chase, Hallow, Worcester, UK) fused silica capillary (Reel 051CO-22, Lot JHA-01A; 0.1 mm I. D., total length 70 cm, 45 cm to the detector) maintained at 25 °C. To activate a new capillary it was rinsed with 1 M NaOH for 60 min and with ultra-pure water for 60 min and then it was conditioned daily before the first sample injection with 0.1 M NaOH for 20 min and with ultra-pure water for 20 min. The capillary was rinsed successively with 0.1 M NaOH for 2 min, ultra-pure water for 2 min and appropriate BGE for 3 min between individual runs. Samples were injected hydrodynamically at a pressure of 50 mbar for 6 s. Detection was carried out at 263 nm for the flavonoids and *p*-coumaric and chlorogenic acid in neutral BGE or at 280 nm for the cinnamic acid derivates at pH 5.4. The migration times corresponding to the electro-osmotic flow (EOF) were determined conventionally by injecting dimethylsulphoxide as the EOF marker.

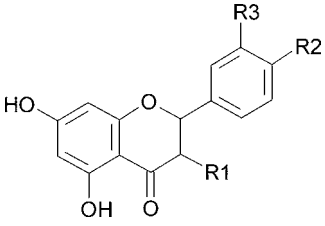
2.3. Chemicals

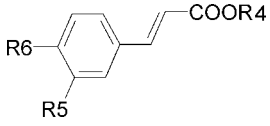
All chemicals used for the preparation of model mixtures and for electrolyte solutions were of analytical grade. The standards of cinnamic acid, ferulic acid, caffeic acid, 3-hydroxycinnamic acid, *p*-coumaric acid, chlorogenic acid, apigenin, hyperoside, luteolin, quercetin and rutin were obtained from Aldrich (Milwaukee, WI, USA), 2-morpholinoethanesulfonic acid (MES), 2-hydroxy-3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid (HEPPSO) and sodium molybdate dihydrate were purchased from Fluka (Buchs, Switzerland). Tris, sodium hydroxide, 1-nitroso-2-naphthol and methanol were obtained from Lachema (Brno, Czech Republic). Ultra pure water used throughout was prepared with Milli-Q system (Millipore, Bedford, MA, USA). The structures of the compounds under study and their literature pK_a values (where available) are shown in Table 1.

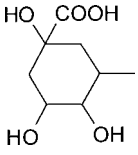
2.4. Standard and electrolyte solutions

Purely aqueous stock solutions (1 g l^{-1}) of ferulic acid, caffeic acid, 3-hydroxycinnamic and cinnamic acid were prepared by dissolving the compounds in water. The final model mixture

Table 1
Structural formulas and ionization constants (pK_{a1}) of separated compounds





Analyte	pK_{a1}	R1	R2	R3
Apigenin	8.54 [28]	H	H	OH
Rutin	7.10 [29]	*O*-Rhamnoglucose	OH	OH
Hyperoside	–	*O*-Galactose	OH	OH
Quercetin	6.74 [29]	OH	OH	OH
Luteolin	–	H	OH	OH
Analyte		R4	R5	R6
Ferulic acid	4.57 [30]	H	OCH_3	OH
Caffeic acid	4.44 [29]	H	OH	OH
3-Hydroxycinnamic acid	–	H	OH	H
Cinnamic acid	4.43 [30]	H	H	H
p-Coumaric acid	4.64 [30]	H	H	OH
Chlorogenic acid	3.59 [30]		OH	OH

