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Short communication

Capillary electrophoretic determination of sanguinarine and chelerythrine in plant extracts and pharmaceutical preparations

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Abstract

Capillary electrophoresis was employed to determine the principal quaternary benzo[*c*]phenanthridine alkaloids, sanguinarine and chelerythrine, in two plant extracts and one oral hygiene product. Phosphate–Tris buffer of pH 2.5 was used as a background electrolyte, limits of detection were 3 $\mu mol\ l^{-1}$ (sanguinarine) and 2.4 $\mu mol\ l^{-1}$ (chelerythrine) using UV detection at 270 nm. The method, which correlated well with HPLC, is suitable for serial determination of sanguinarine and chelerythrine in plant products and pharmaceuticals. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Plant materials; Pharmaceutical analysis; Sanguinarine; Chelerythrine; Alkaloids

1. Introduction

Benzo[*c*]phenanthridinium alkaloids exhibit multiple biological and pharmaceutical effects such as anti-inflammatory [1] and antimicrobial [2] activity; inhibit SH-enzymes, i.e., chelerythrine is a specific protein kinase C inhibitor [3]; inhibit microtubule assembly [4] and interact with DNA [5]. At present, fractions containing benzo[*c*]phenanthridinium alkaloids, mainly sanguinarine and chelerythrine (Fig. 1), isolated from *Sanguinaria canadensis* L. or *Macleaya cordata* (Wild.) Br. R. are used in a number of commercial tooth pastes and oral rinses for their antiplaque effect [6]. Another field of application of these alkaloids are pharmaceutical preparations with cholagogue, choleretic and spasmolytic effects containing extracts from *Chelidonium majus* L. [7]. Very recently a discussion has been re-opened questioning toxicity of benzo[*c*]phenanthridinium alkaloids that has been focused mainly on two topics: (i) epidemic dropsy, a clinical condition resulting from the consumption of mustard oil accidentally or deliberately contaminated by argemone oil, (ii) use of oral hygiene products containing sanguinarine as an antiplaque agent [8,9]. Irrespective of the outcome of the current review of sanguinarine toxicity there is a need for a rapid and simple method allowing simultaneous determination of micromolar concentrations of sanguinarine and chelerythrine, the two alkaloids that always occur together in plants.

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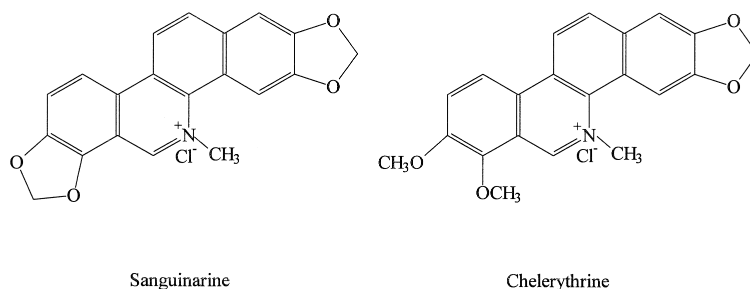

Fig. 1. Molecular structures of sanguinarine and chelerythrine.

To date, the determination of benzo[*c*]phenanthridinium alkaloids in plant extracts and pharmaceuticals has been mostly performed by high-performance thin-layer chromatography [10], high-performance liquid chromatography (HPLC) [11] and isotachophoresis [12,13]. Recently, capillary electrophoresis (CE) has gained in interest, with growing attention to alkaloid analysis in general [14–18], conditions for the separation of sanguinarine in the mixture of berberine alkaloids has recently been described [19]. In the present contribution, we describe the development of a simple and rapid screening method for the simultaneous determination of sanguinarine and chelerythrine by capillary zone electrophoresis. We have compared this method with the HPLC when analyzing two medicinal plant extracts and one commercial oral rinse.

2. Experimental

2.1. Chemicals

The alkaloids sanguinarine chloride, chelerythrine chloride, fagaronine chloride (2-hydroxy-3,8,9-trimethoxy-5-methylbenzo[*c*]phenanthridinium chloride) and chelidonine {(+)-11-hydroxy-2,3,7,8-bis-(methylendioxy) - 5 - methylhexahydrobenzo[*c*]phenanthridine} were obtained from the collection of the Institute of Medical Chemistry and Biochemistry, Olomouc, Czech Republic. QBA fractions of extracts from aerial parts of *Macleaya cordata* (Wild.) Br. R and from the whole plant of *Dicranostigma lactucoides* Hook F. et Thoms, used for analysis, were obtained according to Ref. [20]: dried plant material was extracted with ethanol in a Soxhlet apparatus. After evaporation, the residue was treated with 1% H_2SO_4. The acidic solution was made alkaline with Na_2CO_3 and extracted successively with diethyl ether. After evaporation, the residue was dissolved in acetic acid, diluted with water and filtered to remove nonbasic substances. The solution was then made alkaline with Na_2CO_3 and the bases were extracted successively with diethyl ether. Diethyl ether was evaporated and the residue was dissolved in dilute acetic acid. After the addition of water and conc. HCl, the crystalline chloride QBA fraction was filtered and dried. As for oral rinse, a commercial product Dentosan (Montefarmaco, Pero, Italy) was used for analysis. All other chemicals used were of analytical grade and were purchased from Merck (Darmstadt, Germany). Deionised water (Elga, 18 MΩ cm) was used for preparation of all solutions.

