Determination of Some Phenolic Acids in Majorana hortensis by Capillary Electrophoresis with Online Electrokinetic Preconcentration

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JULY 2008

Impact Factor: 2.91 · DOI: 10.1021/jf8000179 · Source: PubMed

7 AUTHORS, INCLUDING:

Jan Petr
Palacký University of Olomouc
48 PUBLICATIONS **483** CITATIONS
SEE PROFILE

Václav Ranc
Palacký University of Olomouc
44 PUBLICATIONS **278** CITATIONS
SEE PROFILE

Joanna Znaleziona
Palacký University of Olomouc
23 PUBLICATIONS **211** CITATIONS
SEE PROFILE

Radim Knob
Palacký University of Olomouc
22 PUBLICATIONS **151** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Some Phenolic Acids in *Majorana hortensis* by Capillary Electrophoresis with Online Electrokinetic Preconcentration

JAN PETR,* KATEŘINA VÍTKOVÁ, VÁCLAV RANC, JOANNA ZNALEZIONA, VÍTĚZSLAV MAIER, RADIM KNOB, AND JURAJ ŠEVČÍK

Department of Analytical Chemistry, Faculty of Science, Palacký University in Olomouc, Třída Svobody 8, CZ-77146 Olomouc, Czech Republic

An online accumulation/mobilization preconcentration technique based on a dynamic pH junction technique and electrokinetic injection was employed for analysis of phenolic acids (sinapic, ferulic, coumarinic, caffeic, syringic, vanillic, and 4-hydroxybenzoic acid) in extracts from *Majorana hortensis* leaves. Samples were extracted by pressurized solvent extraction with acetone at 150 °C and 15 MPa. The capillary electrophoretic method employed 50 mmol·L^{-1} sodium borate, pH 9.5, as the sample electrolyte, 50 mmol·L^{-1} sodium phosphate, pH 2.5, as the background electrolyte, and 50 mmol·L^{-1} sodium phosphate, pH 2.5, with 60 mmol·L^{-1} sodium dodecyl sulfate as the mobilization electrolyte. The method allowed 720-fold to 5560-fold preconcentration of the phenolic acids during 30 min of electrokinetic accumulation with detection limits from 0.38 to 4.22 ng·mL^{-1}.

KEYWORDS: Online preconcentration; capillary electrophoresis; phenolic acids; electrokinetic accumulation; *Majorana hortensis*; plant extracts

INTRODUCTION

Phenolic acids are secondary plant metabolites having hydroxycinnamic or hydroxybenzoic structures. Their role concerns color and sensory characteristics of plants as well as antioxidant properties of plant-based food (*1–3*). This role in the organoleptic properties of foods (flavor, astringency, and hardness) has been a big interest of analytical and food chemists (*4, 5*). Additionally, the content and profile of phenolic acids, their effect on fruit maturation and prevention of enzymatic browning, and their roles as food preservatives have been evaluated too (*6, 7*). Recent interest comes from their potential protective role against diseases that may be related to oxidative damage, such as coronary heart diseases or cancers (*8–10*).

Bendini et al. (*11*) described several issues of why the phenolic acids are interesting; these cover the levels and chemical structure of antioxidant phenols in different plants, the probable role of plant phenols in the prevention of various diseases, the ability of plant phenols to modulate the activity of enzymes, the ability of certain classes of plant phenols such as flavonoids to bind to proteins (this binding is not related to their direct activity as antioxidants), and the preparation of food supplements.