contained 100 mg l^{-1} of each analyte. Stock solutions containing 2 g l^{-1} of apigenin, hyperoside, luteolin, quercetin, rutin, chlorogenic acid, *p*-coumaric acid or 1-nitroso-2-naphthol were prepared in methanol. The final model mixture A containing 50 mg l^{-1} of each analyte in 25% (v/v) methanol was prepared from the stock solutions of standards. Calibration solutions prepared by dilution of mixture A contained 8–35 mg l^{-1} of the analytes and 10 mg l^{-1} of 1-nitroso-2 naphthol as internal standard. The 1-nitroso-2-naphthol was selected as internal standard since it is well separated from the compounds under study, it is never occurring in plants and it is a phenolic compound like the compounds under study. The BGEs for the separation of cinnamic acid derivates were prepared by dissolving weighed amount of MES in water and adjusting the appropriate pH with Tris. The BGEs for the separation of flavonoids, chlorogenic acid and *p*-coumaric acid contained 25% (v/v) of methanol; they were prepared by dissolving the weighed amount of HEPPSO in 25% (v/v) methanol and adjusting the pH with addition of Tris while using a glass electrode (calibrated with aqueous buffers) for the pH measurement. Therefore the acidity of such BGEs was measured as apparent (pseudo) values denoted as pH^*.

All standard and electrolyte solutions were degassed for 15 min in an ultrasonic bath and filtered through a Millipore Millex-LCR syringe membrane filter (pore size 0.45 μm).

2.5. Plant materials and preparation of plant extracts

M. recutita flowers from plants grown in the Botanical Garden of the Faculty of Pharmacy, Hradec Králové, were collected and dried in August 2003. *H. perforatum* leaves and flowers (in the form of an antidepressant tea, producer MEGAFYT-R, Vrane nad Vltavou, Czech Republic) conforming to pharmacopoeial standards were purchased in a public pharmacy.

2.5.1. M. recutita flowers extract

An amount of 3.5 g of dried and pulverized plant material was mixed with 50 ml of methanol, extracted in an ultrasonic bath for 120 min, the mixture was filtered through dry paper, the filter was washed with 2 × 20 ml of methanol and the united filtrate was evaporated to 10–12 ml (extract A). The completeness of extraction was checked by washing the filter with the retained spent plant residue by 20 ml of methanol, evaporating the filtrate to dryness in a porcelain dish under an infrared lamp and weighing the residue. The extract A was diluted to 25 ml by methanol in a graduated flask and a 2.5 ml aliquot of this extract (extract B) was diluted to 10 ml by water in a graduated flask to contain 25% (v/v) of methanol. This diluted extract was degassed for 15 min in an ultrasonic bath and filtered through a Millipore Millex-LCR syringe membrane filter (pore size 0.45 μm) prior to CE separation (qualitative analysis) under the optimum conditions. The analyte peaks were identified by spiking the extract B with appropriate standard solutions of analytes in 25% methanol. For quantitative analysis a 2 ml aliquot of the extract B was treated with 0.5 ml of methanolic solution containing 0.2 mg ml^{-1} of 1-nitroso-2-naphthol as internal standard and the mixture was diluted to 10 ml with water. For recovery experiments a 2 ml aliquot of preliminarily analysed extract B was treated with the internal standard and with standard solutions of apigenin and luteolin in 25% methanol to increase the concentration of these flavonoids by 50 mg l^{-1} (calculated with respect to the extract A), the mixture was diluted to 10 ml with water and analysed by CE as cited above.

2.5.2. H. perforatum haulm extract

Fifty one grams of pulverised antidepressant tea was macerated overnight in 480 g of 80% (v/v) ethanol, thereafter the mixture was sonicated at −10 °C for 10 min, filtered through dry paper filter, the residue on the filter was washed three times by 80 g of 80% ethanol and the combined filtrate was lyophilized for 180 min; 1 g of the lyophilized extract was dissolved in 100 ml of methanol and a 2.5 ml aliquot of this solution was diluted to 10 ml with water in a graduated flask. The final test solution of *H. perforatum* haulm extract contained 25% (v/v) of methanol. The test solutions for qualitative and quantitative CE analysis and recovery experiments were prepared in the same way as described for the *M. recutita* sample but the standard solutions contained rutin and hyperoside instead of apigenin and luteolin.