2.2. Apparatus and conditions

2.2.1. Capillary electrophoresis

All experiments were performed on a modular system of SpectraPhoresis 100 equipped with a fast scanning SpectraFocus detector (Thermo Separation Products, Fremont, CA, USA). The electrophoretic separations were carried out in an uncoated fused-silica capillary (75 μm I.D.×375 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) with UV

detection over the range 200–320 nm at ambient temperature (25°C). The capillary had an effective length of 45 cm (total length 75 cm). A constant field strength of 400 V cm^{-1} was applied. Sample loading was done by vacuum injection (0.3 s). Sample preparation: QBA fractions of plant extracts were dissolved in water (for concentrations see Table 1) and filtered through a 0.45-μm membrane filter (Millipore, Milford, MA, USA) before loading. The oral rinse was diluted with water (1:1) and filtered as above.

Phosphate–Tris buffer [50 m*M* phosphoric acid, pH adjusted to 2.5 with tris(hydroxymethyl)aminomethane, 50% (v/v) acetonitrile] was used for all experiments as background electrolyte. At the beginning of each working day, the capillary was washed with water, potassium hydroxide (0.1 *M*), water, and separation buffer for 5 min, and after each electrophoretic run the capillary was washed with water, potassium hydroxide, water and separation buffer for 2 min. These standard conditions were used for all electrophoretic experiments. A calibration curve method was used for the quantitative determination of alkaloids, corrected peak areas at 270 nm being used for quantification. Five parallel determinations were run for each sample.

2.2.2. High-performance liquid chromatography

The HPLC analysis was carried out on a Shimadzu Class LC-10AT (Kyoto, Japan) apparatus equipped with a 250×4 mm steel column filled with Nucleosil 120-5 C_{18} (Macherey–Nagel, Düren, Germany) sorbent, particle size 5 μm, and a diode array SPD-M10Avp detector (Shimadzu). The solvent system was methanol–water–triethylamine (78:22:0.1, v/v/v) adjusted to pH 6.15 with phosphoric acid, flow-rate 1 ml/min, temperature 40°C, detection at 270 nm. Sample preparation: QBA fractions of plant extracts were dissolved in the mobile phase (for concentrations see Table 1), the solution was filtered through a 0.45 μm membrane filter and 20-μl volumes were injected. The oral rinse was diluted with the mobile phase (1:1), the filtration and injection as above. A calibration curve method was used for quantitative determination of alkaloids. Five parallel determinations were run for each sample.

3. Results and discussion

The three-dimensional plot of capillary electropherograms of a standard mixture of sanguinarine, chelerythrine, chelidonine and fagaronine (Fig. 2) displays a good separation of all alkaloids. The last two can be used as internal standards for determination of sanguinarine and chelerythrine. Linearity response and detection limits were examined with authentic compounds in water solution. A linear

Table 1
Sanguinarine (SA) and chelerythrine (CHE) content in analyzed QBA fractions of plant extracts and oral rinse determined by capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) expressed as concentrations±SD (mg ml^{-1})

QBA fraction (mg ml^{-1})	CE		HPLC	
	SA	CHE	SA	CHE
Macleaya cordata (0.0973)	0.0629 ±0.0009	0.0239 ±0.0004	0.0623 ±0.0002	0.0214 ±0.0001
Dicranostigma lactucoides (0.0497)	0.0059 ±0.0009	0.0445 ±0.0004	0.0053 ±0.0002	0.0424 ±0.0001
Dicranostigma lactucoides (0.3128)	0.0366 ±0.0009	0.2800 ±0.0004	0.0380 ±0.0002	0.2865 ±0.0001
Oral rinse[a]	1.1068 ±0.0118	0.9686 ±0.0096	1.0960 ±0.0066	0.9380 ±0.0042

[a] Containing 3 g of fluid extract from *Sanguinaria canadensis* in 100 ml.

