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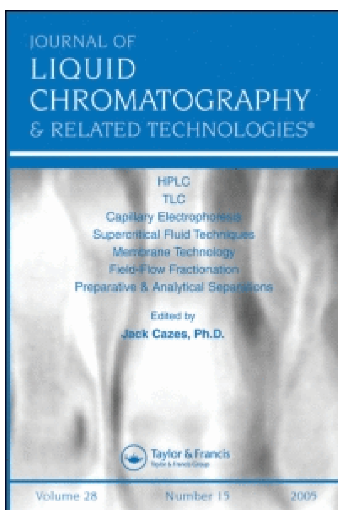

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The Quantitative Determinations of Glycyrrhizic Acid, Glycyrrhetinic Acid, Morphine, and Sodium Benzoate in Compound Liquorice Tablets by HPCE

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The Quantitative Determinations of Glycyrrhizic Acid, Glycyrrhetinic Acid, Morphine, and Sodium Benzoate in Compound Liquorice Tablets by HPCE

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ABSTRACT

Capillary zone electrophoresis (CZE) was used to quantitatively determine the contents of glycyrrhizic acid, glycyrrhetinic acid, morphine, and sodium benzoate in compound liquorice tablets. The detection wavelength was 228 nm and 14 kV voltage was applied, and the 50 mM sodium borate was used as the background electrolyte, in which hydrochlorothiazide served as an internal standard and the temperature was 24–25°C. There were good linear relations between the concentrations and the peak-area ratios of glycyrrhizic acid, glycyrrhetinic acid, morphine, and sodium benzoate, respectively. The average recoveries of glycyrrhizic acid, glycyrrhetinic acid, morphine, and sodium benzoate were 98.2%, 97.3%, 97.1%, and 97.5%, respectively. The method was simple, quick, and accurate.

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Key Words: Glycyrrhizic acid; Glycyrrhetinic acid; HPCE.

INTRODUCTION

Compound liquorice tablets (CLTs) are frequently used as an expectorant, having wide applications in the clinics in China.[1] They contain 0.1125 g of extractum glycyrrhizae (EG), 0.004 g of powdered opium (PO), 0.002 g of camphor (CP), 0.002 g of star anise oil (SAO), and 0.002 g of sodium benzoate (SB) in one tablet. So far nothing has been reported about the simultaneous determinations of its four important constituents by HPCE.[2,3,4,5] Owing to CLTs containing many complex constituents, it was difficult for HPLC to assay, simultaneously, its main constituents such as glycyrrhizic acid (GHIA), glycyrrhetinic acid (GHEA), morphine (MP), and SB, etc. In addition, the columns are expensive and large amounts of mobile phases are needed for analyses. The total assay time was often nearly an hour for a Chinese herbal medicine to be analyzed by HPLC. So we developed an HPCE method to assay the contents of GHIA, GHIA, GHEA, MP, and SB in CLTs. Their chemical structures are shown in Fig. 1.