2.6. Quantitative analysis, calibration curves and figures of merit

Calibration curves relating the analyte peak area/internal standard peak area ratio to concentration of the analyte were measured with calibration solutions containing 8–35 mg l^{-1} of the analytes (five different concentrations), 10 mg l^{-1} of 1-nitroso-2 naphthol as internal standard and 25% (v/v) of methanol. Each calibration curve was measured five times under optimised CE conditions, i.e., with 25 mM HEPPSO/Tris of pH^* 7.4 containing 2 mM sodium molybdate in aqueous 25% (v/v) methanol as the BGE (25 kV, 0.1 mm I.D. silica capillary, total length 70 cm, effective length 45 cm, UV detection at 263 nm, hydrodynamic sampling at a pressure of 50 mbar for 6 s). At the same time the migration times of the analytes were determined and the repeatability of the peak areas at concentration level of 20 mg l^{-1} of the analytes was evaluated. The limit of detection (LOD) values were calculated from the linear regression data as 3.3SDi/slope where SDi stands for the standard deviation of intercept; similarly the limit of quantification (LOQ) values were estimated as 10SDi/slope [24]. The accuracy of the results was evaluated by the added-found (recovery) experiments; to estimate the recovery the plant extracts, initially analysed by CE, were treated with the analytes to increase their concentration by 50 mg l^{-1} and thereafter they were subjected to additional CE assay.

3. Results and discussion

3.1. Effect of molybdate on the electrophoretic migration of cinnamic acid derivates

To demonstrate any effect of molybdate on the separation of cinnamic, 3-hydroxycinnamic, ferulic and caffeic acid proper BGE of pH 5–6 ensuring solely ionization of the carboxylic

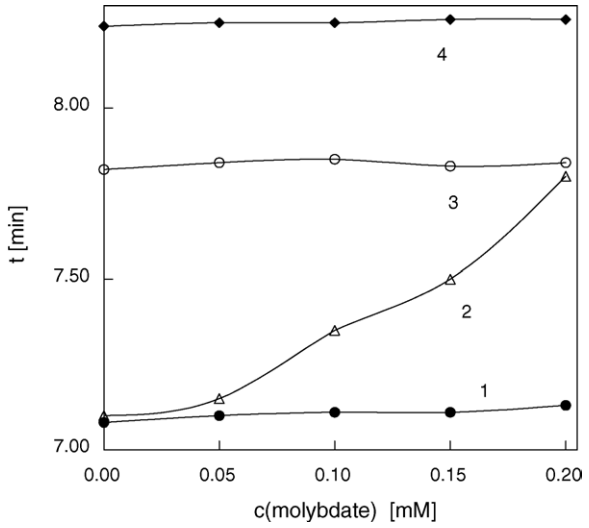


Fig. 1. Effect of the concentration of sodium molybdate in the BGE on the migration times of cinnamic acid derivates; ferulic acid (1), caffeic acid (2), 3-hydroxycinnamic acid (3), cinnamic acid (4) (100 mg l^{-1} of each). BGE: 25 mM MES of pH 5.4 (adjusted with Tris); applied voltage 25 kV; temperature 25 °C; hydrodynamic sampling at pressure of 50 mbar for 6 s; detection at 280 nm.

group of the model compounds had to be selected initially; MES (with pK_a 6.1) was found to be suitable while phosphate or acetate were disqualified to avoid any risk of their possible chemical interaction with molybdate. Effects of MES concentration (10–100 mM), pH (5.3–6.4, adjusted with Tris) and molybdate concentration (0.05–0.2 mM) on migration times of the model compounds were investigated. Relatively low concentration of molybdate ensures that at pH >5 the Mo(VI) exists predominantly in the form of mononuclear MoO_4^{2-} species [25,26]. Optimal peak shape and symmetry and good separation for all the analytes except of the caffeic acid–ferulic acid couple together with reasonably short migration times was attained with 25 mM MES of pH 5.4–5.6. The effect of molybdate concentration on the migration behaviour of the compounds under study in 25 mM MES of pH 5.4 is shown in Fig. 1. The mean migration time corresponding to the EOF was 5.73 min in this electrolyte.

