Journal of Chromatography A, 653 (1993) 184–188
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 428

Short Communication

Determination of glycyrrhizin and glycyrrhetinic acid in traditional Chinese medicinal preparations by capillary electrophoresis

Hong-Ren Chen and Shuenn-Jyi Sheu*

Department of Chemistry, National Taiwan Normal University, Taipei (Taiwan)

(First received May 25th, 1993; revised manuscript received July 13th, 1993)

ABSTRACT

A simple, rapid, accurate and reproducible capillary electrophoretic method was developed for the assay of glycyrrhizin and glycyrrhetinic acid in traditional Chinese medicinal preparations. The buffer solution used in this method was acetonitrile and 0.02 *M* sodium dihydrogenphosphate solution adjusted to pH 7.5 with 0.05 *M* sodium hydroxide. The linear calibration range was 0.04–2.00 mg/ml ($r = 0.9988$) for glycyrrhizin and 0.007–0.35 mg/ml ($r = 0.9985$) for glycyrrhetinic acid and recoveries were 98.1–101.3% for glycyrrhizin and 98.5–101.4% for glycyrrhetinic acid. The relative standard deviations were 1.02% ($n = 6$) for glycyrrhizin and 0.91% ($n = 6$) for glycyrrhetinic acid. The content of these two acids in Glycyrrhizae Radix and Glycyrrhizae Radix-containing Chinese medicinal preparations was successfully determined within 10 min.

INTRODUCTION

Glycyrrhizae Radix is a Chinese herbal drug commonly used as an expectorant, detoxicant and spleen tonic and to restore vitality, reduce fever, arrest coughing, comfort the stomach, alleviate urgency and potentiate the effect of various other herbs. It is known to contain mainly glycyrrhizin (20β-carboxy-11-oxo-30-norolean-12-en-3β-yl-2-O-β-D-glucopyranuronosyl-α-D-glucopyranosiduronic acid) and its aglycone, glycyrrhetinic acid [1]. It is widely found in Chinese medicinal preparations such as tonic, surdorific, coordinative, vitality-regulating, blood-regulating, chill-dispelling and moistening formulas [2]. At present, the best method of measuring the equivalence of Glycyrrhizae Radix-containing Chinese medicinal preparations is to determine the content of glycyrrhizin by HPLC [3]. However, owing to complicated components in Chinese medicinal formulas, the use of HPLC is restricted by its lengthy analysis time (about 50 min), poor resolution and the fact that the chromatographic column is easily contaminated and hard to clean. Capillary electrophoresis (CE) is a recently developed method that has the following advantages: analysis time is short, only a small sample is required, thorough cleaning of the column is easy and autosampling is possible [4]. Used in the analysis of Chinese herbs, it gives very good results [5–9]. This study has also found that using CE to analyse various Glycyrrhizae Radix-containing preparations can

* Corresponding author.

0021-9673/93/$06.00

offer very satisfactory results. Hence, it is a suitable method for analyses of traditional Chinese medicinal preparations, especially when analysis of large numbers of samples is required and for quality control in pharmaceutical plants.

EXPERIMENTAL

Reagents and materials

Glycyrrhizin and glycyrrhetinic acid were purchased from Tokyo Kasei (Tokyo, Japan) and sodium dihydrogenphosphate from Wako (Osaka, Japan). Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all buffers and sample solutions. Acetonitrile and methanol were of HPLC grade. Glycyrrhizae Radix-containing Chinese medicinal preparations were provided by Sun-Ten Pharmaceutical (Taipei, Taiwan).

Preparation of Chinese medicinal preparation extracts

A 1.0-g sample of a Chinese medicinal preparation was extracted with 70% methanol (7.0 ml) by ultrasonic agitation at room temperature for 15 min, then centrifuged at 1500 *g* for 10 min. Extraction was repeated three times. The extracts were combined and filtered through a No. 1 filter paper. After the addition of a 1.5 ml of internal standard solution (2.5 mg of cinnamic acid in 1 ml of 70% methanol), the Chinese medicinal preparation extract was diluted to 25 ml with 70% methanol. This solution was passed through a 0.45-μm filter and *ca.* 0.8 nl (5 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 70 cm $\times$ 75 μm I.D. uncoated capillary (Millipore, USA) with the detection window placed at 62.5 cm. The conditions were as follows: sampling time, 5 s hydrostatic; run time, 10 min; applied voltage, 25 kV (constant voltage, positive to negative polarity); temperature, 25.0–25.5°C. The electrolyte was a buffer solution that contained acetonitrile and 0.02 *M* sodium dihydrogenphosphate solution, which was adjusted to pH 7.5 with 0.05 *M* sodium hydroxide.

Solution for linearity response

Eight concentrations of glycyrrhizin, which ranged from 0.04 to 2.00 mg/ml, and eight concentrations of glycyrrhetinic acid, which ranged from 0.007 to 0.35 mg/ml, were prepared. Each concentration was analysed three times.