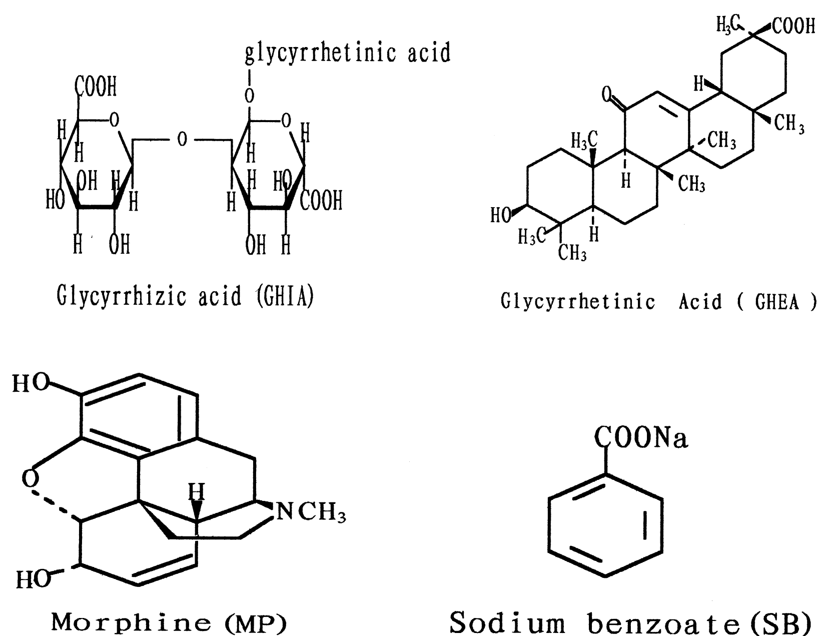

Figure 1. The structures of GHIA, GHEA, MP, and SB.

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The effects of extracting solvents on the electropherograms were investigated and the other experimental conditions were also studied. Four kinds of CLTs, manufactured in different factories in China, were analyzed to have approximately the same results. The method is convenient, accurate, and has both good stability and reproducibility. All the results denoted that this method could be used for quality assessment of CLTs.

EXPERIMENTAL

Reagents and Materials

Ammonium glycyrrhizi nata (AGN), GHEA, MP, and hydrochlorothiazide (HCTZ) standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. SB (refined, content more than 99.8%). Methanol was of the HPLC grade (obtained from the Beijing Changhua Fine Chemical Engineering Factory). CLTs were purchased from a local drugstore. Deionized water was from a system of Labconco, America.

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Apparatus and Conditions

The analysis was performed with a Unimicro CE system from Hyper Quan, Inc., USA, which was equipped with a UV detector operating at 228 nm. A fused-silica capillary tube (75 μm i.d. × 375 μm o.d.) 65 cm long, and the detection window to be set at 50 cm from the injection, was purchased from the Yongnian Optical Fiber Factory, Hebei, China. The experimental conditions were as follows: instrumental sensitivity, 0.01 AUFS, applied voltage, 14 kV (positive to negative polarity); hydrostatic injection time, 10 s (height 7 cm); separation temperature, 24–25°C. The background electrolyte was 50 mM sodium borate solution. The capillary was rinsed with 0.1 M NaOH for 10 min, followed by deionized water for 5 min, and then with BGE for 5 min prior to a days experiment. In between measurements, the capillary was only rinsed with BGE under a high pressure driven for 5 min. The running time was approximately 30 min. All the samples were filtered through a 0.45 μm membrane filter (Millipore, Milford, MA) before the assay.

Preparation of Standard Solution

Amounts of 14.2 mg of AGN (equal to 13.9 mg of GHIA), 20.8 mg of SB, 7.6 mg of GHEA, were accurately weighed and added to 50 mM sodium borate

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solution containing 10% (v/v) methanol to make 25 mL exactly, respectively. Amounts of 7.0 mg of MP was also accurately weighed and diluted with the same solvent mentioned above to 10 mL. The solutions so made were used as the standard stock solutions. Amounts of 0.2537 g of HCTZ were accurately weighed and added to methanol to make 250 mL exactly as the internal standard stock solution.

Preparation of the Sample Solutions of CLTs

Twenty tablets of CLTs and 25.4 mg of HCTZ were added to 50 mM sodium borate solution containing 10% (v/v) methanol to make 250 mL exactly, which was extracted for 30 min in an ultrasonic bath and then filtered to serve as a sample solution.

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RESULTS AND DISCUSSION

Analytical Conditions

Because these four constituents in CLTs have much higher absorption at 228 nm, the detection wavelength was set at this wavelength. In our previous experiments, we selected five kinds of electrolytes, all 14 kV applied and with a concentration of 50 mM NaH_2PO_4, NH_4Ac, $NaHCO_3$, Na_2HPO_4, and $Na_2B_4O_7 \cdot 10H_2O$ solution as the running buffer, respectively. The results denoted, that using the 50 mM sodium borate solution, as BGE was better for separation of CLTs. All the four analytes have hydroxyl groups or carbonyl groups and can be separated in an alkaline borate solution, in principle. When 14 kV voltage was applied, the concentrations of sodium borate were varied among 10, 20, 50, 70 mM. We found, that the zeta potential decreased and the migration time of all the constituents increased as the concentration of sodium borate was gradually increased. At the same time, the separation effects became better at the direction mentioned above. We chose 50 mM sodium borate as BGE because the analysis time was appropriate. As the concentration of BGE (50 mM sodium borate) was fixed, we chose voltage varying from 10 to 20 kV, with increasing 2 kV every step, to separate the sample. We obtained the best separation when 14 kV was applied.