It can be clearly seen that in the absence of molybdate it is impossible to separate ferulic and caffeic acid and that the addition of molybdate considerably increases the migration time of caffeic acid. This happens most probably owing to the formation of Mo(VI)–caffeic acid complex (caffeic acid possesses two aromatic hydroxy groups located in ortho position in its molecule) bearing more negative charge than the single caffeic acid anion. In the presence of 0.2 mM molybdate the peak of caffeic acid becomes asymmetric and its separation from that of 3-hydroxycinnamic acid is poor. Considering the favourable migration time values and quality of separation the optimum concentration of molybdate in the BGE is 0.15 mM. Electropherograms demonstrating separation of a model mixture of cinnamic acid derivates in the absence and in the presence of molybdate in the BGE are shown in Fig. 2.

3.2. Separation and determination of flavonoids, p-coumaric acid and chlorogenic acid

Due to the absence of carboxylic moiety in their molecules the flavonoids are substantially weaker acids than hydroxyphenylcarboxylic acids; e.g., the pK_a value of the phenolic group of the most simple 7-hydroxyflavone was found to be 7.39 [27]. In view of this fact HEPPSO (with pK_a 7.5) was selected as suitable BGE for investigating the effect of Mo(VI) on the migration of flavonoids. Because of limited solubility of the analytes under study in water the BGE and all test solutions contained 25% (v/v) of methanol.

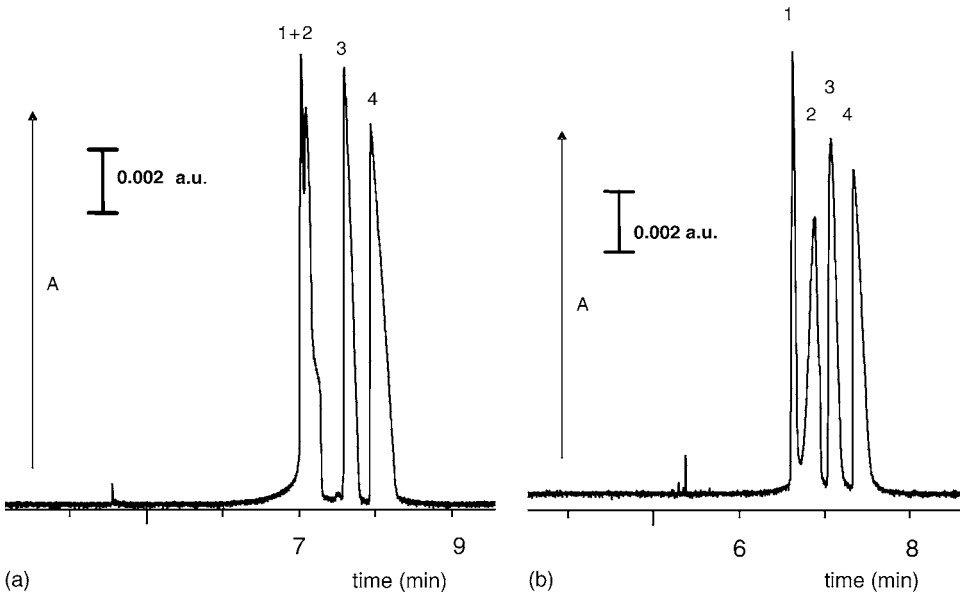


Fig. 2. (a) Electrophoreogram of standard solution of cinnamic acid derivatives with BGE without Mo(VI); ferulic acid (1), caffeic acid (2), 3-hydroxycinnamic acid (3) and cinnamic acid (4) (100 mg l^{-1} of each); BGE: 25 mM MES of pH 5.4 (adjusted with Tris); applied voltage 25 kV; temperature 25 °C; hydrodynamic sampling at pressure of 50 mbar for 6 s; detection at 280 nm; (b) the same analytes and CE conditions but the BGE contains also 0.15 mM sodium molybdate.

