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Journal of Chromatography A, 717 (1995) 271–277

JOURNAL OF  
CHROMATOGRAPHY A

# Application of $\beta$ -cyclodextrin for the analysis of the main alkaloids from *Chelidonium majus* by capillary electrophoresis

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## Abstract

Capillary zone electrophoresis (CZE) with multi-wavelength detection was used for the separation of the main alkaloids from *Chelidonium majus*. Optimum separation was achieved with a fused-silica capillary tube (70 cm  $\times$  50  $\mu$ m I.D.) and a running electrolyte at pH 5.5 prepared from 40 mM citric acid and 80 mM Na<sub>2</sub>HPO<sub>4</sub> solutions. Addition of  $\beta$ -cyclodextrin (12.5 mmol/l) to the buffer system was found to be essential for the separation. The applied voltage was 25 kV and the capillary temperature was kept constant at 25°C.

## 1. Introduction

*Chelidonium majus* L. (Papaveraceae) is a herbaceous perennial plant indigenous to Europe and Asia. The aerial parts are ingredients of many herbal preparations used mainly for the treatment of gall, stomach and intestinal ailments [1]. The main constituents of *C. majus* have been shown to be berberine, chelidonine, coptisine, homochelidonine, protopine and stylophine, along with minor constituents such as allocryptopine, chelerythrine and sanguinarine [1] (Fig. 1). Some of these alkaloids exhibit interesting pharmacological and biological activities [1]. So far, analyses for these compounds have been accomplished using TLC and HPLC [2–5]. In this study, a capillary zone electrophoresis (CZE) method for the rapid separation and determination of the main alkaloids in *C. majus* extracts was developed. The influence of pH, the ionic strength of the buffer, the applied

voltage, temperature and the cyclodextrin concentration on the separation is discussed.

## 2. Experimental

### 2.1. Materials

Methanol, Na<sub>2</sub>HPO<sub>4</sub> and citric acid were purchased from Merck (Darmstadt, Germany),  $\beta$ -cyclodextrin from Serva (Heidelberg, Germany), berberine, protopine, chelidonine and sanguinarine from Sigma (Deisenhofen, Germany) and allocryptopine from Professor J. Slavik (Brno, Czech Republic). Stylophine and coptisine were isolated from plant material. Plant material of *C. majus* (aerial parts) was purchased from Leopold Bichler (Innsbruck, Austria); commercial preparation A, Choleodoron drops, from Weleda (Vienna, Austria); and commercial preparation B, Cholagogum-Nattermann drops, from Nattermann (Cologne, Germany). Voucher specimens have been de-

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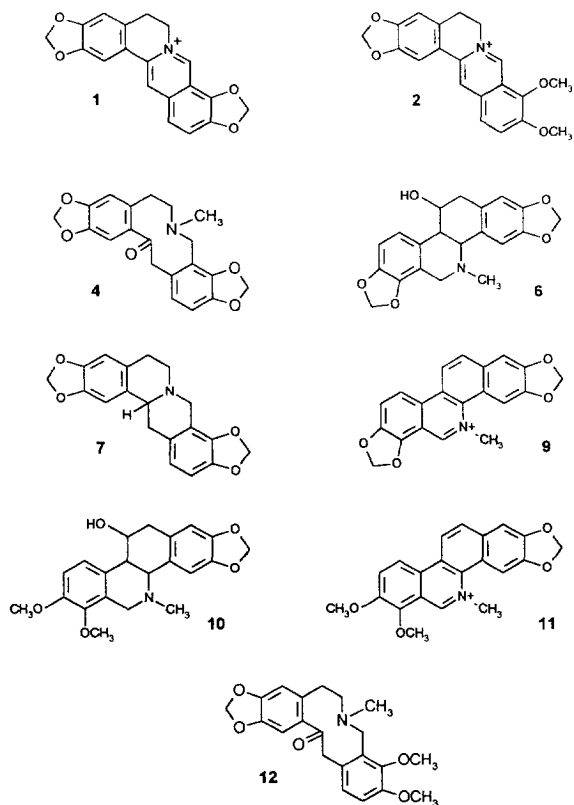


Fig. 1. Structures of coptisine (1), berberine (2), protopine (4), chelidonium (6), stylopine (7), sanguinarine (9), homochelidonium (10), chelerythrine (11) and allocryptopine (12).

posited at the Institute of Pharmacognosy, University of Innsbruck (Austria).

