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Short communication

# Analysis of aromatic plant acids by capillary zone electrophoresis

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**Abstract**

The use of capillary zone electrophoresis is described for the separation and identification of selected mixtures of aromatic plant acids. The influence of voltage, temperature, buffer systems and buffer pH on the migration order and migration times was investigated. The best separation was obtained with a fused-silica capillary column (64.5 cm×50 μm I.D.), a running buffer of 20 m*M* sodium phosphate (pH 7.0), an applied voltage of 25 kV and a temperature of 40°C, combined with diode array detection. Using a methanolic-aqueous extract of *Epilobium angustifolium* L. as an example, the possibility of using the method developed for the identification of plant acids in plant material is demonstrated. Ferulic, gallic, protocatechuic, cinnamic, caffeic, gentisic and chlorogenic acids could be identified. © 1998 Elsevier Science B.V.

*Keywords:* Organic acids; Benzoic acids; Cinnamic acids

## 1. Introduction

Aromatic plant acids are widespread secondary plant metabolites and have many different physiological properties. Apart from the variety of roles in the plant kingdom, some plant acids possess chemotaxonomic importance as well as pharmacological properties. Therefore, analysis of them is of considerable interest. Most aromatic plant acids exist as derivatives of benzoic acid or cinnamic acid. The existing methods used for the analysis of aromatic plant acids are generally high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC) [1–6]. However, capillary electrophoresis is an efficient method with additional possibilities for the analysis of medicinal plants [7–9]. Because of the high-resolution separation, simplicity of operation, versatility and sensitivity, capillary zone electrophoresis (CZE) is a widely used mode and is attractive in this area. Several aromatic plant acids were separated by isotachophoresis, micellar electrokinetic capillary chromatography (MECC) and reversed electroosmotic flow capillary electrophoresis [10–12]. As in plant extracts, when many different compounds (charged and noncharged) are present, CZE is an ideal tool for the separation of charged from neutral solutes. Charged compounds are separated based on variations in mass-to-charge ratios. Neutral solutes coelute with the electroosmotic flow (EOF). In this paper, the separation and identification of a mixture of common plant acids (Table 1) are studied, using CZE combined with diode array detection (DAD). Primarily, the investigations aimed at the application of the developed CZE system for the analysis of free aromatic acids in plant extracts. The influence of

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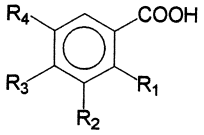
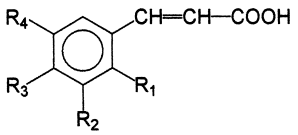
Table 1
Selected plant acids, their UV maxima and migration times ($t_m$).




**I**




**II**

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $UV_{max}$ (nm) | $t_m$ (min) |
|---|---|---|---|---|---|---|
| *Benzoic acid derivatives I* | | | | | | |
| Syringic (1) | H | $CH_3O$ | $CH_3O$ | OH | 260.5 | 6.90 |
| Veratrum (2) | H | $CH_3O$ | $CH_3O$ | H | 250.5/282.5 | 7.30 |
| Vanillic (3) | H | $CH_3O$ | OH | H | 250.5/284.5 | 7.51 |
| Protocatechuic (4) | H | OH | OH | H | 248.5/286.5 | 7.79 |
| Anisic (5) | H | H | $CH_3O$ | H | 246.5 | 8.27 |
| Gentisic (6) | OH | H | H | OH | 230.5/318.5 | 8.93 |
| Benzoic (7) | H | H | H | H | 224.5/258.5 | 9.58 |
| *Cinnamic acid derivatives II* | | | | | | |
| Chlorogenic (8) | H | H | OH | OH | 300.5/322.5 | 8.16 |
| Sinapic (9) | H | $CH_3O$ | OH | $CH_3O$ | 296.5 | 9.61 |
| Ferulic (10) | H | $CH_3O$ | OH | H | 278.5/304.5 | 10.46 |
| Caffeic (11) | H | OH | OH | H | 284.5/300.5 | 11.17 |
| *p*-Coumaric (21) | H | H | OH | H | 276.5 | 12.03 |
| *m*-Coumaric (13) | H | OH | H | H | 268.5 | 12.19 |
| *o*-Coumaric (14) | OH | H | H | H | 266.5/308.5 | 12.35 |
| Cinnamic (15) | H | H | H | H | 264.5 | 13.96 |

different separation conditions (voltage, temperature, buffer) is described and the CZE method was extended to the separation and identification of a number of aromatic plant acids in *Epilobium angustifolium* [13,14].

## 2. Experimental

### *2.1. Apparatus*

CZE was performed on a Hewlett-Packard $H^{3D}$ capillary electrophoresis system, serial No. 3320 G00139, using a fused-silica capillary column (64.5 cm×50 μm I.D.). Detection was at 280 nm and by DAD, applied voltages were 5–30 kV, and the temperatures used were 30 and 40°C. Analytes were injected in the hydrostatic mode using 50.0 mbar for 2 s. Data were processed on a HP Vectra 486/66 XM. For daily preconditioning, the capillary tubes were flushed with 1 *M* NaOH (10 min), 0.1 *M* NaOH (10 min) and water (10 min). To maintain the capillary conditions, capillary tubes were flushed with buffer at pH 7 for 2 min.

### *2.2. Reagents*

Sodium phosphate buffers (20 m*M*), pH 5–8.5, 20 m*M* sodium borate buffer, pH 8.5, and 0.1 and 1 *M* sodium hydroxide solutions were obtained from Fluka, Buchs, Switzerland. Water was of HPLC grade (Merck, Darmstadt, Germany). The plant acids (Table 1, 1–15) were obtained from Sigma (St. Louis, MO, USA). The purchased plant acids were dissolved in 70% aqueous methanol at a concentration of 1.5 mg/ml.