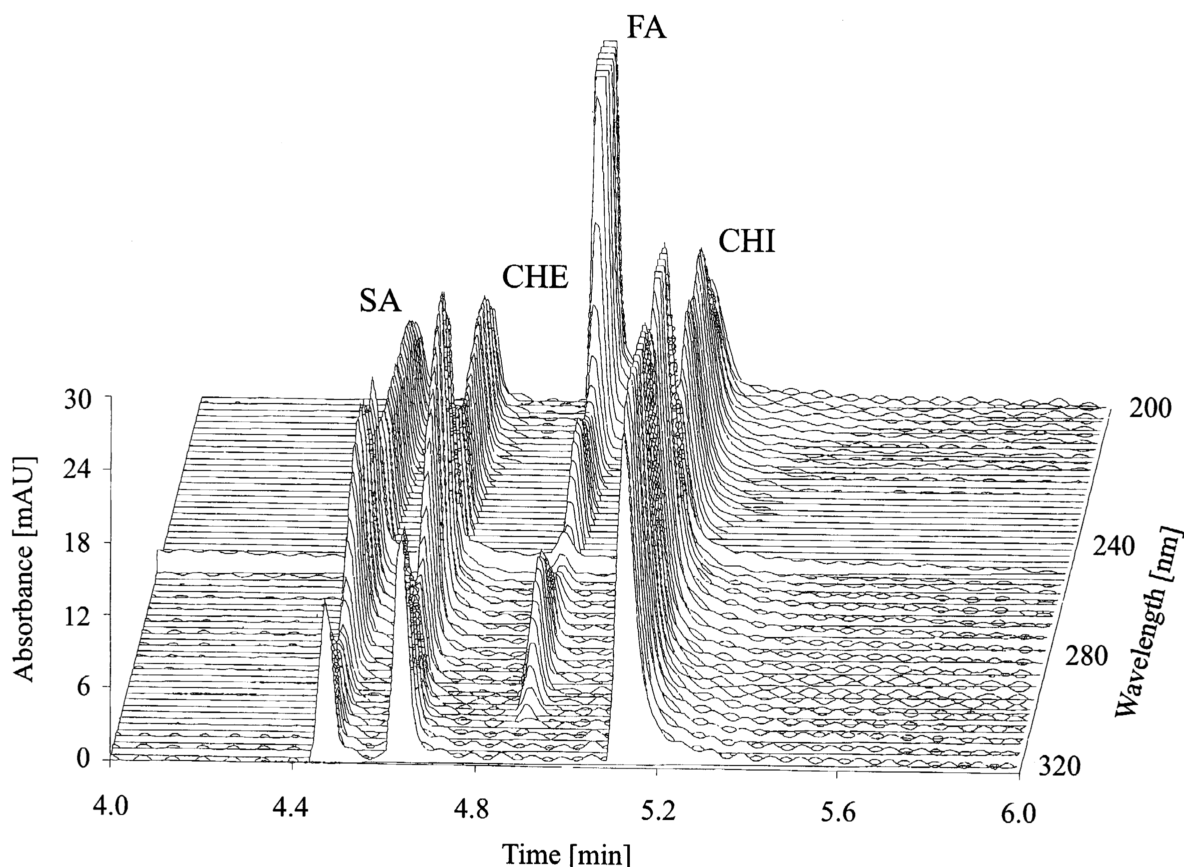

Fig. 2. Three-dimensional plot of separation of authentic compounds of sanguinarine (SA), chelerythrine (CHE), fagaronine (FA) and chelidonine (CHI). Experimental conditions: phosphate–Tris buffer [pH 2.5, 50 m*M*, 50% (v/v) acetonitrile], V=400 V cm^{-1}, ambient temperature.

correlation from 20 to 500 μmol l^{-1} was found for sanguinarine (y=2.373x+0.0065, R^2=0.9988) and chelerythrine (y=2.983x+0.0019, R^2=0.9991), based on peak areas. The limits of detection at 270 nm for sanguinarine and chelerythrine were 3 μmol l^{-1} and 2.4 μmol l^{-1}, respectively. The run-to-run reproducibilities were tested at 100 μmol l^{-1} (n=8). The relative standard deviations for the migration times of both alkaloids were 1.8% and for the concentration were 4.9% for sanguinarine and 4.4% for chelerythrine.

In HPLC, a linear correlation over the concentration range as above was found both for sanguinarine (y=18.35x−92.61, R^2=0.9999) and chelerythrine (y=14.91x−29.97, R^2=0.9999), based on peak areas. The limit of detection for both alkaloids was 3 μmol l^{-1}. The relative standard deviations (run-to-run reproducibilities measured as above) for the concentration were 5.9% for sanguinarine and 4.6% for chelerythrine.