Each task listed above needs an analytical methodology suitable for separation of phenolic compounds usually from a complicated matrix and their identification and quantification (*12–14*). These days, the use of chromatographic methods, both gas chromatography and high-performance liquid chromatography, together with mass spectrometry has been well established (*15–19*). Capillary electrophoresis (CE) represents another alternative for fast and highly efficient separation of compounds of interest. CE usually suffers from a lack of detection sensitivity with use of a common UV−vis detector. On the other hand, CE provides a simple solution by using one of the online preconcentration techniques (*20–22*). Online preconconcentration of phenolic acids was described by isotachophoresis and stacking. Urbánek et al. (*23*) introduced a "background electrolyte−sample−background electrolyte" system with a methanolic sample and a background electrolyte consisting of β-hydroxy-4-morpholinopropanesulfonic acid (MOPSO), tris(hydroxymethyl)aminomethane (Tris), and boric acid at pH 9.0 for plant extracts with limits of detection (LODs) around 50 ng·mL^{-1}. Hamoudová et al. (*24*) used the same system for red wine

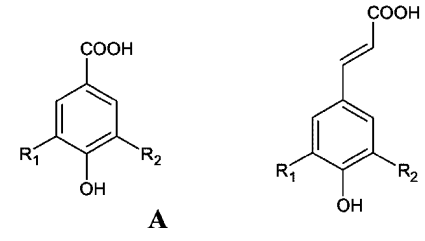

Figure 1. Phenolic acid structures: (**A**) $R_1 = R_2 = H$, 4-hydroxybenzoic acid; $R_1 = CH_3$, $R_2 = H$, syringic acid; $R_1 = H$, $R_2 = OCH_3$, vanillic acid; (**B**) $R_1 = R_2 = H$, coumarinic acid; $R_1 = H$, $R_2 = OH$, caffeic acid; $R_1 = OCH_3$, $R_2 = H$, ferulic acid; $R_1 = OCH_3$, $R_2 = OCH_3$, sinapic acid.

* To whom correspondence should be addressed. Phone: +420585634416. Fax: +420585634433. E-mail: petrjan1@gmail.com.

10.1021/jf8000179 CCC: $40.75
Published on Web 05/13/2008

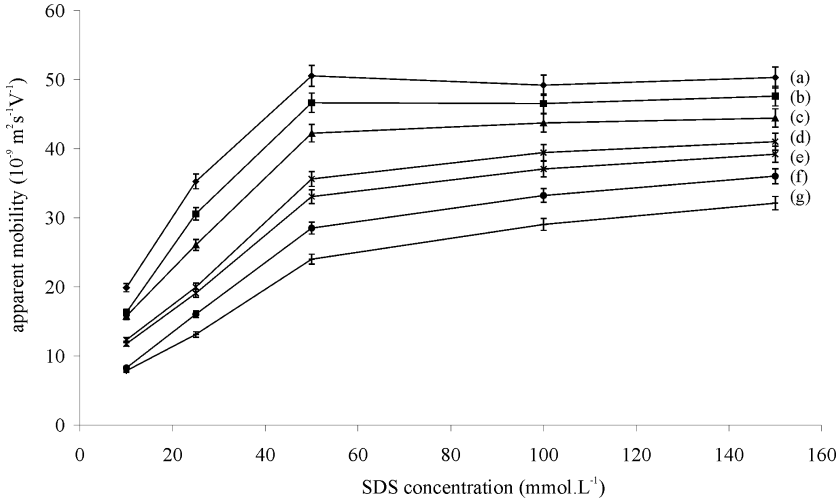

Figure 2. Influence of the SDS concentration on the apparent mobilities of the studied compounds: (a) sinapic, (b) ferulic, (c) coumarinic, (d) caffeic, (e) syringic, (f) vanillic, and (g) 4-hydroxybenzoic acid. Conditions: sample electrolyte, 50 mmol · L^{-1} sodium borate, pH 9.5; junction electrolyte, 50 mmol · L^{-1} sodium phosphate, pH 2.5; mobilization electrolyte, 50 mmol · L^{-1} sodium phosphate, pH 2.5, with SDS; electrokinetic injection, 10 min at −10 kV; separation voltage, −10 kV; standard concentration, 50 ng · mL^{-1}.