The Identifying Test

To dilute every standard stock solution to a reasonable concentration, with addition of the internal standard solution to prepare a mixed solution,

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the solution was injected for 10 s and recorded with the electropherogram. The sample solution was analyzed under the same condition mentioned above. By comparing the migration times of peaks in the sample electropherogram with that of the standards, we drew the conclusions as in Fig. 2(d).

Calibration Curves and Detection Limits of GHIA, GHEA, MP, and SB

Aliquots of a suitable volume (mL) of the standard stock solutions were pipetted and each transferred to a 10-mL volumetric flask. To each flask, were added 1.0 mL of the internal standard solution and 50 mM sodium borate to make exactly 10 mL, which were used to graph calibration curves. After each concentration was repeated twice, their average peak-area ratios were taken for calculation. Calibration curves: peak-area ratio, y vs. concentration, x, mg/mL were plotted over the range of 0.1390–2380, 0.0304–0.1520, 0.0140–0.2240, 0.0208–0.3328 mg/mL for GHIA, GHEA, MP, and SB, respectively. The regression equations of the four curves and their correlation coeffients were as follows: GHIA, $y = 1.6234 \times -0.7215$ ($r = 0.9892$); GHEA, $y = 1.7459 \times -0.0491$ ($r = 0.9981$); MP, $y = 34.9469 \times -0.9933$ ($r = 0.9921$); and SB, $y = 14.2302 \times +0.7467$ ($r = 0.9965$), respectively. The detection limits (S/N = 3) of the four constituents were: 36 μg/mL, 11 μg/mL, 5 μg/mL and 7 μg/mL for GHIA, GHEA, MP, and SB, respectively.

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The Extracting Solvents

In order to optimize the extracting method, we selected water, 50% (v/v) ethanol, 0.1 M potassium hydroxide, and 50 mM sodium borate solution containing 10% (v/v) methanol as the extracting solvent, respectively. The reason why the 4 solvents were selected for extraction was that we intended to find the best extracting solvent to extract the 4 analytes thoroughly at the same time. The contents of the four constituents in CLTs extracted by the different solvents for four replicates were listed in Table 1. Because EG contained many hydrophilic constituents, water was firstly selected to extract CLTs with better results. The 50%(v/v) ethanol was secondly selected to extract both hydrophilic and partly hydrophobic constituents in CLTs losing two peaks. The 0.1 M KOH served as the third solvent to investigate the content changes of GHIA and GHEA under the hydrolysis conditions, but the results were not good. The 50 mM sodium borate solution containing 10% (v/v) methanol was