Solution for recovery studies

Fixed amounts of glycyrrhizin and glycyrrhetinic acid standard were added to three samples of Chinese medicinal preparations of known acids content and the mixture was extracted and analysed using the proposed procedure.

RESULTS AND DISCUSSION

Analytical conditions

Glycyrrhizin and glycyrrhetinic acid contain a carboxyl group and should be analysed as anions in a capillary electrophoresis system. Hence we used sodium dihydrogenphosphate solutions with different pH values as buffer solutions. We found that a 0.02 *M* sodium dihydrogenphosphate solution that was adjusted to pH 7.5 with 0.05 *M* sodium hydroxide gave the best result. At higher pH, the separation was good but the analysis time was longer, whereas at lower pH the peak of glycyrrhizin was found to be overlapped by other peaks. However, further improvements were required to minimize the noise. Addition of acetonitrile to the buffer solution made the peaks sharper, the baseline smoother and also produced a better separation. After a series of trials, a concentration of 20% acetonitrile was selected; a lower concentration of acetonitrile gave only a slight improvement and higher concentrations prolonged the analysis time. An electrolyte containing 80% 0.02 *M* sodium dihydrogenphosphate (pH 7.5) and 20% acetonitrile was found to produce the best resolution. Fig. 1 is an electropherogram showing the separation of the authentic glycyrrhizin and

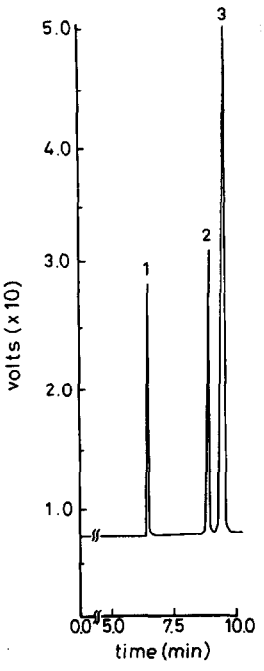

Fig. 1. Capillary electropherogram of a mixture of glycyrrhizin and glycyrrhetinic acid. Peaks: 1 = glycyrrhetinic acid; 2 = internal standard (cinnamic acid); 3 = glycyrrhizin.

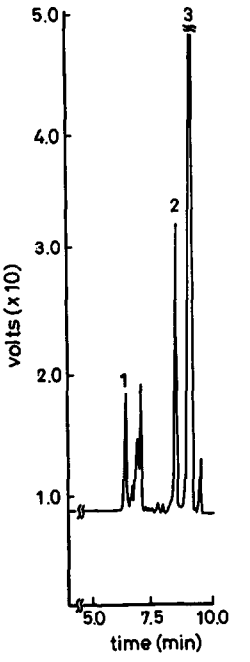

Fig. 2. Capillary electropherogram of the extract of a Glycyrrhizae Radix sample. Symbols as in Fig. 1.

glycyrrhetinic acid with migration times of 6.5 min for glycyrrhetinic acid, 8.8 min for the internal standard and 9.5 min for glycyrrhizin. The analysis of these two constituents can be completed within 10 min. As the methanol–water extracts of the crude drug and Chinese medicinal preparations were injected directly and analysed, the results were as good as those obtained with pure chemical samples without any interference with other peaks, and the analyses could also be completed within 10 min, as shown in Figs. 2 and 3.

Calibration graph for glycyrrhizin and glycyrhetinic acid

Calibration graphs (peak-area ratio, y, *vs.* concentration, x, mg/ml) were constructed in the range 0.04–2.00 mg/ml for glycyrrhizin and 0.007–0.35 mg/ml for glycyrrhetinic acid. The regression equations of these curves and the correlation coefficients were calculated as follows:

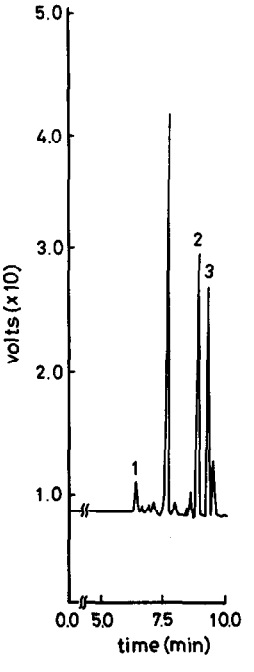

Fig. 3. Capillary electropherogram of a Chinese medicinal preparation, *Chia-wei-hsiao-yao-san*. Symbols as in Fig. 1.

Glycyrrhizin $y = 2.348x - 0.1033$ ($r = 0.9988$)

Glycyrrhetinic acid $y = 4.386x - 0.01125$ ($r = 0.9985$)

System suitability test

The reproducibility (relative standard deviation) of this proposed method, on the basis of peak-area ratios for six replicate injections, was 1.02% (intra-day) and 1.12% (inter-day) for glycyrrhizin and 0.91% (intra-day) and 1.07% (inter-day) for glycyrrhetinic acid.