## 2.2. Sample preparation

Powdered aerial parts (5.307 g) of *C. majus* were extracted with a Soxhlet apparatus using methanol as solvent. For qualitative and quantitative analyses, the extract obtained was evaporated to dryness (1.500 g) and the residue was dissolved in 50 ml of methanol. For qualitative analyses prior to injection, the methanolic solution was purified through a BondElut  $C_{18}$  cartridge using methanol as solvent. A 5-ml volume of the commercial products A and B were evaporated to dryness, the residues were dissolved in 1 ml of  $H_2SO_4$  (2%), the solutions were extracted with 1 ml of dichloromethane

(four times) and the aqueous phase again evaporated to dryness. The residue (41.2 mg) of preparation A was dissolved in 1 ml of methanol and that of preparation B in 4 ml of methanol, the solutions were filtered and evaporated to dryness (10.7 mg residue) and the residue was dissolved in 1.00 ml of methanol.

## 2.3. Calibration

Calibration graphs were obtained from standard solutions containing the alkaloids berberine (2), protopine (4) and chelidonium (6) at concentrations between 0.0375 and 1.500 mg/ml (solvent, methanol).

## 2.4. Analytical method

The experiments were performed with a Spectra Phoresis 1000 system (Thermo Separation Products, Fremont, CA, USA) equipped with a high-speed scanning UV-Vis detector, an automatic injector, a temperature-controlled column cartridge with a fused-silica capillary (70 cm  $\times$  50

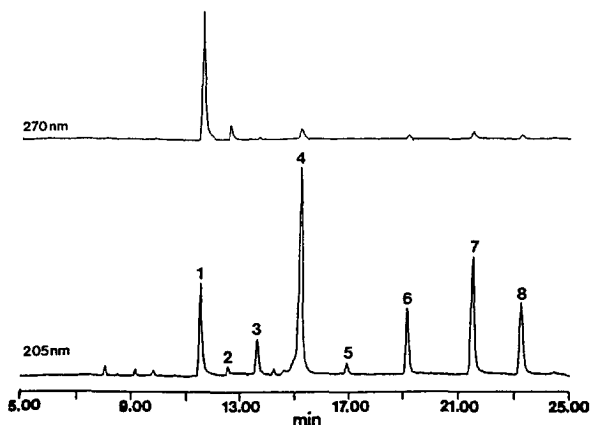


Fig. 2. Electropherograms of a methanolic extract of the aerial parts of *C. majus*. Peaks represent coptisine (1), berberine (2), protopine (4), chelidonium (6) and stylopine (7). Running electrolyte, citric acid- $Na_2HPO_4$  buffer prepared from 40 mM citric acid and 80 mM  $Na_2HPO_4$  solutions (pH 5.5) and  $\beta$ -cyclodextrin (12.5 mmol/l); column, fused silica (70 cm  $\times$  50  $\mu$ m I.D.), injection, vacuum mode, 2 s; voltage, 25 kV; detection, UV at 205 and 270 nm; temperature, 25°C.