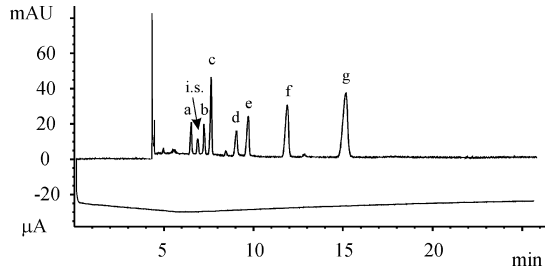

Figure 3. Example of analysis of phenolic acid standards: (a) sinapic, (b) ferulic, (c) coumarinic, (d) caffeic, (e) syringic, (f) vanillic, and (g) 4-hydroxybenzoic acid. Conditions: sample electrolyte, 50 mmol · L^{-1} sodium borate, pH 9.5; junction electrolyte, 50 mmol · L^{-1} sodium phosphate, pH 2.5; mobilization electrolyte, 50 mmol · L^{-1} sodium phosphate, pH 2.5, with 60 mmol · L^{-1} SDS; electrokinetic injection, 10 min at −10 kV; separation voltage, −10 kV; standard concentration, 50 ng · mL^{-1}.

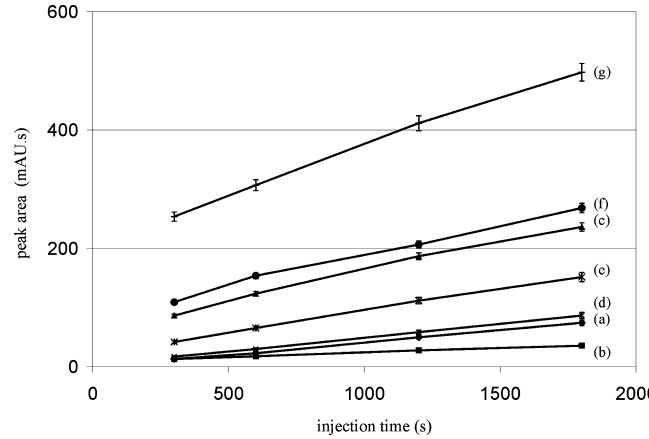

Figure 4. Influence of the injection duration on the peak areas of the studied compounds: (a) sinapic, (b) ferulic, (c) coumarinic, (d) caffeic, (e) syringic, (f) vanillic, and (g) 4-hydroxybenzoic acid. Conditions: sample electrolyte, 50 mmol · L^{-1} sodium borate, pH 9.5; junction electrolyte, 50 mmol · L^{-1} sodium phosphate, pH 2.5; mobilization electrolyte, 50 mmol · L^{-1} sodium phosphate, pH 2.5, with 60 mmol · L^{-1} SDS; electrokinetic injection at −10 kV; separation voltage, −10 kV; standard concentration 50 ng · mL^{-1}.

samples. Šafra et al. (*25*) published an ITP-CE system with column coupling for methanolic extracts and obtained LODs from 5 to 61 ng·mL^{-1}. Deng et al. (*26*) and Kubáň et al. (*27*) used water as a background for the samples, which resulted in stacking conditions. Šafra et al. (*28*) described using a methanolic sample for 40-fold sensitivity enhancement (LODs between 10−66 ng·mL^{-1}).

The aim of our work was to develop a method for determination of some phenolic acids (sinapic, ferulic, coumarinic, caffeic, syringic, vanillic, and 4-hydroxybenzoic acid; for the structures see **Figure 1**) by capillary electrophoresis with online preconcentration via an electrokinetic accumulation/mobilization technique after pressurized solvent extraction. This research followed similar studies on determination of phenolics for purposes of studying antioxidant properties of plants and plant products, such as that published, e.g., by Jirovský et al. (*18*) or by Ayaz et al. (*19*). *Majorana* sp. were chosen as common food supplies that contain these phenolic acids.

MATERIALS AND METHODS

Chemicals. Boric acid, phosphoric acid, sodium hydroxide, and sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO). Standards of phenolic acids, syringic, sinapic, ferulic, vanillic, coumarinic, 4-hydroxybenzoic, and caffeic acid, were also obtained from Sigma. All of the chemicals were of analytical grade purity. *Majorana hortensis* leaves for foods were obtained from the supermarket Globus (Olomouc, Czech Republic).

