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Analysis of the components of *Paeonia radix* by capillary zone electrophoresis

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ABSTRACT

The methanol extract of *Paeonia radix* was analysed by capillary zone electrophoresis in 100 m*M* borate buffer (pH 10.5). Monoterpene glycosides (paeoniflorin and oxypaeoniflorin) were migrated first, well separated from each other, then gallic acid and its derivatives. The monoterpene glycosides were quantified with high reproducibility using 3,4-dimethoxycinnamic acid as an internal standard. The data obtained from various *Paeonia radix* samples were highly correlated to those obtained by high-performance liquid chromatography.

INTRODUCTION

Capillary zone electrophoresis (CZE), introduced by Mikkers *et al.*[1], allows the separation of ionic components of samples in a capillary tube with excellent resolution by the combined effects of electrophoresis and electroosmosis. Electrophoresis separates ions based on differences in charge and size, whereas the flow of carrier induced by electroosmosis drives the separated ions from one end of the tube to the other. As electroosmotic flow is plug flow and heat evolved during analysis is efficiently dissipated from the capillary wall, diffusion of separated components is minimized and a high column efficiency is attainable. This capillary method also allows accurate and reproducible quantification of components by on-tube detection.

CZE has been applied to various ionic compounds, including inorganic anions[1] and cations[2], organic acids[1,2–5], dansylated amino acids[6], ammonium salts[7], pyridinium salts[2], catecholamines[8], nucleosides[9], drug metabolites[10] and proteins[11]. However, there have been no papers dealing with crude drugs. Therefore, we have undertaken a study of the application of this powerful method to the determination of the components of crude drugs. This paper presents some preliminary results obtained for *Paeonia radix*, which has been traditionally used as a sedative, lenitive or antispasmolytic agent.

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EXPERIMENTAL

Samples of *Paeonia radix* were gifts from Tochimoto Tenkaido (Suehiro-cho, Kita-ku, Osaka, Japan). The authentic specimens of paeoniflorin and oxypaeoniflorin were isolated by chromatography of the methanol extract of *Paeonia radix* on a column of silica gel with chloroform–methanol as eluent and purified by recrystallization from chloroform. All other chemicals were of the highest grade commercially available.

Extraction of the components

Methanol (10 ml) was addd to thin slices of each crude drug sample (100 mg), and the mixture was heated under reflux for 30 min. The methanol extract was removed and the residue was heated twice more with additional batches (10 ml each) of methanol. The extracts were combined and evaporated to dryness under reduced pressure. The residue was reconstituted with methanol, and the solution was analysed.

Procedure for CZE

Each of two laboratory-made PTFE blocks having hollows of 1.5-ml capacity, in which platinum wires (10 mm × 1 mm O.D.) as electrodes were fixed, was filled with 100 m*M* borate buffer (pH 10.5) and an 80-cm portion of 50-μm I.D. fused-silica capillary tubing (Scientific Glass Engineering, Melbourne, Australia) newly cut off from a roll, containing the same solution as the carrier, was bridged between these blocks. A Matsusada Precision Devices Model HER-30PI apparatus was used to supply high voltages. The platinum wires were connected to the anode and the cathode (grounded) via shielded cables. Part of the polyethylene coating of the tube was removed by burning at a position 15 cm from the cathode, and the transparent portion was fixed to a slit (50 μm × 700 μm) which was screwed on a specially made PTFE holder. This holder was placed in the cell housing of a JASCO UVIDEC 100-V UV monitor and the absorbance at 254 nm was recorded.

Prior to each run, the tube was rinsed with 0.1 *M* sodium hydroxide solution, and conditioned by rinsing with the carrier solution using a small injector. The tube was also rinsed with methanol after every five runs. A sample solution was introduced into the tube by moving the anodic end of the tube into the sample solution and its level was maintained for 5 s 5 cm higher than the level of the cathode solution. By this syphonic procedure several nanolitres of the sample solution were introduced into the tube. After introduction of the sample solution the anodic end was quickly returned to the anode solution, and its level was adjusted to that of the cathode solution. Analysis was performed by applying 20 kV in the constant-voltage mode.