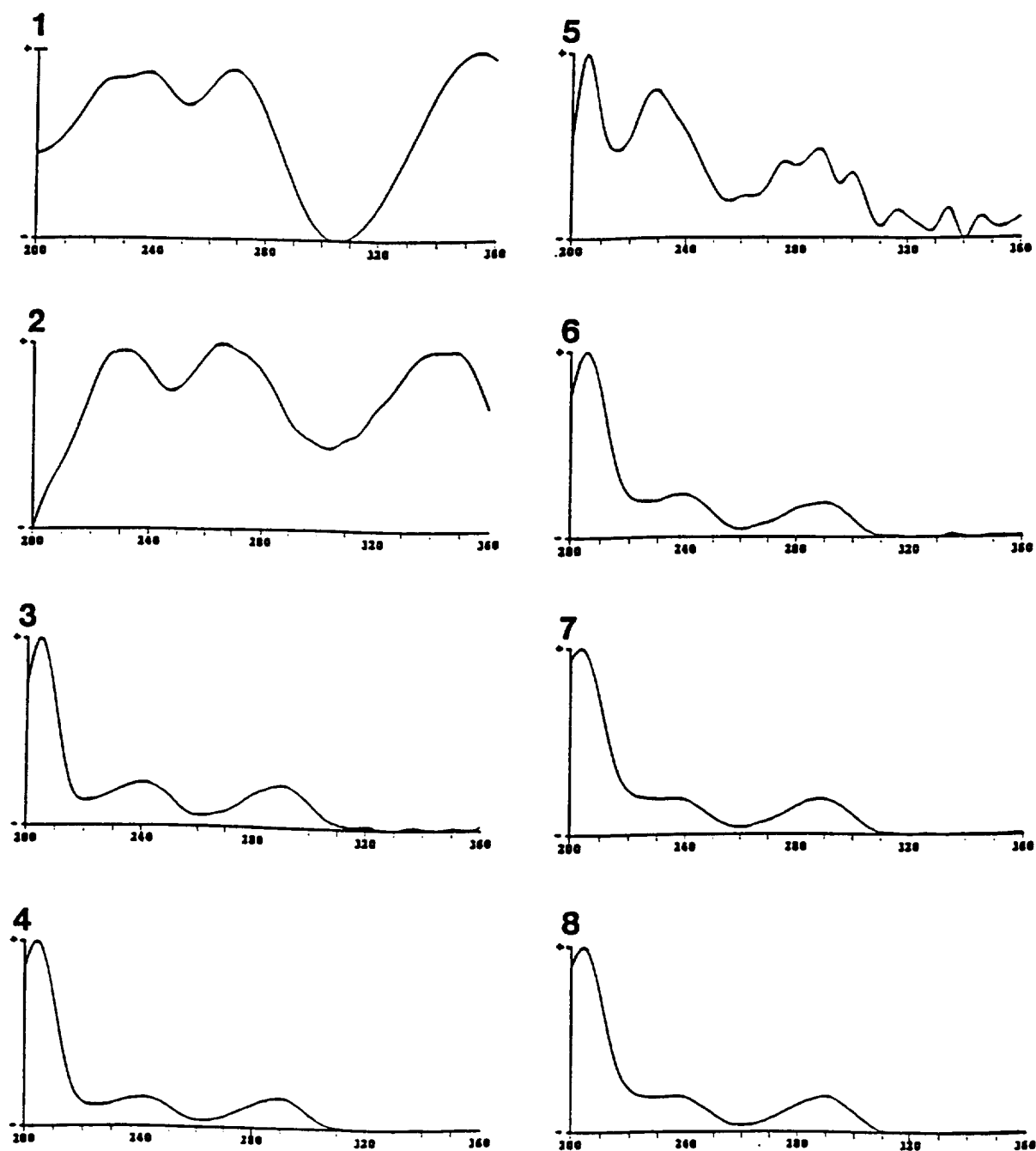


Fig. 3. UV spectra of the alkaloids 1-8 recorded on-line by the high-speed scanning detector.