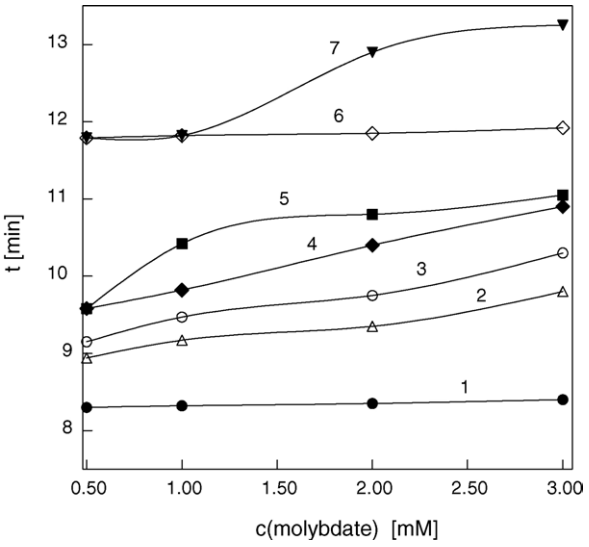


Fig. 3. Effect of the concentration of sodium molybdate in the BGE on the migration times of flavonoids, *p*-coumaric and chlorogenic acid; apigenin (1), rutin (2), hyperoside (3), quercetin (4), luteolin (5), *p*-coumaric acid (6) and chlorogenic acid (7) (50 mg l^{-1} of each). BGE: 25 mM HEPPSO of pH* 7.4 (adjusted with Tris) in 25% (v/v) methanol; applied voltage 25 kV; temperature 25 °C; hydrodynamic sampling at pressure of 50 mbar for 6 s; detection at 263 nm.

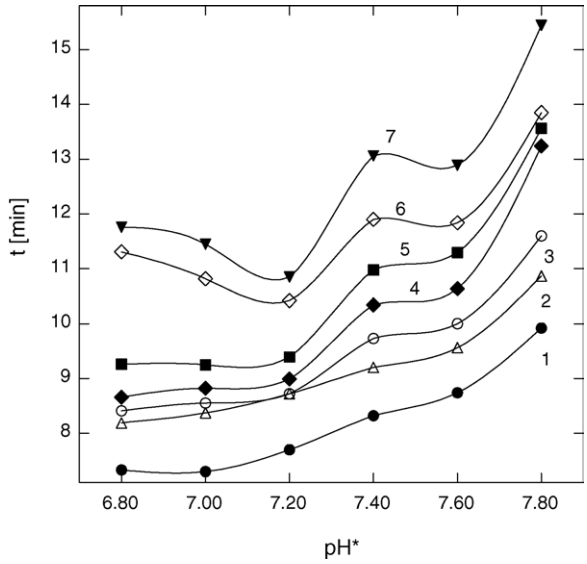


Fig. 4. Effect of pH* on the migration times of flavonoids, *p*-coumaric and chlorogenic acid; Apigenin (1), rutin (2), hyperoside (3), quercetin (4), luteolin (5), *p*-coumaric acid (6) and chlorogenic acid (7) (50 mg l^{-1} of each). BGE: 25 mM HEPPSO in 25% (v/v) methanol containing 2 mM sodium molybdate; applied voltage 25 kV; temperature 25 °C; hydrodynamic sampling at pressure of 50 mbar for 6 s; detection at 263 nm.