RESULTS AND DISCUSSION

In CZE, the main factors affecting separation are the nature of the inner wall of the capillary tube and the composition and pH of the carrier solution. Several kinds of materials are used for such narrow-bore tubes (mostly 25–100 μm), including glass, fluorinated polyethylene (FPE) and fused silica. All of them are negatively charged when in contact with electrolyte. Glass and FPE are advantageous for handling, because capillary tubes made of these materials, especially FPE, are flexible. However,

they absorb UV light strongly, and this causes problems with detection. In contrast, fused silica does not absorb in the UV region, where various organic and inorganic compounds have absorption bands. Fragility is overcome by coating polyimine resin on the surface of the tube, as in gas–liquid chromatography. For these reasons, a fused-silica tube coated with polyimine was used in this work. The high negativity of the inner wall induced rapid electroosmotic flow, which facilitated transportation of the separated components, making the analysis time short.

The composition and pH of the carrier may be varied so that the best separation is obtained for each sample. However, in this preliminary work we used 100 m*M* borate buffer (pH 10.5), because this carrier permitted the separation of glycosides in addition to anionic components[12].

Fig. 1 shows a typical electropherogram of the methanol extract of *Paeonia radix*. Peaks 1 and 2, appearing at 8.14 and 10.94 min, respectively, were assigned to paeoniflorin and oxypaeoniflorin, based on comparison of their migration times with those of authentic samples. Although paeoniflorin is a neutral compound, its carbohydrate moiety reacts with the borate ion in the carrier to form an anionic complex. This complex was slightly held back by electrophoresis to give a peak at 8.14 min, slightly retarded from the methanol peak (7.21 min), which was driven only by electroosmotic flow.

Oxypaeoniflorin is a derivative of paeoniflorin, having a phenolic hydroxyl

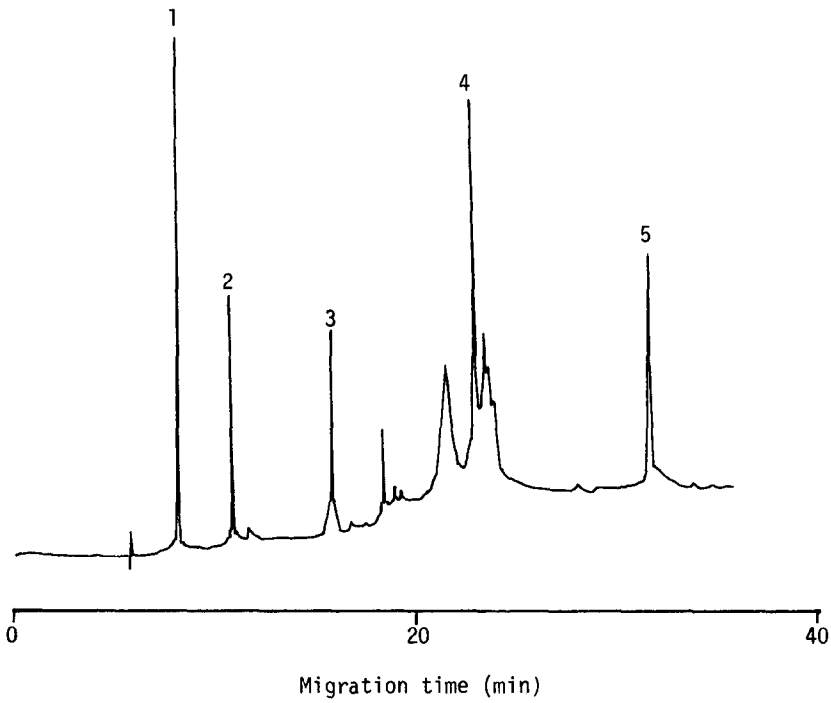

Fig. 1. Typical electropherogram of the methanol extract of *Paeonia radix*. Capillary, fused silica (80 cm × 50 μm I.D.); carrier, 100 m*M* borate buffer (pH 10.5); applied voltage, 20 kV; detection, UV (254 nm). Peaks: 1 = paeoniflorin; 2 = oxypaeoniflorin; 3 = methyl gallate; 4 = tannic acid; 5 = gallic acid.