Capillary Electrophoresis. Separations were performed on the capillary electrophoresis system HP ^{3}DCE (Agilent Technologies, Waldbronn, Germany) with diode array detection; the detection wavelength was 200 nm. Uncoated fused silica capillaries (Polymicro Technologies, Phoenix, AZ), 50 μm i.d., with a total capillary length of 48.5 cm and an effective length of 40.0 cm were used in these experiments. The capillary cassette was thermostated at 20 °C; the applied accumulation and separation voltage was −10 kV.

The capillary was washed daily before the first experiment with 0.1 mol·L^{-1} NaOH (10 min), deionized water (20 min), and then sodium phosphate buffer (10 min). The capillary was washed with 0.1 mol·L^{-1} NaOH (2 min), deionized water (3 min), and then sodium phosphate buffer (5 min) between the individual analysis runs. Electrolytes were prepared by dissolution of appropriate amounts of the acid in deionized water (18 MΩ cm^{-1}, Elga Bucks, U.K.), and the pH was adjusted by 50% (w/w) sodium hydroxide to the desired value; then in the case of mobilization the electrolyte sodium dodecyl sulfate was added. The stock standard solutions of phenolic acids were prepared in 10 μg·mL^{-1}

Table 1. Validation Results

	sinapic acid	ferulic acid	coumarinic acid	caffeic acid	syringic acid	vanillic acid	4-hydroxybenzoic acid
			Precision of Corrected Migration Times [a]				
intraday RSD (%)	1.3	0.9	1.4	1.8	1.9	1.6	2.8
interday RSD (%)	7.6	8.9	11.0	9.7	10.3	9.6	12.1
			Precision of Corrected Peak Areas[a]				
intraday RSD (%)	2.5	3.2	1.9	6.5	6.3	6.6	6.9
interday RSD (%)	12.3	14.5	13.2	15.1	14.6	13.8	14.9
			Recovery, Correlation Coefficients				
recovery (%)	94 ± 2	94 ± 3	97 ± 2	96 ± 3	96 ± 1	98 ± 1	93 ± 3
correlation coefficient	0.984	0.993	0.986	0.993	0.981	0.985	0.976
			Detection and Quantification Limits, Enrichment Factor				
LOD of developed method (ng/mL)	2.23	4.22	0.71	2.04	1.14	0.62	0.38
LOQ of developed method (ng/mL)	7.43	14.07	2.37	6.80	3.80	2.07	1.27
LOD of MEKC method[a] (μg/mL)	1.61	3.29	2.87	3.32	2.89	2.35	2.14
enrichment factor	723	780	4048	1627	2544	3762	5565

[a] For details, see the Materials and Methods.

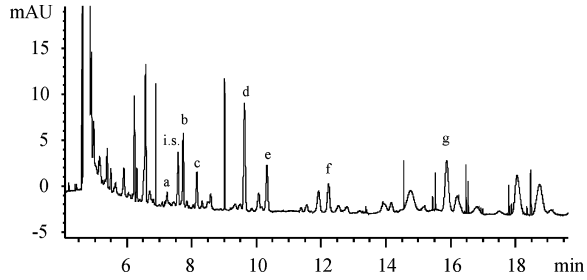

Figure 5. Example of analysis of the *Majorana* extract: (a) sinapic, (b) ferulic, (c) coumarinic, (d) caffeic, (e) syringic, (f) vanillic, and (g) 4-hydroxybenzoic acid. Conditions: sample electrolyte, 50 mmol·L^{-1} sodium borate, pH 9.5; junction electrolyte, 50 mmol·L^{-1} sodium phosphate, pH 2.5; mobilization electrolyte, 50 mmol·L^{-1} sodium phosphate, pH 2.5, with 60 mmol·L^{-1} SDS; electrokinetic injection, 30 min at −10 kV; separation voltage, −10 kV.

and then mixed and diluted to the desired concentrations. All of the measurements were performed five times if not stated otherwise.

