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Determination of coptisine, berberine and palmatine in traditional Chinese medicinal preparations by capillary electrophoresis

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ABSTRACT

A capillary electrophoresis method for the determination of coptisine, berberine and palmatine in traditional Chinese medicinal preparations was established. The buffer solution used in this method was 0.2 *M* sodium acetate solution–acetonitrile (1:1). The linear calibration range was 0.003–0.128 mg/ml ($r = 0.9998$) for coptisine, 0.016–0.640 mg/ml ($r = 0.9999$) for berberine and 0.006–0.240 mg/ml ($r = 0.9999$) for palmatine. The recovery was 98.1–101.9% for coptisine, 98.0–100.4% for berberine and 99.7–101.0% for palmatine. The relative standard deviation was 0.96% (intraday) and 2.56% (interday) for coptisine, 1.50% (intraday) and 2.68% (interday) for berberine and 1.12% (intraday) and 2.22% (interday) for palmatine. This method is simple, rapid and reproducible. The contents of these three alkaloids in 23 Coptidis Rhizoma- and/or Phellodendri Cortex-containing Chinese medicinal preparations could easily be determined within 8 min without any pretreatment or any inference.

INTRODUCTION

Coptidis Rhizoma and Phellodendri Cortex are commonly used Chinese herbal drugs with the effects of clearing heat, drying up dampness, purging toxicosis and detoxification [1]. The former is known to contain coptisine, berberine and palmatine (Fig. 1) [2] and the latter contains mainly berberine and palmatine [3]. They may combine with other herbs to form tonic, surface-internal, coordinative, blood regulating, fire purging and astringent formulas [4]. At present, the best method of evaluating the quality of Coptidis Rhizoma- and/or Phellodendri Cortex-containing Chinese medicinal preparations is to determine the contents of coptisine, berberine and palmatine by HPLC [5]. However, owing to complicated components in Chinese medicinal formulas, the use of HPLC is restricted by its lengthy analysis time (at least 30 min until the next injection) and the presence of potentially interfering alkaloidal peaks and because the chromatographic column is easily to contaminated and hard to clean. CE is a recently developed technique that requires a short analysis time, uses a small amount of sample, and can be used for autosampling; in addition, the capillary can easily be thoroughly cleaned. Used in the analysis of Chinese herbs, it gives very good results [6–9]. This study has also found that the use of the CE to analyse various Coptidis Rhizoma- and/or Phellodendri Cortex-containing formulas can offer very satisfactory results. Hence, it is a suitable method for analyses of Chinese medicinal preparations, especially when large numbers of samples are involved and for quality control in pharmaceutical plants.

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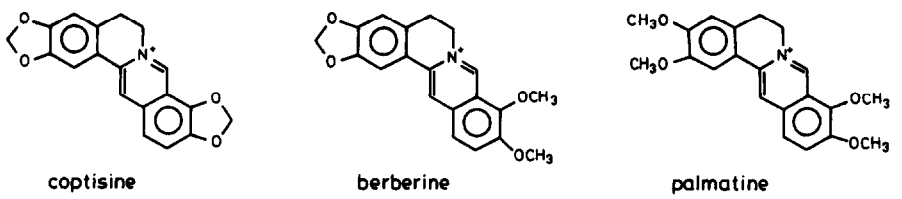

Fig. 1. Structures of marker substances.

EXPERIMENTAL

Reagents and materials

Berberine chloride was purchased from Sigma (St. Louis, MO, USA), coptisine chloride from Nacalai (Kyoto, Japan) and sodium acetate from Osaka (Osaka, Japan). Palmatine was isolated from *Phellodendron amurense* Pupr. [3]. Methyltriphenylphosphonium iodide was prepared from triphenyl phosphine and methyl iodide [10]. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all buffer and sample solutions. Methanol and acetonitrile were HPLC grade. Coptidis Rhizoma- and/or Phellodendri Cortex-containing Chinese medicinal preparations were provided by a Chinese pharmaceutical company in Taipei, Taiwan.

Preparation of Chinese medicinal preparations extracts

A 0.5-g sample of Chinese medicinal preparation was extracted with 70% aqueous methanol (3 ml) by stirring at room temperature for 30 min, then centrifuged at 1500 *g* for 5 min. Extraction was repeated three times. The extracts were combined and filtered through a No. 1 filter paper. After the addition of a 1.0 ml of internal standard solution (6.0 mg of methyltriphenylphosphonium iodide in 1 ml of 70% aqueous methanol), the Chinese medicinal preparation extract was diluted to 10 ml with 70% aqueous methanol. This solution was passed through a 0.45-μm filter and *ca.* 1.7 nl (10-s hydrostatic sampling) of the filtrate were injected into the capillary electrophoresis system directly.