3.2.1. Effect of concentration of sodium molybdate

The effect of 0.5–3.0 mM sodium molybdate on the selectivity of separation in the BGEs with 25 mM HEPPSO of pH* 7.4 (adjusted with Tris) is shown in Fig. 3; according to the Mo(VI) equilibrium data [25,26] the Mo(VI) should exist in the form of MoO_4^{2-} under these conditions.

In contrast to influence on caffeic acid (cf. Fig. 1) in this instance the influence of Mo(VI) on the migration of flavonoids was observed only at substantially higher concentrations of molybdate in the BGE. With 0.5 mM molybdate in the BGE apigenin was separated from the other flavonoids while rutin, hyperoside, quercetin and luteolin migrated as three incompletely separated peaks (quercetin and luteolin formed a single zone); chlorogenic acid and *p*-coumaric acid migrated together as single peak. The optimum molybdate concentration ensuring the separation of all compounds down to the baseline was found to be 2 mM. Further increase in molybdate concentration to 3 mM influenced significantly the mobility of quercetin which resulted in incomplete separation of quercetin from luteolin. Moreover the peak of chlorogenic acid became extremely asymmetric. In accordance with chemical structures of apigenin and *p*-coumaric acid (both lacking o-diphenol moiety in their molecules) the migration times of these analytes were not affected by the addition of molybdate to the BGE.

3.2.2. Effect of concentration and pH of the BGE*

Buffers containing 10–100 mM HEPPSO were investigated. For 10 mM HEPPSO in the BGE the migration times of the analytes were shorter but the separation efficiency was poor. At >25 mM HEPPSO the migration times increased considerably and therefore 25 mM HEPPSO was employed. To estimate the effect of pH* on the separation of flavonoids the BGEs containing 25 mM HEPPSO and 2 mM sodium molybdate with pH* values ranging between 6.8 and 7.8 were tested (the results are depicted in Fig. 4).

In the pH* range 6.8–7.2 only apigenin, chlorogenic acid and *p*-coumaric acid were sufficiently separated; the zones of the other analytes were separated imperfectly. At pH* 7.4–7.6 good resolution was achieved for all seven analytes. The R_S (resolution factor) values determined at pH* 7.4 were 2.36 for the rutin–hyperoside and quercetin–luteolin couples of peaks; the R_S was 2.48 for the hyperoside–quercetin peaks. Further increase in pH did not lead to improvement of resolution; the migration times increased and at pH 7.8 poor separation of quercetin, luteolin and *p*-coumaric acid was achieved. Electropherogram demonstrating separation of a model mixture of flavonoids, chlorogenic acid and *p*-coumaric acid in the presence of molybdate in the BGE is shown in Fig. 5.

Full separation of the model mixture of seven analytes takes <13 min (see Fig. 5). The mean migration time corresponding to the EOF was 3.30 min in the optimal buffer of pH* 7.4 containing 25 mM HEPPSO and 2 mM sodium molybdate. In the electrolyte system without molybdate the flavonoids were not separated and chlorogenic acid migrated before *p*-coumaric acid. Chlorogenic acid and *p*-coumaric acid were added to the model mixture merely to show that the peaks of these acids are detected behind that of the "slowest" flavonoid luteolin. This is caused by the presence of additional ionized (carboxylic) group in the structure of these two model compounds.

3.2.3. Quantitative analysis, calibration curves and figures of merit

The linear regression parameters of calibration curves, migration times determined and corresponding R.S.D. values are