group in the *para* position of the benzoyl group in paeoniflorin. Because of the dissociation of the phenolic hydroxyl group, it was more strongly held back by electrophoresis than paeoniflorin. The peaks eluting more slowly than 15 min were those of gallic acid and its derivatives. Among these peaks the fastest (peak 3, 15.57 min) was that of methyl gallate, presumably formed during the extraction process. The broadened multiple peaks in the range 20.5–24.5 min (peak 4 at the centre) were due to tannic acid analogues and the peak at 31.71 min (peak 5) was gallic acid. Analysis of tannic acids suffers from a number of problems arising from its susceptibility to air oxidation. Extensive studies of tannic acid analysis are in progress, using a number of samples from various sources apart from *Paeonia radix*, and the results will appear elsewhere.

As both paeoniflorin and oxypaeoniflorin are considered to be the effective components of this crude drug, they were quantified using 3,4-dimethoxycinnamic acid as an internal standard. For both monoterpene glycosides the plot of the peak response relative to the internal standard (IS) *versus* concentration was a straight line passing through the origin at least in the range 2–20 ng/nl, as shown in Fig. 2.

The relative standard deviations (R.S.D.) for paeoniflorin (n = 8) at 5 and 20 ng/nl were 2.2 and 3.0%, respectively. The R.S.D. values for oxypaeoniflorin (n = 8)

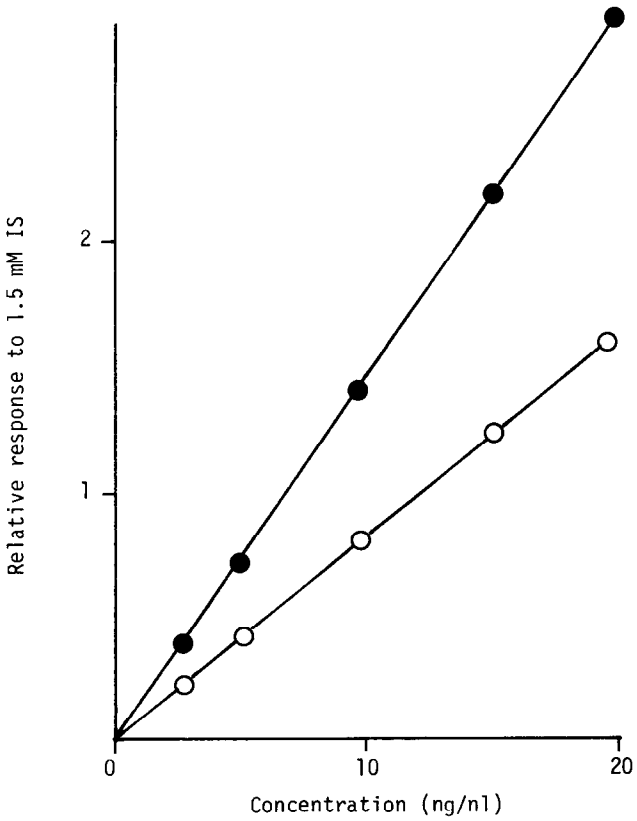

Fig. 2. Calibration graphs for (○) paeoniflorin and (●) oxypaeoniflorin.