Apparatus and conditions

All analyses were carried out on a Waters Quanta 4000 capillary electrophoresis system equipped with a UV detector set at 254 nm and a 80 cm × 75 μm I.D. uncoated capillary (Millipore, USA) with the detection window placed at 72.5 cm. The conditions were as follows: sampling time, 10 s hydrostatic; run time, 8 min; applied voltage, 25 kV (constant voltage, positive to negative polarity); temperature, 24.5–25.0°C. The electrolyte was a buffer solution consisting of 0.2 *M* sodium acetate solution–acetonitrile (1:1).

Solution for linearity response

Seven solutions of coptisine, berberine and palmatine, which ranged from 0.003 to 0.128 mg/ml for coptisine, 0.016 to 0.640 mg/ml for berberine and 0.006 to 0.240 mg/ml for palmatine, were prepared. Each concentration was analysed three times.

Solution for recovery studies

Different amounts of coptisine, berberine and palmatine standard were added to two samples of Chinese medicinal preparations of known alkaloid content and the mixtures were extracted and analysed using the proposed procedure.

RESULTS AND DISCUSSION

Analytical conditions

In the studies of Coptidis Rhizoma and Phellodendri Cortex crude drugs [7,8], we found that a mixture of sodium acetate solution and organic solvent (methanol or acetonitrile) could give a good resolution of the quaternary alkaloids (for Coptidis Rhizoma, they were coptisine, berberine, epiberberine, palmatine, columbamine, berberastine, jatrorrhizine and magnoflorine; for Phellodendri Cortex, they were berberine, palmatine, jatrorrhizine, magnoflorine and phellodendrine). However, surveys of a number of

commercial Coptidis Rhizoma- and/or Phellodendri Cortex-containing Chinese medicinal preparations, showed that usually only berberine, palmatine and coptisine existed in the text solutions. Actually, one of these three compounds is now used as the marker substance for the evaluation of different Chinese medicinal preparations by HPLC [5]. Therefore, it would be desirable to find a far simpler method that will lead to a shorter analysis time and could be applied to many various Coptidis Rhizoma- and/or Phellodendri Cortex-containing formulas, though it may only be able to separate coptisine, berberine and palmatine well.

From the analysis of quaternary alkaloids [7,8], we knew that carboxylate was a good counter ion for the positively charged nitrogen of the alkaloids, and organic solvent (methanol or acetonitrile) could make the peaks sharper. Hence, we tried to prepare buffer solutions with sodium acetate solutions and methanol (or acetonitrile). After a series of experiments, it was found that 0.2 *M* sodium acetate solution–acetonitrile (1:1) could resolve coptisine, berberine and palmatine within 8 min. At a lower concentration of sodium acetate (0.1 *M*), the peaks were found to be partially overlapped. At lower concentrations of acetonitrile (40, 30, 20 and 10%), the resolution was not good, Acetonitrile was found to be better than methanol in this experiment.

An electrolyte consisting of 0.2 *M* sodium acetate solution–acetonitrile (1:1) was chosen as the buffer solution of this study. Fig. 2 is an electropherogram showing the separation of the authentic compounds with migration times of 6.2 min for the internal standard (methyltriphenylphosphonium iodide), 6.4 min for coptisine, 6.6 min for berberine and 7.0 min for palmatine. As the methanol–water extracts of Chinese medicinal preparations were injected directly and analysed, the results were as good as those obtained with pure chemical samples without interference with each peak and the analysis could also be completed within 8 min, as shown in Fig. 3.