The results of standard addition recovery studies of glycyrrhizin and glycyrrhetinic acid from sample composites of Chinese medicinal preparations are shown in Table I. The ranges of recovery were 98.1–101.3% for glycyrrhizin and 98.5–101.4% for glycyrrhetinic acid. The tailing factors of the three peaks (internal standard, glycyrrhizin and glycyrrhetinic acid) are very close to 1.

Determination of glycyrrhizin and glycyrrhetinic acid in Chinese medicinal preparations

When the test solutions of Chinese medicinal preparation extracts were analysed by CE under the selected conditions, the calculated contents of glycyrrhizin and glycyrrhetinic acid as shown in Table II were obtained. There was no interference with any peak of the extracts in various Chinese medicinal preparations (total 21 preparations were successfully examined). These data indicate that the proposed CE method is suitable for the determination of glycyrrhizin and glycyrrhetinic acid in Chinese medicinal preparations. Moreover, this analytical method not only needs no pretreatment, but also offers autosampling. In addition to its speed and accuracy, it allows a second injection within 12 min with a thoroughly cleaned column. Therefore, it should

TABLE II

THE CONTENT OF GLYCYRRHIZIN AND GLYCYRRHETINIC ACID IN GLYCYRRHIZAE RADIX AND CHINESE MEDICINAL PREPARATIONS (mg/g) ($n = 6$)

Sample	Glycyrrhizin	Glycyrrhetinic acid
Glycyrrhizae Radix	52.06	1.56
Chia-wei-hsiao-yao-san	5.43	0.38
Kuei-pi-tang	3.51	0.22
Pai-tu-san	2.05	0.13

TABLE I

RECOVERY OF GLYCYRRHIZIN AND GLYCYRRHETINIC ACID FROM VARIOUS CHINESE MEDICINAL PREPARATIONS ($n = 6$)

Sample [9][a]	Amount added (mg)		Recovery (%)	
	Glycy[b]	Gly · acid[c]	Glycy	Gly · acid
Chia-wei-hsiao-yao-san (bupleurum and peony formula)	0.1219	0.05	101.3	101.4
Kuei-pi-tang (ginseng and longan combination)	0.1219	0.05	99.2	100.8
Pai-tu-san (rehmannia and lonicera formula)	0.1219	0.05	98.1	98.5

[a] Name and composition of the Glycyrrhizae Radix-containing Chinese herbal formulas: *Chia-wei-hsiao-yao-san:* Angelicae Radix, Paeoniae Radix, Atractylodis Rhizoma, Poria, Bupleuri Radix, Moutan Radicis Cortex, Gardeniae Fructus, Glycyrrhizae Radix, Zingiberis Rhizoma, Menthae Herba; *Kuei-pi-tang:* Astragali Radix, Ginseng Radix, Atractylodis Rhizoma, Poria, Zizyphi Spinosi Semen, Longanae Arillus, Angelicae Radix, Zingiberis Rhizoma, Zizyphi Fructus, Polygalae Radix, Glycyrrhizae Radix, Saussureae Radix; *Pai-tu-san:* Rehmanniae Radix, Platycodi Radix, Forsythiae Fructus, Moutan Radics Cortex, Trichosanthis Radix, Scrophvlariae Radix, Lonicerae Flos, Bupleuri Radix, Glycyrrhizae Radix, Phellodendri Cortex, Menthae Herba, Paeoniae Radix, Gypsum Fibrosum, Arctii Fructus.

[b] Glycy = Glycyrrhizin.

[c] Gly · acid = Glycyrrhetinic acid.

be especially useful for bulky samples and also for quality control in the pharmaceutical plants.

ACKNOWLEDGEMENT

Financial support from the National Science Council (NSC 82-0420-B-003-012-M13), Taiwan, is gratefully acknowledged.

REFERENCES

1 H.Y. Hsu, Y.P. Chen, S.J. Sheu, C.H. Hsu, C.C. Chen and H.C. Chang, *Chinese Materia Medica —A Concise Guide*, Modern Drug Press, Taipei, 1984, pp. 31–32.
2 S.Y. Huang, *A Collective Commentary to Herbal Formulas*, Ba-Teh Education and Culture Press, Taipei, 1987, pp. 1–10.
3 M. Harada, Y. Ogigara, Y. Kano, A. Akahori, Y. Ichio, O. Miura and H. Suzuki, *Iakuhin Kenkyu*, 19 (1988) 852.
4 B.L. Karger, A.S. Cohen and A. Guttman, *J. Chromatogr.*, 492 (1990) 585.
5 Y.M. Liu and S.J. Sheu, *J. Chromatogr.*, 600 (1992) 370.
6 Y.M. Liu and S.J. Sheu, *J. Chromatogr.*, 623 (1992) 196.
7 Y.M. Liu and S.J. Sheu, *J. Chromatogr.*, 634 (1993) 329.
8 Y.M. Liu, S.J. Sheu, S.H Chiou, H.C. Chang and Y.P. Chen, *Planta Medica.*, in press.
9 Y.M. Liu and S.J. Sheu, *J. Chromatogr.*, 637 (1993) 219.