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Short Communication

Determination of quaternary alkaloids from Coptidis Rhizoma by capillary electrophoresis

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ABSTRACT

A simple and rapid method for the simultaneous determination of eight quaternary alkaloids (coptisine, berberine, epiberberine, palmatine, columbamine, berberastine, jatrorrhizine and magnoflorine) in coptis rhizome by capillary electrophoresis was developed A buffer solution composed of 85% 0 1 *M* sodium acetate solution and 15% methanol was found to be the most suitable electrolyte for this separation, whereby the levels of the eight quaternary alkaloids in the crude drug Coptidis Rhizoma could be easily determined

INTRODUCTION

Coptidis Rhizoma (huang-lien) is a commonly used Chinese herbal drug indicated as a bitter-tasting gastric and intestinal regulative, and is known to contain seven protoberberinium salts (coptisine, berberine, epiberberine, palmatine, columbamine, berberastine and jatrorrhizine) and a quaternary aporphine salt(magnoflorine) (Fig 1) as its major bioactive components [1–7]

Several methods have been reported for the determination of some of these eight quaternary alkaloids, including thin-layer chromatography [5, 8–12], micellar chromatography [13], electron microscopic analysis [14–16] and high-performance liquid chromatography (HPLC) [17–22] However, none of these methods is entirely adequate because their accuracy, degree of separation or sensitivity is unsatisfactory and above all their resolution is limited to at the most six [20] (excluding columbamine and magnoflorine, the former was found to overlap with jatrorrhizine) of the eight quaternary alkaloids

We describe here the development of a simple and rapid method for the simultaneous determination of these eight quaternary alkaloids in samples of crude Coptidis Rhizoma by capillary electrophoresis

EXPERIMENTAL

Reagents and materials

Berberine chloride was purchased from Sigma (St Louis, MO, USA), coptisine chloride from Nacalai (Kyoto, Japan) and palmatine chloride from Wako (Osaka, Japan) Sodium acetate was obtained from Osaka (Osaka, Japan) and benzyltriethylammonium chloride from Merck (Darmstadt, Germany) Epiberberine, columbamine, berberastine and jatrorrhizine were isolated from coptis rhizome [7,19] Magnoflorine was isolated from

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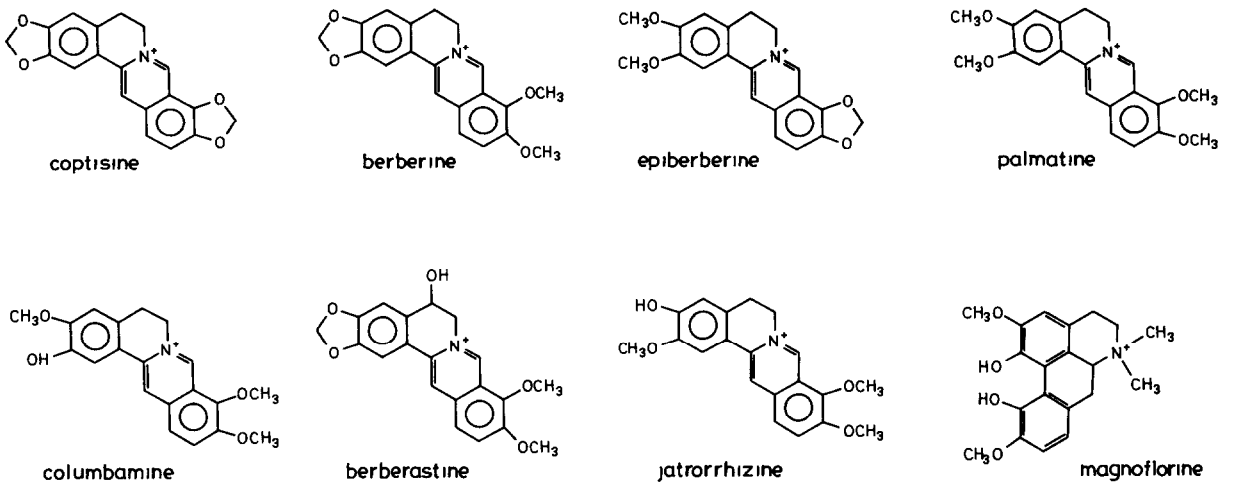

Fig 1 The molecular structures of the eight alkaloids in Coptidis Rhizoma

Phellodendri Cortex [23] Coptidis Rhizoma was purchased from the Chinese herbal market in Taipei (Taiwan)

Preparation of Coptidis Rhizoma extracts

A 0 2-g sample of pulverized Coptidis Rhizoma was extracted with 50% ethanol (7 ml) by stirring at room temperature for 30 min, then centrifuged at 1500 *g* for 10 min The extraction was repeated three times The extracts were combined and filtered through a No 1 filter-paper After the addition of a 2 5-ml aliquot of internal standard solution (6 mg of benzyltriethylammonium chloride in 1 ml of water), the Coptidis Rhizoma extract was diluted to 25 ml with 50% ethanol This solution was passed through a 0 45-μm filter and *ca* 2 4 nl (8-s hydrostatic sampling) of the filtrate was injected into the capillary electrophoresis system directly