Calibration graphs for coptisine, berberine and palmatine

Calibration graphs (peak-area ratio, y, *vs.* concentration, x, mg/ml) were constructed in the range 0.003–0.128 mg/ml for coptisine, 0.006–

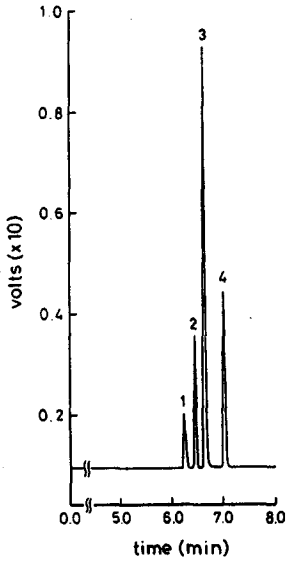

Fig. 2. Capillary electropherogram of authentic compounds of coptisine, berberine and palmatine. Peaks: 1 = internal standard (methyltriphenylphosphonium iodide), 0.600 mg/ml; 2 = coptisine, 0.048 mg/ml; 3 = berberine, 0.240 mg/ml; 4 = palmatine, 0.090 mg/ml.

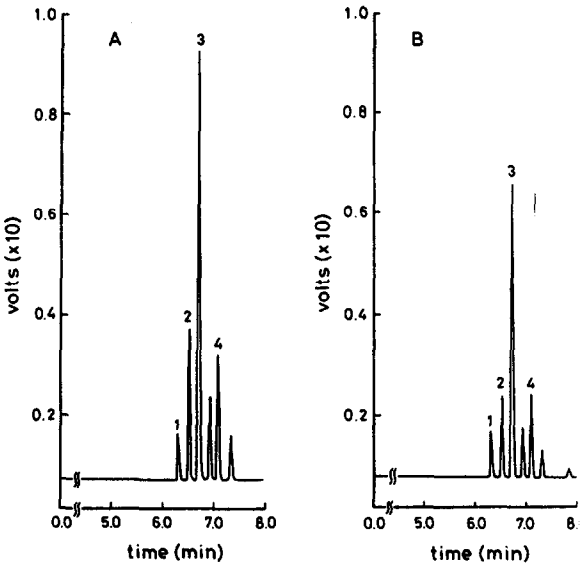

Fig. 3. Capillary electropherograms of Chinese medicinal preparations: (A) *Huang-lien-tang*; (B) *Pu-chi-hsiao-tu-yin*. Peaks as in Fig. 2.

0.240 mg/ml for berberine and 0.006–0.240 mg/ml for palmatine. The regression equations of these curves and their correlation coefficients were calculated as follows:

Coptisine $y = 30.84x + 0.01$ ($r = 0.9998$)
Berberine $y = 32.14x - 0.03$ ($r = 0.9999$)
Palmatine $y = 33.41x - 0.01$ ($r = 0.9999$)

System suitability test

The reproducibility (relative standard deviation) of this proposed method, on the basis of peak-area ratios for six replicate injections, was 0.96% (intraday) and 2.56% (interday) for coptisine, 1.50% (intraday) and 2.68% (interday) for berberine and 1.12% (intraday) and 2.22% (interday) for palmatine.

The results of standard addition recovery studies of coptisine, berberine and palmatine from sample composites of Chinese medicinal preparations are calculated. The recovery was 98.1–101.9% for coptisine, 98.0–100.4% for berberine and 99.7–101.0% for palmatine. All

TABLE I

CONTENTS OF COPTISINE, BERBERINE AND PALMATINE IN COPTIDIS RHIZOMA-CONTAINING CHINESE MEDICAL PREPARATIONS (mg/g)

Sample[a]	Coptisine	Berberine	Palmatine	Total
1	0.71	2.45	0.80	3.96
2	0.90	3.43	1.02	5.35
3	0.52	2.18	0.58	3.28
4	1.81	6.52	1.69	10.02
5	2.84	8.80	2.77	14.41
6	0.56	1.84	0.41	2.81
7	1.32	4.10	1.17	6.59
8	0.95	3.60	1.06	5.61
9	2.21	8.99	3.06	14.26
10	0.81	3.57	0.95	5.33
11	0.61	2.19	0.62	3.42
12	0.85	3.15	0.80	4.80