Validation experiments and all the real sample measurements were done under the following conditions: sample electrolyte, 50 mmol·L^{-1} sodium borate, pH 9.5; junction electrolyte, 50 mmol·L^{-1} sodium phosphate, pH 2.5; mobilization electrolyte, 50 mmol·L^{-1} sodium phosphate, pH 2.5, with 60 mmol·L^{-1} SDS; electrokinetic injection, 30 min at −10 kV; separation voltage, −10 kV. Identification was done by using UV spectral matching, migration times, and the standard addition method. The statistical program QC.Expert 2.5 (TriloByte Statistical Software, Pardubice, Czech Republic) was used to evaluate the obtained data. Precision values were determined from calibrations from the peak area divided by the area of an internal standard, phenylalanine (25 ng·mL^{-1}). The intraday precision was determined from the calibration curve between 5 and 50 ng·mL^{-1} (four points, each point measured five times). The calibrations were done for 4 days, and the average RSD values were then calculated. The interday precision was determined by taking all the calibrations made in the 4 days. LODs and limits of quantification (LOQs) were estimated by the software QC.Expert as 3σ and 10σ, respectively. Detection limits for micellar electrokinetic chromatography (MEKC) were determined from separate calibrations employing 50 mmol·L^{-1} sodium phosphate, pH 2.5, with 50 mmol·L^{-1} SDS, 10% (v/v) methanol, and a running voltage of 10 kV (for more details see ref *29*). The enrichment factor was calculated as a ratio between the LODs of the proposed preconcentration technique and the LODs of the MEKC method. Recovery was calculated from the addition of 10 ng·mL^{-1} phenolic acid standard to the plant extract.

***Majorana* Samples.** Extracts from *M. hortensis* L. leaves were prepared by using pressurized solvent extraction (PSE) according to the following setup (optimization of the setup is described in ref *29*): 1.0000 g of dried homogenized plant material was weighed directly into the extraction cartridge (total volume 22 mL) and extracted with acetone at 150 °C and 15 MPa by two static cycles (2−10 min) on the automatic extractor *one* PSE (Applied Separations Inc., Allentown, PA). The extracts were collected in glass vials, evaporated using nitrogen flow, and diluted in water−methanol (90:10, v/v). Then they were filtered through a Microcon YM-10 filter (regenerated cellulose, 10 000, Millipore Corp., Bedford, MA). Finally, the extracts were diluted in 50 mM sodium borate, pH 9.5.

RESULTS AND DISCUSSION

Development and Optimization of the Method. The online accumulation/mobilization preconcentration technique for weak acids introduced by Horáková et al. (*30*) is based on two steps: first, negatively charged analytes in alkaline buffer are electrokinetically introduced into the pH boundary (formed by acidic buffer); here they are neutralized and stacked (accumulation part). After the accumulation, the inlet vial is changed to the vial with acidic buffer with SDS. Partitioning between micelles and the free electrolyte causes mobilization and separation of the analytes.

The influence of the concentration and pH of the sample electrolyte, pH of the junction electrolyte, concentration of SDS, separation voltage, and injection voltage was studied to obtain the best separation and preconcentration conditions.

Concentrations of 100, 75, 50, and 25 mmol·L^{-1} sodium borate electrolyte at pH 9.5 were tested. The higher conductivity of highly concentrated electrolytes (100 and 75 mmol·L^{-1}) was reflected in higher currents during the accumulation that caused instabilities in the process because of heat generation. A 50 mmol·L^{-1} concentration of electrolyte was chosen as the best because higher responses of the acids were given in this electrolyte in comparison with a 25 mmol·L^{-1} concentration (the preconcentration was more effective).

pH, the main characteristic attribute of the junction electrolytes, was studied in both electrolytes. The pH has to be chosen in accordance with the buffering capacity of the electrolyte; phosphoric acid has pK_a values of 2.16, 7.21, and 12.32, and boric acid has a pK_a equal to 9.27 (*31*). Therefore, the following pH values were studied: 50 mM sodium phosphate, pH 2.0, 2.5, and 3.0; 50 mM sodium borate, pH 9.0, 9.5, and 10.0. pH values of 2.0 and 3.0 caused instabilities in the currents, and intraday repeatability of the peak areas was worse than 20%. A pH value of 10 resulted in small injected amounts. pH values of 9.5 and 9.0 seemed

to be equal for preconcentration. A pH value of 9.5 was chosen because the value is closer to the pK_a value (higher buffering capacity).