$\mu\text{m}$  I.D.), an autosampler and a printer. The detection wavelengths were 205 and 270 nm. All experiments were carried out at 25°C at a con-

stant voltage of 25 kV. Temperatures between 10 and 40°C were used to study the effect of temperature on the resolution. When studying

the effect of the electric field on the resolution, voltages between 15 and 30 kV were used. Injections were made using the vacuum mode for 2 s each. The electrolyte solutions (3.9–124.2 mmol/l) were prepared from aqueous solutions of citric acid (40 mmol/l) and  $\text{Na}_2\text{HPO}_4$  (80 mmol/l). The pH of each buffer solution was checked with a pH meter. The effect of  $\beta$ -cyclodextrin on the resolution of adjacent alkaloids was studied by adding 0–15 mmol/l to the running electrolyte. All sample and buffer solutions were filtered through 0.45- $\mu\text{m}$  filters (Sartorius, Göttingen, Germany). Between runs, the capillary was washed with 0.1 M NaOH for 3 min, followed by equilibration with running buffer (3 min).

### 3. Results and discussion

Electropherograms of a methanolic extract of the aerial parts of *C. majus* are shown in Fig. 2. Peaks 1, 2, 4, 6 and 7 were identified as coptisine, berberine, protopine, chelidonine and stylopine, respectively, following comparison of the migration times and UV absorption spectra (Fig. 3) with those of authentic standards. Peaks 3, 5 and 8 could not be identified. However, all compounds on TLC gave a positive reaction with Dragendorff reagent, indicating the presence of alkaloids [6]. Chelerythrine and sanguinarine were present only in trace amounts and therefore not detectable in the electropherograms of the extracts. Baseline separation of these compounds could be achieved by using a fused-silica capillary tube with a citric acid–phosphate buffer (pH 5.5, prepared from 40 mM citric acid and 80 mM  $\text{Na}_2\text{HPO}_4$  solutions) as running electrolyte containing  $\beta$ -cyclodextrin (12.5 mmol/l). The applied voltage was 25 kV and the capillary was thermostated at 25°C. UV detection was performed at 205 and 270 nm.

Allocriptopine, a minor *Chelidonium* alkaloid, co-eluted with protopine. In the chosen electrolyte system the alkaloids are positively charged and migrate to the cathode. Optimi-

zation of the parameters was carried out by investigating the influence of electrolyte composition, pH, electric field, ionic strength, temperature and  $\beta$ -cyclodextrin concentration on the separation efficiency.

The parameter with the greatest influence on the separation of the *C. majus* alkaloids 1–8 was found to be the  $\beta$ -cyclodextrin concentration in the electrophoretic buffer system (Fig. 4). Without  $\beta$ -cyclodextrin, three of seven alkaloid pairs remained unseparated. Excellent resolution of adjacent alkaloid pairs ( $R_s > 4$ ) was obtained by adding 12.5 mmol/l of  $\beta$ -cyclodextrin to the buffer system. Above this concentration  $\beta$ -cyclodextrin was no longer soluble in the electrolyte buffer. Below a cyclodextrin concentration of 7.5 mmol/l, alkaloids 5 and 6 change their order of elution. Enhancement of the selectivity by the use of cyclodextrins is usually

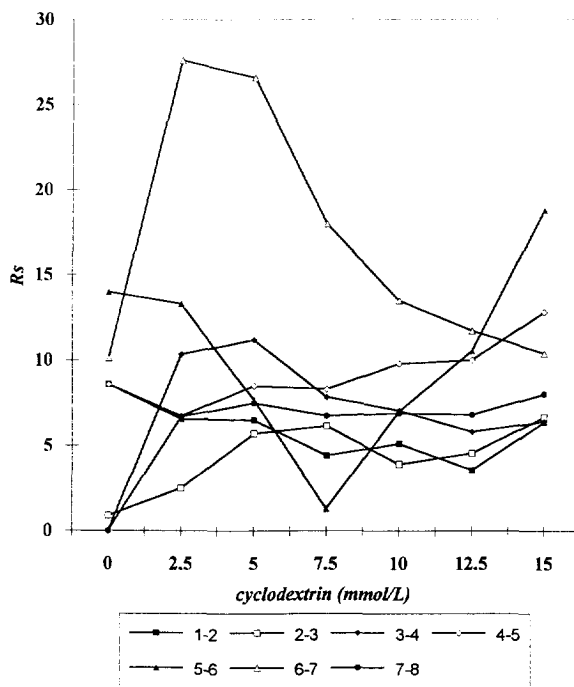


Fig. 4. Effect of  $\beta$ -cyclodextrin (0–15 mmol/l) on resolution ( $R_s$ ) of the alkaloid pairs 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 and 7–8. Other conditions as in Fig. 2.