### *2.3. Sample preparation*

A 3-g amount of powdered herb from *Epilobium angustifolium* L. was extracted with 50 ml of 50% aqueous methanol for 1 h at 50°C. After filtration, the solution was evaporated to dryness in vacuo and the residue was dissolved in 5 ml of methanol.

## 3. Results and discussion

Using the CZE technique under the conditions investigated, separation of all benzoic acid- and cinnamic acid derivatives (except for the separation of *o*- and *p*-coumaric acid) was achieved within 15 min (Figs. 1 and 2). Retention times for the plant acids with only a hydroxyl group (except gallic acid) were higher than of those with substituted aromatic rings. In the case of compounds with the same substitution pattern, methoxylated acids migrated faster than the corresponding hydroxylated ones. The analytical conditions had been varied previously to optimize the separation. Changes in buffer pH, voltages, temperature and the nature of the running electrolyte had an influence on the total migration time and on the separation of all plant acids. Migration times decreased with increasing pH values. The best separation was achieved at pH 7. Alterations above and below pH 7 did not optimize the separation. Increasing the voltage from 5 to 30 kV gave non-linear decreases in migration times. The migration order of the plant acids did not change with increasing voltage. Variations of temperature from 30 to 40°C showed that migration times of the plant acids studied decreased with increasing temperature. On changing the non-complexing electrophoretic sodium phosphate buffer to borate buffer (pH 8.5), the plant acids with vicinal hydroxyl groups could not be separated from the others under the same conditions (20 m*M* buffers, 30 kV, 40°C) in one run. This behaviour is attributed to borate complexation. It is well known that certain polyhydroxy compounds react with borate ions to form complexes that are negatively charged. For the optimum detection and for identification of analysed plant acids, DAD with a wavelength range from 200 to 400 nm was appropriate. The determined wavelength to give maximum absorbance for all of the analytes is shown in Table 1. Finally, optimum separation was obtained using the parameters mentioned in Figs. 1 and 2. With respect to these results, it can be concluded that the developed CZE method is a valuable alternative to HPLC for the separation of aromatic plant acids. Using this method, ferulic, gallic, protocatechuic, cinnamic, caffeic, gentisic and chlorogenic acids could be identified from *Epilobium angustifolium* (Fig. 3). The plant acids were iden-

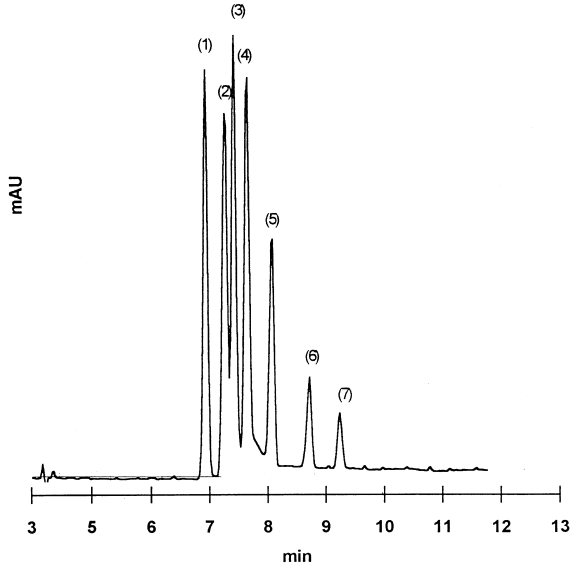



Fig. 1. CZE separation of benzoic acid derivatives. Capillary, 64.5 cm×50 μm I.D.; applied voltage, 25 kV; running electrolyte, 20 m*M* $Na_3PO_4$ (pH 7); temperature, 40°C; detection, 280 nm (mixed wavelength for all plant acids). For peak identification, see Table 1.

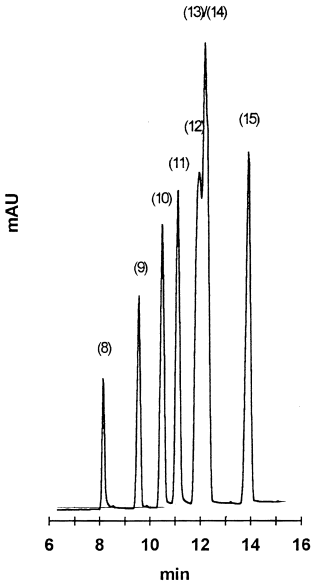



Fig. 2. CZE separation of cinnamic acid derivatives. For the conditions used, see Fig. 1. For peak identification, see Table 1.

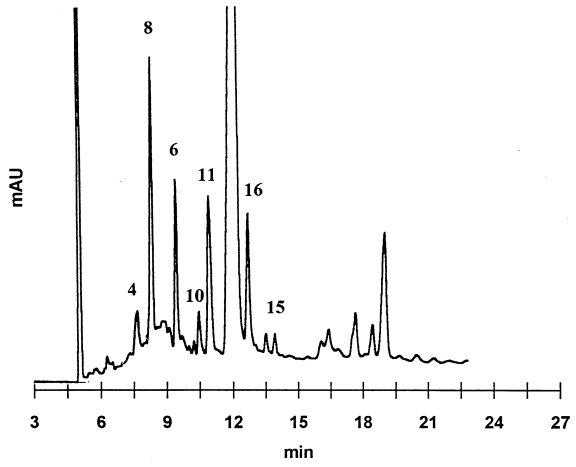



Fig. 3. Electropherogram of an extract from *Epilobium angustifolium*. For the conditions used, see Fig. 1. For peak identification, see Table 1. Peak 16=gallic acid.

tified by comparison with authentic compounds on the basis of migration time and their on-line UV spectra (DAD).

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