The influence of the SDS concentration was examined in the range 10−150 mmol·L^{-1}. Because the separation mechanism was partitioning between micelles and the free electrolyte, this parameter was very important. It was observed that the highest SDS concentration led to the fastest and the most efficient separation (**Figure 2**); 100 mmol·L^{-1} SDS was chosen because it gave results similar to those of 150 mmol·L^{-1} SDS but with less additive consumption. However, in the case of analysis *Majorana* extracts an incomplete separation of phenolic acids from the matrix was observed. This situation needed a titration of the SDS concentration, resulting in using 60 mmol·L^{-1} SDS solutions. Within these conditions, it was possible to separate both phenolic acids and matrix components.

The last studied parameter was the accumulation and separation voltage. In both tasks, higher voltage provided higher current (see the current profile in **Figure 3**) that was reflected in instabilities of the accumulation and separation process. Currents lower than 50 μA, which corresponded to voltages lower than −15 kV, enabled reproducible separation, while higher currents were reflected in the formation of spikes because of Joule heating. A voltage of −10 kV was chosen as a compromise between the system stability and the loaded amount of the analytes. The influence of the injection duration within −10 kV was studied in the range of 5−30 min with 50 ng·mL^{-1} standards (**Figure 4**). The peak areas increased linearly; 30 min was chosen because it provides the highest responses of the acids. Differences in the injection time did not have a significant effect on the migration times. Longer injections were not tested because they suppress one of the applauded characteristics of CE—the speed.

An example of separation of the phenolic acid standards is displayed in **Figure 3**. The internal standard phenylalanine at a concentration of 25 ng·mL^{-1} was added to the standard mixture of phenolic acids for accurate concentration determination.

Analysis of *Majorana* Extracts. The suggested online preconcentration technique was tested on an analysis of real samples—PSE extracts of *M. hortensis* leaves. Validation of the electrophoretic method was done before determination of the phenolic acid content. All the calculations were done using phenylalanine as the internal standard. The most important parameters are listed in **Table 1**. Calibrations were linear between 2 and 100 ng·mL^{-1} (six points, $n = 5$), with correlation coefficients between 0.976 and 0.993. No significance of an absolute parameter was found at the 0.05 confidence level. As can be seen from **Table 1**, the method allowed determination of phenolic acids in units of nanograms per milliliter (LODs were 0.4−4.2 ng·mL^{-1}), which was a 723-fold to 5565-fold improvement of the detection limits in comparison with those of the simple MEKC method. Moreover, the LODs were approximately 10 times lower than the LODs already published for electrophoretic techniques.

An example of analysis of the *Majorana* extract is displayed in **Figure 5**. Analyzed leaves contained 11 ± 2 μg·g^{-1} sinapic acid, 61 ± 12 μg·g^{-1} ferulic acid, 7 ± 2 μg·g^{-1} coumarinic acid, 78 ± 11 μg·g^{-1} caffeic acid, 16 ± 4 μg·g^{-1} syringic acid, 15 ± 4 μg·g^{-1} vanillic acid, and 29 ± 5 μg·g^{-1} 4-hydroxybenzoic acid. These results reflected the antioxidant potential of the *Majorana* sample. The phenolic acid content in *Lamiaceae* is quite broad (*16, 17, 32–34*) because the different growth regions affected the amount of phenolics. The effects of growing are of interest to researchers now, and the capillary electrophoretic method presented in this paper can be used to contribute to this task. In our opinion, the method can also be used for analyzing a broad variety of PSE extracts from plant materials for determination of the phenolic acids. Moreover, longer injection times can lead to lower detection and quantification limits, which can be important in some special cases, e.g., metabolic studies.