attributable to their ability to include selectively a wide variety of guest molecules in their hydrophobic cavity [7]. It can be assumed that differences in the stability of the inclusion complexes for structurally related solutes provide the mechanism to improve the separation of the *C. majus* alkaloids.

The pH of the electrolyte is known to have a strong influence on the mobilities of alkaloids, depending on the  $pK_a$  values of these weak bases [7]. Sufficient ionization of the alkaloids can only be achieved in an acidic medium. Resolutions ( $R_s$ ) of adjacent alkaloid pairs at five pH values of the citric acid–phosphate buffer in the range 4.5–6.5 are shown in Fig. 5. An acceptable resolution ( $R_s$ ) for all adjacent pairs of alkaloids was observed at pH 5.5. At pH 4.5 the alkaloid pair 4–5 remained unseparated. The pairs 6–7 and 7–8 were also unseparable at pH 6.5.

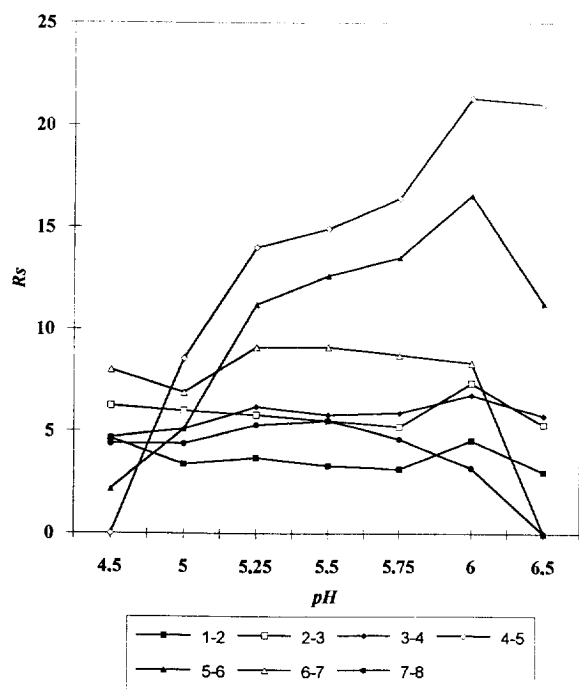


Fig. 5. Effect of pH (4.5–6.5) on resolution ( $R_s$ ) of the alkaloid pairs 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 and 7–8. Other conditions as in Fig. 2.

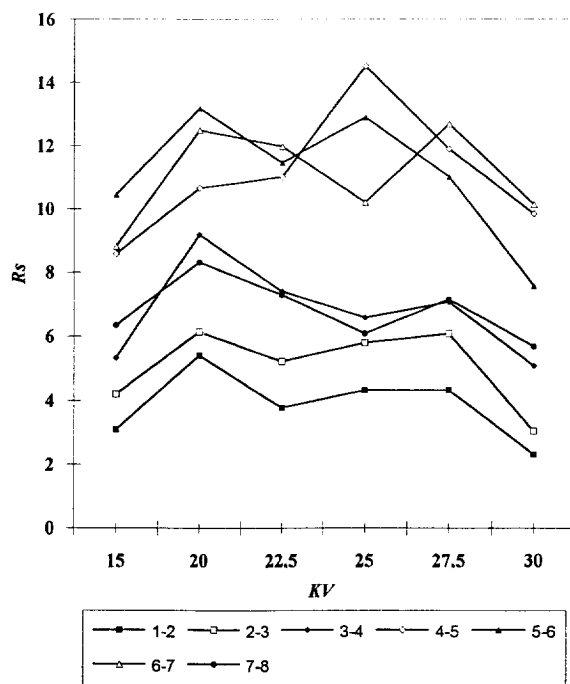


Fig. 6. Effect of voltage (15–30 kV) on resolution ( $R_s$ ) of the alkaloid pairs 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 and 7–8. Other conditions as in Fig. 2.