[a] Names and compositions of the Coptidis Rhizoma-containing Chinese medicinal formulas: **1** = *Chai-hsien-tang*: Pinelliae Tuber, Trichosanthis Fructus, Bupleuri Radix, Coptidis Rhizoma, Scutellariae Radix, Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma, Zizyphi Fructus; **2** = *Ching-wei-tang*: Moutan Radicis Cortex, Angelicae Radix, Rehmanniae Radix, Coptidis Rhizoma, Cimicifugae Rhizoma; **3** = *Ching-yin-li-ke-tang*: Lonicerae Flos, Forsythiae Fructus, Schizonepetae Herba, Menthae Herba, Ledebouriellae Radix, Scrophulariae Radix, Coptidis Rhizoma, Platycodi Radix, Scutellariae Radix, Niter, Rhei Rhizoma, Gardeniae Fructus, Arctii Fructus, Glycyrrhizae Radix; **4** = *Huang-lien-tang*: Coptidis Rhizoma, Glycyrrhizae Radix, Zingiberis Siccatum Rhizoma, Ginseng Radix, Cinnamomi Ramulus, Zizyphi Fructus, Pinelliae Tuber; **5** = *Ko-ken-huang-lien-huang-chin-tang*: Puerariae Radix, Coptidis Rhizoma, Scutellariae Radix, Glycyrrhizae Radix; **6** = *Pan-hsia-hsieh-hsin-tang*: Pinelliae Tuber, Scutellariae Radix, Coptidis Rhizoma, Zingiberis Siccatum Rhizoma, Ginseng Radix, Glycyrrhizae Radix, Zizyphi Fructus; **7** = *Pan-hsieh-liu-chun-tzu-tang*: Pinelliae Tuber, Zingiberis Siccatum Rhizoma, Scutellariae Radix, Atractylodis Rhizoma, Coptidis Rhizoma, Citri Leiocarpae Exocarpium, Ginseng Radix, Glycyrrhizae Radix, Poria, Ostreae Testa; **8** = *Pu-chi-hsiao-tu-yin*: Scutellariae Radix, Coptidis Rhizoma, Baphicacanthis Rhizoma et Radix, Forsythiae Fructus, Arctii Fructus, Scrophulariae Radix, Glycyrrhizae Radix, Platycodi Radix, Cimicifugae Rhizoma, Bupleuri Radix, Lashiosphaera, Citri Leiocarpae Exocarpium, Menthae Herba, Bombyx Batryticatus; **9** = *San-huang-hsieh-hsin-tang*: Rhei Rhizoma, Scutellariae Radix, Coptidis Rhizoma; **10** = *Shao-yao-tang*: Paeoniae Radix, Cinnamomi Cortex, Saussureae Radix, Scutellariae Radix, Glycyrrhizae Radix, Angelicae Radix, Rhei Rhizoma, Arecae Semen, Coptidis Rhizoma; **11** = *Tien-wang-pu-hsin-tan*: Rehmanniae Radix, Ginseng Radix, Poria, Polygalae Radix, Scrophulariae Radix, Salviae Miltiorrhizae Radix, Platycodi Radix, Thujae Orientalis Semen, Asparagi Radix, Ophiopogonis Tuber, Zizyphi Spinosi Semen, Angelicae Radix, Schizandrae Fructus, Cinnabaris, Acori Rhizoma, Coptidis Rhizoma; **12** = *Tzu-sheng-ming-mu-tang*: Angelicae Radix, Paeoniae Radix, Ligustici Rhizoma, Rehmanniae Radix, Platycodi Radix, Ginseng Radix, Gardeniae Fructus, Coptidis Rhizoma, Angelicae Dahuricae Radix, Viticis Fructus, Chrysanthemi Flos, Glycyrrhizae Radix, Junci Caulis Medulla, Camelliae Folium.

the tailing factors of the four peaks (internal standard, coptisine, berberine and palmatine) are very close to 1.

Determination of coptisine, berberine and palmatine in Chinese medicinal preparations

When the test solutions of Chinese medicinal preparations extracts were analysed by CE under the selected conditions, the calculated contents of coptisine, berberine and palmatine as shown in Tables I–III were obtained. There was no interference with any peak of the extracts in various Chinese medicinal preparations (including Coptidis Rhizoma containing, Phellodendri Cortex containing as well as Coptidis Rhizoma and Phellodendri Cortex containing). These data indicate that the proposed CE method is relatively suitable for the determination of coptisine, berberine and palmatine in Chinese medicinal preparations. Moreover, this analytical method not only needs no pretreatment, but also offers autosampling. In addition to its rapid and accurate performance, it allows the next injection to be given within 8 min with a thoroughly cleaned capillary too. Therefore, it should be especially useful for bulky samples and also quality control in pharamaceutical plants.