LITERATURE CITED

(1) Maga, J. A. Simple phenol and phenolic compounds in food flavor. *Crit. Rev. Food Sci. Nutr.* **1978**, *10*, 323–372.

(2) Nergiz, C.; Unal, K. Determination of phenolic acids in virgin olive oil. *Food Chem.* **1991**, *39*, 237–240.

(3) Moure, A.; Cruz, J. M.; Franco, D.; Domínguez, J. M.; Sineiro, J.; Domínguez, H.; Núñez, M. J.; Parajó, J. C. Natural antioxidants from residual sources. *Food Chem.* **2001**, *72*, 145–171.

(4) Peleg, H.; Naim, M.; Rouseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J. Sci. Food Agric.* **1991**, *57*, 417–426.

(5) Canas, S.; Belchior, A. P.; Spranger, M. I.; Bruno-de-Sousa, R. High performance liquid chromatography for analysis of phenolic acids, phenolic aldehydes, and furanic derivatives in brandies. Development and validation. *J. Sep. Sci.* **2003**, *26*, 496–502.

(6) Robbins, R. J. Phenolic acids in foods: an overview of analytical methodology. *J. Agric. Food Chem.* **2003**, *51*, 2866–2887.

(7) Nurmi, A.; Nurmi, T.; Mursu, J.; Hiltunen, R.; Voutilainen, S. Ingestion of oregano extract increases excretion of urinary phenolic metabolites in humans. *J. Agric. Food Chem.* **2006**, *54*, 6916–6923.

(8) Masaki, H.; Okamoto, N.; Sakaki, S.; Sakurai, H. Protective effects of hydroxybenzoic acids and their esters on cell damage induced by hydroxyl radicals and hydrogen peroxides. *Biol. Pharm. Bull.* **1997**, *20*, 304–308.

(9) Gomes, C. A.; Girao da Cruz, T.; Andrade, J. L.; Milhazes, N.; Borges, F.; Marques, M. P. M. Anticancer activity of phenolic acids of natural or synthetic origin: a structure-activity study. *J. Med. Chem.* **2003**, *46*, 5395–5401.

(10) Nichenametla, S. N.; Taruscio, T. G.; Barney, D. L.; Exon, J. L. A review of the effects and mechanisms of polyphenolics in cancer. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 161–183.

(11) Bendini, A.; Cerretani, L.; Carrasco-Pancorbo, A.; Gómez-Caravaca, A. M.; Segura-Carretero, A.; Fernández-Gutiérrez, A. Lercker, G. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules* **2007**, *12*, 1679–1719.

(12) Robbins, R. J. Phenolic acids in foods: an overview of analytical methodology. *J. Agric. Food Chem.* **2003**, *51*, 2866–2887.

(13) Stalikas, C. D. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci.* **2007**, *30*, 3268–3295.

(14) Naczk, M.; Shahidi, F. Extraction and analysis of phenolics in food. *J. Chromatogr., A* **2004**, *1054*, 95–111.

(15) Waksmundzka-Hajnos, M. Chromatographic separations of aromatic carboxylic acids. *J. Chromatogr., B* **1998**, *717*, 93–118.

(16) Fiamegos, Y. C.; Nanos, C. G.; Vervoort, J.; Stalikas, C. D. Analytical procedure for the in-vial derivatization-extraction of phenolic acids and flavonoids in methanolic and aqueous plant extracts followed by gas chromatography with mass-selective detection. *J. Chromatogr., A* **2004**, *1041*, 11–18.

(17) Kivilompolo, M.; Obůrka, V.; Hyötyläinen, T. Comparison of GC-MS and LC-MS methods for the analysis of antioxidant phenolic acids in herbs. *Anal. Bioanal. Chem.* **2007**, *388*, 881–887.