The electric field strength and temperature are of minor importance for obtaining complete separation (Figs. 6 and 7). Within the temperature range 15–30°C and at applied voltages of 15–30 kV the resolution of adjacent alkaloid pairs was without exception higher than 2.

Good resolution of adjacent pairs of alkaloids was obtained with an ionic strength of the running electrolyte of 62.1 mmol/l (Fig. 8). Lowering the citric acid– $\text{Na}_2\text{HPO}_4$  buffer (pH 5.5) concentration caused a decrease in resolution of all alkaloid pairs. A higher concentration of the buffer system resulted in a worse separation of the compound pair 5–6.

Determination of the major alkaloids in the crude methanolic extracts of the aerial parts of *C. majus* and two pharmaceutical preparations (A and B) was performed by using the external standard method. Calibration was carried out

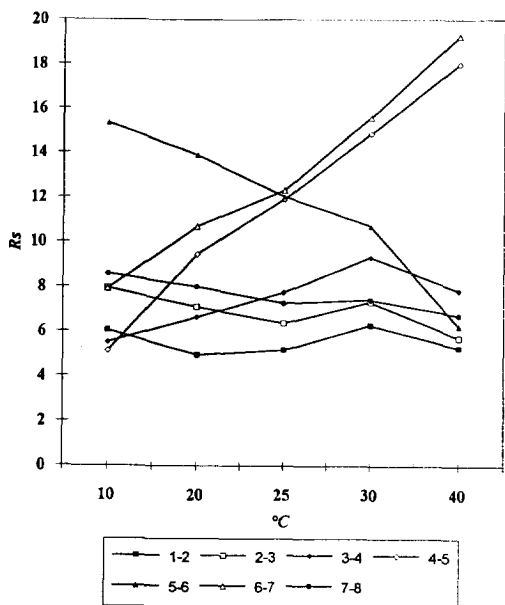


Fig. 7. Effect of capillary thermostating temperature (10–40°C) on resolution ( $R_s$ ) of the alkaloid pairs 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 and 7–8. Other conditions as in Fig. 2.

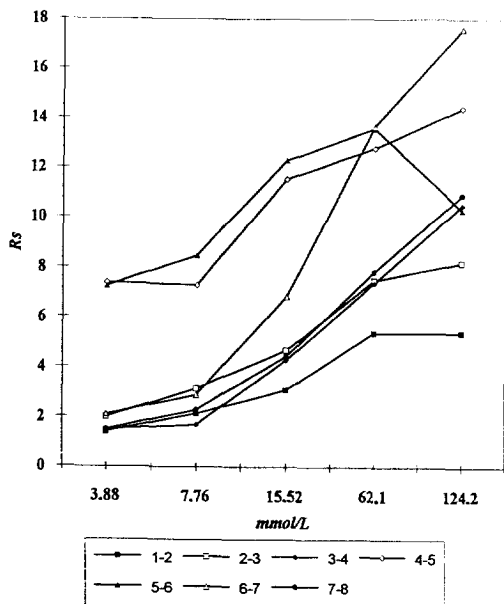


Fig. 8. Effect of buffer concentration on resolution ( $R_s$ ) of the alkaloid pairs 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 and 7–8. Ionic strength: citric acid– $\text{Na}_2\text{HPO}_4$  buffer, 3.9–124.2 mmol/l. Other conditions as in Fig. 2.