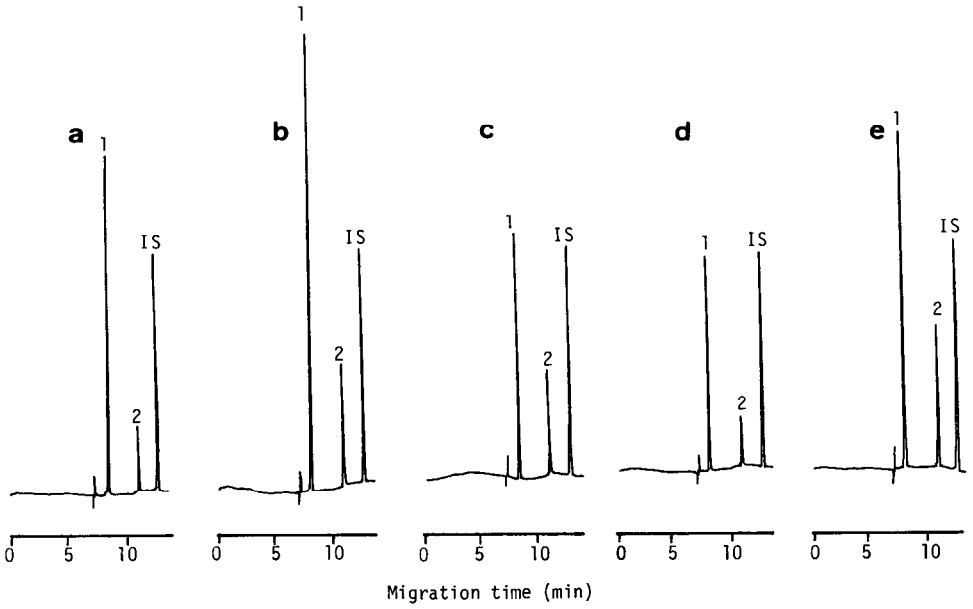

Fig. 3. Comparison of the electropherograms of the methanol extracts of *Paeonia radix* obtained from various habitats (a, Gunma; b, Nara; c, Hokkaido; d, China; e, Korea). Peaks after 15 min are omitted. The extract from each *Paeonia radix* sample (100 mg) was reconstituted with 500 μl of methanol containing 3,4-dimethoxycinnamic acid as an internal standard (IS) to a concentration of 1.5 m*M*. Analytical conditions and assignment of peaks 1 and 2 as in Fig. 1.

were higher (4.2 and 2.8%, respectively), but sufficiently low for practical analysis. The R.S.D. values of the migration times were higher (paeoniflorin 4.6%, oxypaeoniflorin 5.1%), because temperature control was not applied in this work. The use of an efficient thermostat would have made improved the reproducibility of the migration time and hence the peak response. Rinsing with an alkali solution and methanol was essential, otherwise the migration times of paeoniflorin and oxypaeoniflorin became longer on repetition of analysis, owing to the reduction in the rate of electroosmotic

TABLE I

CONTENTS OF PAEONIFLORIN AND OXYPAEONIFLORIN IN *PAEONIA RADIX* SAMPLES FROM VARIOUS HABITATS

Habitat	*Content (%)*	
	Paeoniflorin[a]	*Oxypaenoflorin*
Gunma	3.27 (3.55)	0.16
Nara	4.32 (4.86)	0.28
Hokkaido	2.36 (2.57)	0.24
China	2.11 (2.11)	0.12
Korea	3.29 (3.61)	0.32

[a] Values in parentheses were obtained by HPLC[13].

flow by adsorption of the components on the inner wall of the capillary. By this rinsing the repeatability of analysis were ensured, the change in migration time being less than 5% even after 100 analyses.

Fig. 3 shows electropherograms for several *Paeonia radix* samples obtained from various habitats, and Table I gives the contents of paeoniflorin and oxypaeoniflorin determined by the present method.

We shall not discuss the difference in habitats as these data were obtained from only one sample for each habitat, but it should be noted that the values obtained for paeoniflorin were in good agreement with those determined by high-performance liquid chromatography (HPLC) (column, μBondapak C_{18}; eluent, acetonitrile–water–acetic acid, 15:85:1) according to the literature[13], which is considered to be the standard method.

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