(18) Jirovský, D.; Horáková, D.; Kotouček, M.; Valentová, K.; Ulrichová, J. Analysis of phenolic acids in plant materials using HPLC with amperometric detection at a platinum tubular electrode. *J. Sep. Sci.* **2003**, *26*, 739–742.
(19) Ayaz, F. A; Hayirlioglu-Ayaz, S.; Alpay-Karaoglu, S.; Grúz, J.; Valentová, K.; Ulrichová, J.; Strnad, M. Phenolic acid contents of kale (*Brassica oleracea* L. var. *acephala* DC.) extracts and their antioxidant and antibacterial activities. *Food Chem.* **2008**, *107*, 19–25.
(20) Simpson, S. L.; Quirino, J. P.; Terabe, S. On-line sample preconcentration in capillary electrophoresis: fundamentals and applications. *J. Chromatogr., A* **2008**, *1184*, 504–541.
(21) Malá, Z.; Křivánková, L.; Gebauer, P.; Boček, P. Contemporary sample stacking in CE: A sophisticated tool based on simple principles. *Electrophoresis* **2007**, *28*, 243–253.
(22) Lin, C.-H.; Kaneta, T. On-line sample concentration techniques in capillary electrophoresis: Velocity gradient techniques and sample concentration techniques for biomolecules. *Electrophoresis* **2004**, *25*, 4058–4073.
(23) Urbánek, M.; Pospíšilová, M.; Polášek, M. On-line coupling of capillary isotachophoresis and zone electrophoresis for the assay of phenolic compounds in plant extracts. *Electrophoresis* **2002**, *23*, 1045–1052.
(24) Hamoudová, R.; Urbánek, M.; Pospíšilová, M.; Polášek, M. Assay of phenolic compounds in red wine by on-line combination of capillary isotachophoresis with capillary zone electrophoresis. *J. Chromatogr., A* **2004**, *1032*, 281–287.
(25) Šafra, J.; Pospíšilová, M.; Spilková, J. Determination of phenolic acids in Herba Epilobi by ITP-CE in the column-coupling configuration. *Chromatographia* **2006**, *64*, 37–42.
(26) Deng, Y.; Fan, X.; Delgado, A.; Nolan, C.; Furton, K.; Zuo, Y.; Jones, R. D. Separation and determination of aromatic acids in natural water with preconcentration by capillary zone electrophoresis. *J. Chromatogr., A* **1998**, *817*, 145–152.
(27) Kubáň, P.; Štěrbová, D.; Kubáň, V. Separation of phenolic acids by capillary electrophoresis with indirect contactless conductometric detection. *Electrophoresis* **2006**, *27*, 1368–1375.
(28) Šafra, J.; Pospíšilová, M.; Kavalírová, A. Development of a stacking-CZE method for the analysis of phenolic acids. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1022–1024.
(29) Vítková, K.; Ranc, V.; Petr, J.; Maier, V.; Čáp, L.; Barták, P. Determination of some phenolic acids in *Majorana hortensis*: a comparison of separation techniques Manuscript in preparation.
(30) Horáková, J.; Petr, J.; Maier, V.; Tesařová, E.; Veis, L.; Armstrong, D. W.; Gaš, B.; Ševčík, J. On-line preconcentration of weak electrolytes by electrokinetic accumulation in CE: Experiment and simulation. *Electrophoresis* **2007**, *28*, 1540–1547.
(31) Lide, D. R. Ed. *CRC Handbook of Chemistry and Physics*; CRC Press: Boca Raton, FL, 2004.
(32) Štěrbová, D.; Matějíček, D.; Vlček, J.; Kubáň, V. Combined microwave-assisted isolation and solid-phase purification procedures prior to the chromatographic determination of phenolic compounds in plant materials. *Anal. Chim. Acta* **2004**, *513*, 435–444.
(33) Zgórka, G.; Glowniak, K. Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. *J. Pharm. Biomed. Anal.* **2001**, *26*, 79–87.
(34) Sun, R.-C.; Sun, X.-F.; Zhang, S.-H. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *J. Agric. Food Chem.* **2001**, *49*, 5122–5129.

Received for review January 3, 2008. Revised manuscript received March 3, 2008. Accepted March 18, 2008. Financial support of this research by Research Project MSM6198959216 and Grant 203/07/P233 is gratefully acknowledged.

JF8000179