Table 1

Regression equations and correlation coefficients for berberine (2), protopine (4) and chelidonium (6)

Compound	Regression equation	$r^2$
2	$y = 25.73 + 575.46x$	0.997
4	$y = 137.43 + 3356.34x$	0.997
6	$y = 21.82 + 3298.63x$	0.997

with compounds 2, 4 and 6. The graphs obtained were linear in the range 37.5–1500  $\mu\text{g/ml}$  (Table 1). The detection limit was approximately 10  $\mu\text{g/ml}$ . Coptisine (1) was determined using the calibration graph for berberine (2), which according to the literature [4] has a similar response factor. Compounds 3, 5, 7 (stylopine) and 8 were determined by using the calibration graph for protopine (4). The results are given in Table 2. The relative standard deviations for the quantitative analyses of the methanolic extract (six experiments) and the commercial preparations A and B were between 1.2 and 9.3% for all compounds studied. For analyses of the commercial preparations a clean-up procedure was necessary.

In conclusion, the developed CZE method can be used successfully for the qualitative and quantitative determination of alkaloids from *C. majus*. Further, it can be applied to fingerprint analyses of methanolic extracts of *Berberis vulgaris* L., *Hydrastis canadensis* L. and *Jatheorrhiza palmata* (LAM) Miers (Fig. 9), plants which have a similar alkaloid pattern to *C. majus*. Further investigations are in progress concerning peak identification and optimization of the CZE separation of the major alkaloids of these plants.

#### Acknowledgement

The authors thank Professor J. Slavik (Brno, Czech Republic) for kindly supplying a sample of allocryptopine.

Table 2  
Determination of alkaloids 1–8

Compound	Concentration (g per 100 g)		
	Sample 1	Sample 2	Sample 3
1	0.717 (2.52)	0.046 (3.52)	0.048 (2.50)
2	0.075 (9.27)	–	–
3	0.039 (7.74)	0.019 (11.9)	0.048 (2.50)
4	0.219 (3.77)	0.047 (3.95)	0.176 (4.91)
5	–	–	–
6	0.049 (2.24)	0.063 (4.54)	0.501 (1.19)
7	0.092 (4.23)	–	–
8	0.045 (3.30)	–	–
Total	1.24	0.18	0.73

Data are means of six replicates; relative standard deviations (%) are given in parentheses. Sample 1, methanolic extract of the aerial parts of *C. majus*; samples 2 and 3, phytopharmaceutical preparations A and B, respectively.

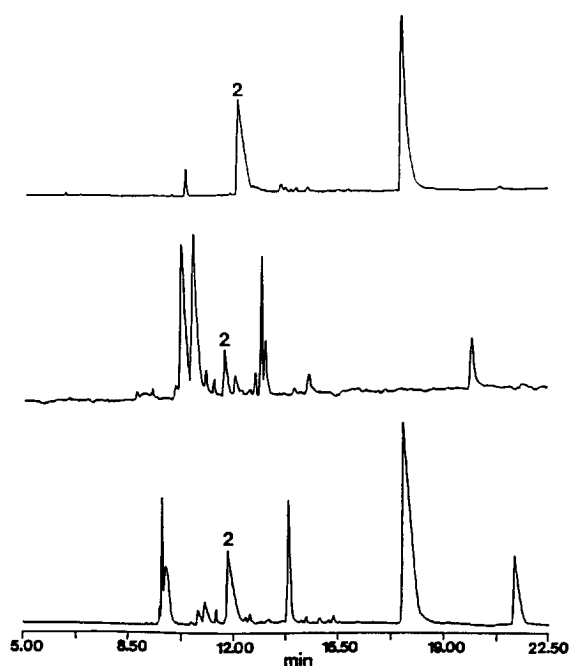


Fig. 9. Electropherograms of methanolic extracts of the aerial parts of (top) *Hydrastis canadensis*, (middle) *Jatrorhiza palmata* and (bottom) *Berberis vulgaris*. UV detection at 205 nm; other conditions as in Fig. 2. Peak 2 = berberine.

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