Apparatus and conditions

All analysis were carried out on a Waters Quanta 4000 capillary electrophoresis system equipped with a UV detector set at 254 nm and a 100 cm × 100 μm I D uncoated capillary (Millipore, USA) with the detection window placed at 92 5 cm The conditions were as follows sampling time, 8 s hydrostatic, run time, 15 min, applied voltage, 25 kV (constant voltage, positive to negative polarity, electroosmotic flow, *ca* 7 2 cm/min), temperature, 27 5–28 0°C The electrolyte was a buffer solution consisting of 85% 0 1 *M* sodium acetate solution and 15% methanol The electrolyte was filtered through a 0 45-μm filter before use

RESULTS AND DISCUSSION

By eluting with a mixture of acetonitrile and a buffer solution that consisted of 15 ml of acetic acid, 3 g of sodium acetate, 1 g of sodium dodecylsulphate and 0 5 ml of diethylamine in 300 ml of water, we have developed an HPLC method for the separation of the eight quaternary alkaloids [24] However, there are still some problems the analysis time is too long, the composition of mobile phase is too complicated, and the retention times of peaks are unstable Recently, capillary electrophoresis has been applied to the determination of the components of crude drugs [25,26] with good results Hence, we tried to use it in our analysis

The eight quaternary alkaloids of Coptidis Rhizoma do not differ much in their molecular masses, and each carries a single positive charge So, it is necessary to find a counter-ion that can undergo different interactions with the positively charged nitrogen of the alkaloids and can also lead to an effective separation We tested many negative ions and found that carboxylates gave the best results At appropriate concentrations, sodium oxalate, sodium citrate and sodium acetate can each give good resolution However, only sodium acetate can sepa-

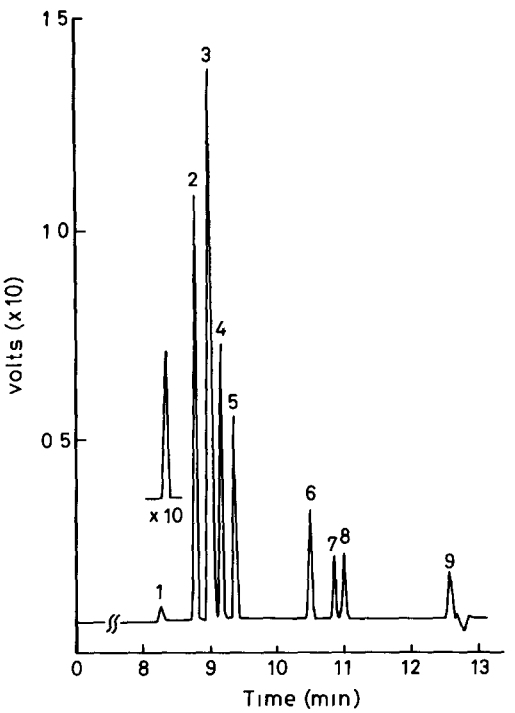

Fig 2 Capillary electropherogram of a mixture of quaternary alkaloids usually present in Coptidis Rhizoma Peaks 1 = internal standard (benzyltriethylammonium chloride), 1 44 ng, 2 = coptisine, 0 56 ng, 3 = berberine, 1 10 ng, 4 = epiberberine, 0 36 ng, 5 = palmatine, 0 29 ng, 6 = columbamine, 0 26 ng, 7 = berberastine, 0 13 ng, 8 = jatrorrhizine, 0 07 ng, 9 = magnoflorine, 0 17 ng

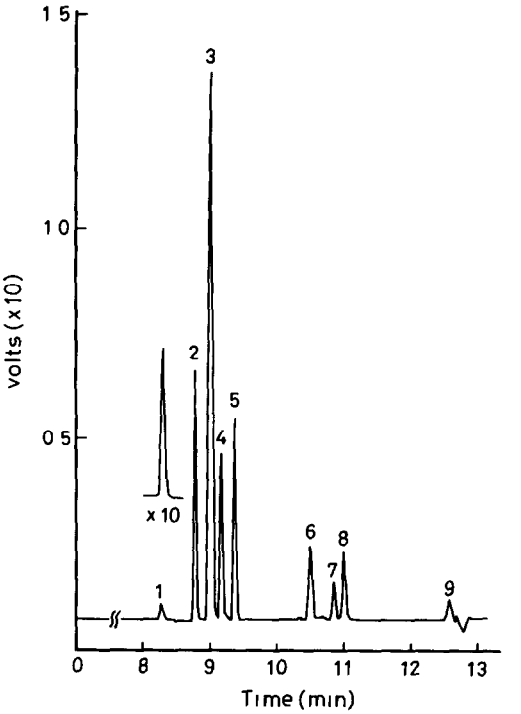

Fig 3 Capillary electropherogram of the extract of a Coptidis Rhizoma sample Peaks 1 = internal standard, 1 44 ng, 2 = coptisine, 0 33 ng, 3 = berberine, 1 08 ng, 4 = epiberberine, 0 21 ng, 5 = palmatine, 0 27 ng, 6 = columbamine, 0 16 ng, 7 = berberastine, 0 09 ng, 8 = jatrorrhizine, 0 07 ng, 9 = magnoflorine, 0 07 ng