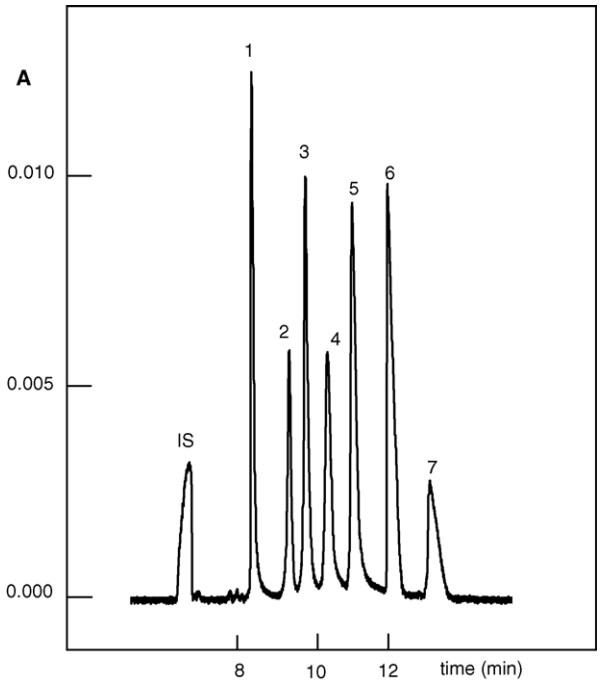


Fig. 5. Electrophoreogram of standard mixture of flavonoids and phenolic acids with BGE containing Mo(VI); apigenin (1), rutin (2), hyperoside (3), quercetin (4), luteolin (5), *p*-coumaric acid (6) and chlorogenic acid (7) (50 mg l^{-1} of each) and 1-nitroso-2-naphthol (IS; 10 mg l^{-1}); BGE: 25 mM HEPPSO + 2 mM sodium molybdate of pH* 7.4 (adjusted with Tris) in 25% (v/v) methanol; applied voltage 25 kV; temperature 25 °C; hydrodynamic sampling at pressure of 50 mbar for 6 s; detection at 263 nm.

summarized in Table 2. It can be seen that the repeatability is fairly good since the R.S.D. values of migration times and peak areas do not exceed 2.1%. Even though the internal standard was not so suitable to this work for its relatively poor theoretical plate (peak broadening), the results still were acceptable because all area R.S.D. were under 2.5%. Our LOQ value for apigenin (2.5 mg l^{-1}) compares well with that of 11.5 mg l^{-1} attained by CE with BGE of pH 10 based on 20 mM borate [24]. On the other hand our LOD values for apigenin, luteolin and quercetin (cf. Table 2) are about 10 times higher than those reported by Wang and Huang [12] who attained unusually wide (for CZE) rectilinear calibration ranges (extending over three orders of concentration variable) with CE at pH 8.9 (35 mM borate) and UV detection at 250 nm.

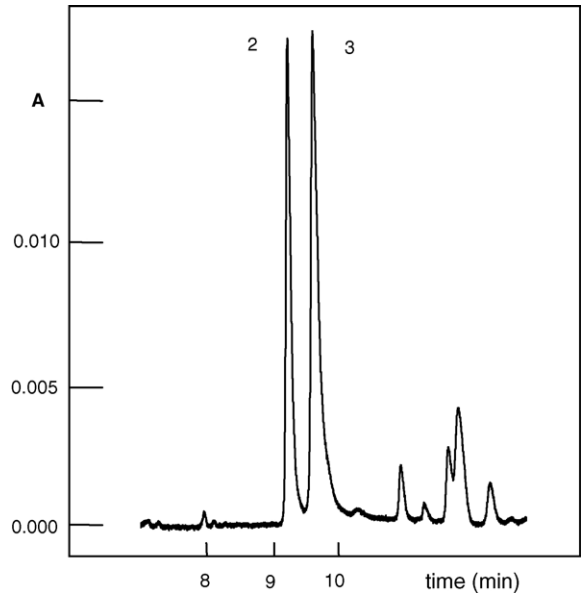


Fig. 6. Electrophoreogram of native *Hypericum perforatum* haulm extract: (2) rutin, (3) hyperoside; For separation conditions see Fig. 5.

3.2.4. Determination of the flavonoids in the plant extracts

The CE method developed has been applied to the determination of major flavonoids in *M. recutita* flowers extract (apigenin and luteolin) and *H. perforatum* haulm extract (rutin and hyperoside). The electropherogram of the latter native extract containing rutin and hyperoside as its major naturally occurring flavonoid components is shown in Fig. 6.