Both CE and HPLC methods were then applied for quantitative analysis of sanguinarine and chelerythrine in plant products (Fig. 3); the results are given in Table 1. Both methods yield comparable results, mutual correlation is expressed by equation y=0.9943x+0.0009, R^2=0.9999 (n=16).

The suggested CE method is appropriate for a serial determination of sanguinarine and

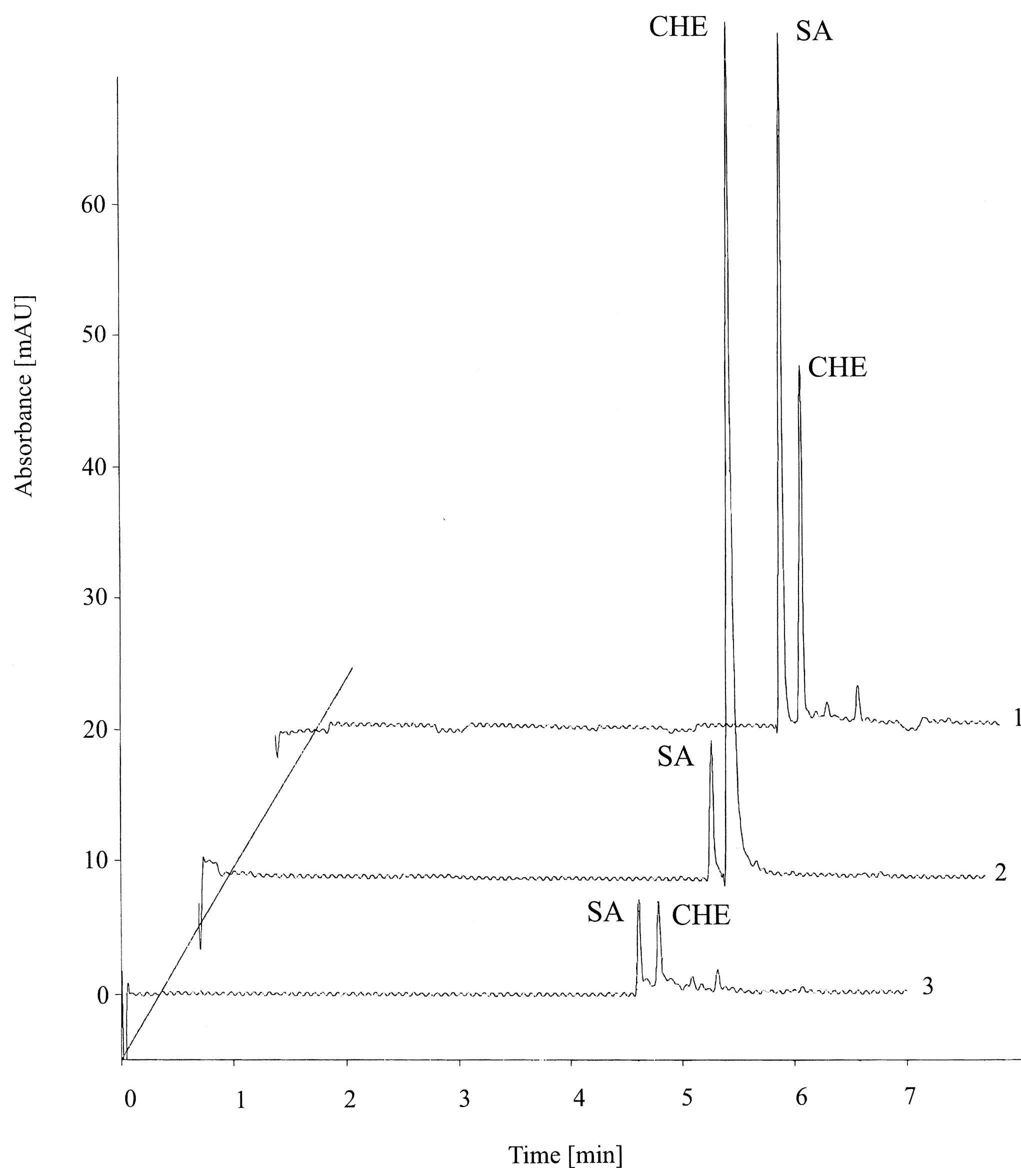

Fig. 3. Capillary electropherograms of *Macleaya cordata* (1) and *Dicranostigma lactucoides* (2) QBA fractions, and of a 1:1 diluted oral rinse (3). SA, sanguinarine; CHE, chelerythrine. Experimental conditions: phosphate–Tris buffer [pH 2.5, 50 m*M*, 50% (v/v) acetonitrile], V=400 V cm^{-1}, λ=260 nm, ambient temperature.

chelerythrine in plant products and pharmaceutical preparations since it is reliable, does not require a pretreatment and analysis time and costs are lower than with HPLC.

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