rate the peak of magnoflorine from that of the solvent

After a series of experiments, it was found that 0 1 *M* sodium acetate could separate all the alkaloids well, especially the coptisine, berberine, epiberberine and palmatine Lower concentrations of acetate resulted in overlapping of these four components, and higher concentrations caused prolonged retention times and gave a high current Addition of methanol to the buffer solution made the peaks sharper and produced a better separation A concentration of 15% methanol was selected smaller amounts had little effect, and higher amounts retarded the peaks

An electrolyte containing 85% 0 1 *M* sodium acetate solution and 15% methanol was found to produce the best resolution Fig 2 is an electropherogram showing the separation of the eight authentic quaternary alkaloids with the following retention times 8 3 min, benzyltriethylammonium chloride (internal standard), 8 8 min, coptisine, 9 0 min, berberine, 9 2 min, epiberberine, 9 4 min, palmatine, 10 5 min, columbamine, 10 8 min, berberastine, 11 0 min, jatrorrhizine, 12 6 min, magnoflorine The measurement of all the constituents can be completed within 13 min As the ethanol–water extract of Coptidis Rhizoma was injected directly and analysed, the results were as good as those obtained with pure chemical samples without interference, and the analysis could be completed within 13 min, as shown in Fig 3

Calibration graphs for quaternary alkaloids

Calibration graphs (peak-area ratio, y, *vs* concentration, x, mg/ml) were constructed in the range 0 80–0 04 mg/ml for berberine, 0 25–0 02 mg/ml for

coptisine, epiberberine, and palmatine, and 0 15–0 01 mg/ml for columbamine, berberastine, jatrorrhizine and magnoflorine The regression equations of these curves and their correlation coefficients were calculated as follows

coptisine $y = 9\ 26x + 0\ 01$ ($r = 0\ 9999$),
berberine $y = 9\ 78x + 0\ 14$ ($r = 0\ 9997$),
epiberberine $y = 10\ 18x + 0\ 10$ ($r = 0\ 9996$),
palmatine $y = 9\ 81x + 0\ 09$ ($r = 0\ 9996$),
columbamine $y = 8\ 62x + 0\ 05$ ($r = 0\ 9991$),
berberastine $y = 6\ 24x + 0\ 00$ ($r = 0\ 9997$),
jatrorrhizine $y = 13\ 87x + 0\ 04$ ($r = 0\ 9994$),
magnoflorine $y = 5\ 53x + 0\ 01$ ($r = 0\ 9999$),

Determination of quaternary alkaloids in Coptidis Rhizoma

The coptis rhizome drug material was extracted at room temperature with water, with 50% methanol and with 50% ethanol We found that 50% ethanol gave the best extraction yield, so the 50% ethanol extract of Coptidis Rhizoma was used as a test solution When the test solution was analysed by capillary electrophoresis under the selected conditions, the electropherogram as shown in Fig 3 was obtained The peaks were identified by comparison with those obtained from authentic samples of the alkaloids By substituting the area ratios of the individual peaks for y in the above equations, we obtained the contents of the individual quaternary alkaloids in the analysed Coptidis Rhizoma ($\pm$S D, $n = 3$) coptisine, 1 95 $\pm$ 0 01%, berberine, 6 60 $\pm$ 0 02%, epiberberine, 0 96 $\pm$ 0 02%, palmatine, 1 57 $\pm$ 0 02%, columbamine, 0 78 $\pm$ 0 01%, berberastine, 0 61 $\pm$ 0 02%, jatrorrhizine, 0 51 $\pm$ 0 01%, magnoflorine, 0 55 $\pm$ 0 01% Suitable amounts of the eight quaternary alkaloids were added to a sample of Coptidis Rhizoma of known alkaloidal content and the mixture was extracted and analysed using the proposed procedure The recoveries of the alkaloids were 96 7–102 5% with relative standard deviations of 1 6–3 3%

From the above results, it can be concluded that the method of simultaneous determination of the quaternary alkaloids in Coptidis Rhizoma by capillary electrophoresis as established in this study has the advantages that only a small amount of sample is required, the analysis time is short, and the electrolyte preparation is simple

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