TABLE II

CONTENTS OF COPTISINE, BERBERINE AND PALMATINE IN PHELLODENDRI CORTEX-CONTAINING CHINESE MEDICINAL PREPARATIONS (mg/g)

Sample[a]	Berberine	Palmatine	Total
13	0.42	0.24	0.66
14	0.61	0.35	0.96
15	0.25	0.15	0.40
16	0.25	0.11	0.36
17	0.51	0.24	0.75

[a] Names and compositions of the Phellodendri Cortex-containing Chinese medicinal formulas: **13** = *Chih-po-pa-wei-wan*: Anemarrhenae Rhizoma, Phellodendri Cortex, Rehmanniae Radix, Batatatis Rhizoma, Corni Fructus, Poria, Alismatis Rhizoma, Moutan Radicis Cortex; **14** = *I-chi-chung-ming-tang*: Astragali Radix, Ginseng Radix, Puerariae Radix, Viticis Fructus, Paeoniae Radix, Phellodendri Cortex, Cimicifugae Rhizoma, Glycyrrhizae Radix; **15** = *Pai-tu-san*: Rehmanniae Radix, Platycodi Radix, Forsythiae Fructus, Moutan Radicis Cortex, Trichosanthis Radix, Scrophulariae Radix, Lonicerae Flos, Bupleuri Radix, Glycyrrhizae Radix, Phellodendri Cortex, Menthae Herba, Paeoniae Radix, Gypsum Fibrosum, Arctii Fructus; **16** = *Pan-hsia-pai-chu-tien-ma-tang*: Pinelliae Tuber, Atractylodis Rhizoma, Poria, Citri Leiocarpae Exocarpium, Atractylodis Lanceae Rhizoma, Hordei Germinatus Fructus, Gastrodiae Rhizoma, Monasco Cum Oryzae Semen, Astragali Radix, Ginseng Radix, Alismatis Rhizoma, Phellodendri Cortex, Zingiberis Siccatum Rhizoma, Zingiberis Rhizoma; **17** = *Tzu-yin-chiang-huo-tang*: Angelicae Radix, Paeoniae Radix, Asparagi Radix, Ophiopogonis Tuber, Atractylodis Rhizoma, Rehmanniae Radix, Citri Leiocarpae Exocarpium, Phellodendri Cortex, Anemarrhenae Rhizoma, Glycyrrhizae Radix.

TABLE III

CONTENTS OF COPTISINE, BERBERINE AND PALMATINE IN COPTIDIS RHIZOMA AND PHELLODENDRI CORTEX-CONTAINING CHINESE MEDICAL PREPARATIONS (mg/g)

Sample[a]	Coptisine	Berberine	Palmatine	Total
18	0.62	2.92	0.91	4.45
19	1.03	5.51	1.43	7.97
20	1.44	3.38	2.19	7.01
21	4.42	17.79	5.30	27.51
22	1.32	4.16	1.34	6.82
23	0.85	3.39	0.86	5.10

[a] Names and compositions of the Coptidis Rhizoma and Phellodendri Cortex-containing Chinese medicinal formulas: **18** = *Chai-hu-ching-kan-tang*: Bupleuri Radix, Angelicae Radix, Paeoniae Radix, Ligustici Rhizoma, Rehmanniae Radix, Coptidis Rhizoma, Scutellariae Radix, Phellodendri Cortex, Gardeniae Fructus, Forsythiae Fructus, Platycodi Radix, Arctii Fructus, Trichosanthis Radix, Menthae Herba, Glycyrrhizae Radix; **19** = *Chih-cho-ku-pen-wan*: Nelumbinis Stamen, Coptidis Rhizoma, Phellodendri Cortex, Alpiniae Oxyphyllae Fructus, Amomi Semen, Pinelliae Tuber, Poria, Polyporus, Glycyrrhizae Radix; **20** = *Huang-lien-chieh-tu-tang*: Coptidis Rhizoma, Phellodendri Cortex, Scutellariae Radix, Gardeniae Fructus; **21** = *Pai-tou-weng-tang*: Pulsatillae Radix, Coptidis Rhizoma, Phellodendri Cortex, Fraxini Cortex; **22** = *San-huang-shih-kao-tang*: Gypsum Fibrosum, Scutellariae Radix, Coptidis Rhizoma, Phellodendri Cortex, Gardeniae Fructus, Ephedrae Herba, Sojae Semen Praeparatum, Zingiberis Rhizoma, Zizyphi Fructus, Camelliae Folium; **23** = *Wen-ching-yin*: Angelicae Radix, Rehmanniae Radix, Paeoniae Radix, Ligustici Rhizoma, Coptidis Rhizoma, Scutellariae Radix, Phellodendri Cortex, Gardeniae Fructus.

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