The peaks of the analytes were identified by spiking the test solutions successively with the flavonoid standard solutions resulting in the increase of heights of the appropriate peaks. Quantification was conducted by utilizing calibration curves of the individual analytes and the content of these flavonoids was calculated as their percentage in the dry mass of the plant material analysed. The results of the CE analyses of the plant extracts and the recovery data (together with the R.S.D. values) are reported in Table 3. Reasonably high and reproducible recovery values indicate that the CE method using molybdate as complex-forming additive in neutral BGE is suitable for the assay of flavonoids in medicinal herbs.

Table 2
Migration times (t_m), CE calibration parameters and figures of merit for flavonoids and hydroxyphenylcarboxylic acids determined by the proposed CE method

Analyte	t_m (min) (R.S.D.; $n = 5$)	Equation	r	Area R.S.D. ($n = 5$)[a]	LOD (mg l^{-1})	LOQ (mg l^{-1})
Apigenin	8.32 (1.5)	$y = 0.372x + 0.184$	0.9991	1.6	0.8	2.5
Rutin	9.21 (0.9)	$y = 0.198x + 0.046$	0.9998	0.4	0.4	1.2
Hyperoside	9.73 (1.8)	$y = 0.352x + 0.164$	0.9987	1.6	0.9	2.6
Quercetin	10.34 (0.7)	$y = 0.384x - 0.066$	0.9995	1.8	0.9	2.8
Luteolin	10.98 (2.1)	$y = 0.501x + 0.241$	0.9993	2.1	0.8	2.5
p-Coumaric acid	11.90 (0.4)	$y = 0.506x + 0.289$	0.9994	0.9	0.4	1.1
Chlorogenic acid	13.06 (1.8)	$y = 0.147x + 0.120$	0.9986	2.5	1.6	4.7

x: analyte concentration (mg l^{-1}); y: peak area ratio (analyte/internal standard).
[a] Calculated from peak areas for 20 mg l^{-1} of each analyte.

Table 3
Results of CE assay of major flavonoids in dried herbs and estimation of recoveries

Analyte	Found (mg l^{-1})	Found (%) (R.S.D.; $n = 3$)	Added (mg l^{-1})	Found[a] (mg l^{-1})	Recovery (%) (R.S.D.; $n = 3$)
Matricaria recutita flower					
Apigenin	402.5	0.92 (4.7)	50.0	48.5	97.1 (2.4)
Luteolin	35.0	0.08 (3.8)	50.0	47.9	95.8 (3.5)
Hypericum perforatum haulm					
Rutin	21.0	1.03 (0.9)	50.0	48.1	96.2 (1.0)
Hyperoside	16.7	0.82 (2.1)	50.0	48.7	97.4 (1.7)

The found (mg l^{-1}) data are related to the plant extract analysed; the (found %) data are related to the mass of the original dried plant material.
[a] Found of added.

4. Conclusions

Addition of molybdate to BGEs affects substantially electrophoretic migration of compounds with *o*-dihydroxy functionalities due to the formation of anionic complexes involving Mo(VI) as central ion whereas, as expected, the mobility of monohydroxy compounds is virtually not influenced. This effect seems to be similar to that of borate but it occurs at substantially lower pH and at lower concentration of Mo(VI) compared to B(III) since the complex Mo(VI) species with o-dihydroxy ligands are generally more stable than analogical B(III) ones [13]. The option of using neutral or slightly acidic BGEs containing molybdate for the separation of aromatic polyhydroxy compounds might be beneficial because in alkaline BGEs with B(III) as complex-forming additive the risk of oxidation of such analytes by atmospheric oxygen increases considerably. Investigations focused on possible effect of Mo(VI) on the CE separation of aliphatic polyhydroxy compounds are in progress.

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