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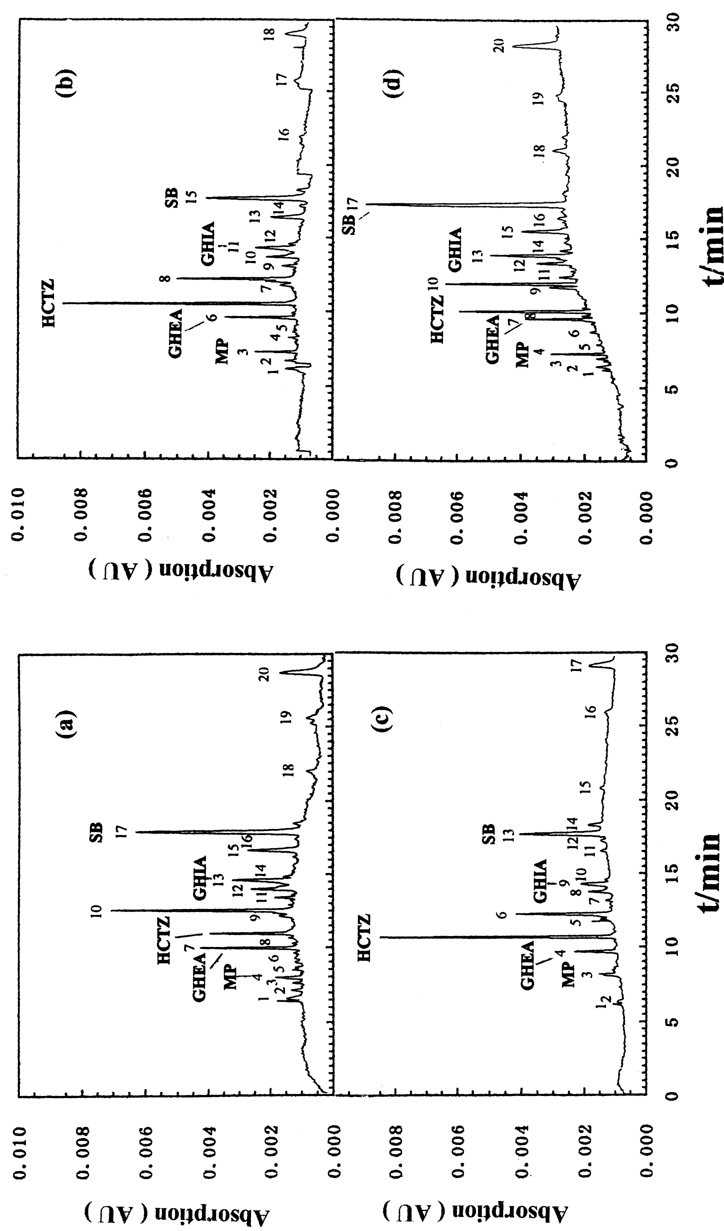

Figure 2. Electropherograms of samples extracted by different solvents. (a) Water; (b) 50% (v/v) ethanol; (c) 0.1 M potassium hydroxide; (d) 50 mM sodium borate. Experimental conditions: applied voltage, 14 kV; buffer, 50 mM sodium borate; hydrostatic injection, 10 s (height, 7 cm); UV detection at 228 nm; instrumental sensitivity, 0.01 AUFS.

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Table 1. The contents of the 4 constituents of CLTs extracted by different solvents ($n = 4$).

	X (R.S.D.%)			
Solvent	H_2O	50% Ethanol	0.1 M KOH	50 mM Borate
GHIA (mg/tablet)	11.03 (2.9)	6.91 (2.6)	6.59 (2.3)	16.20 (2.9)
GHEA (mg/tablet)	5.27 (3.3)	3.30 (2.4)	1.73 (3.4)	6.37 (2.5)
MP (mg/tablet)	0.39 (2.5)	0.42 (2.6)	0.38 (3.1)	0.51 (2.7)
SB (mg/tablet)	2.08 (2.2)	0.43 (3.2)	1.05 (2.3)	2.12 (2.8)

selected as the extracting solvent to obtain both many peaks and high contents of the 4 contituents. Figure 2 presents the electropherograms showing the effects of different extracting solvents on the number of peaks and the peak-height. Figure 2 (b) and Fig. 2 (c) had less peaks than that of the other two. Figure 2 (a) and Figure 2 (d) were very much the same. As far as the BGE was concerned, using 50 mM sodium borate solution containing 10% (v/v) methanol as the extracting solvent was good for the separation, meanwhile, the analytes in CLTs were extracted thoroughly.

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Method Suitability Tests

A mixed standard solution containing 0.5560 mg/mL GHIA, 0.1216 mg/mL GHEA, 0.0560 mg/mL MP, and 0.0832 mg/mL SB was injected to determine the theoretical plate numbers as follows: 38,567 of GHIA, 29,000 of GHEA, 38,563 of MP, 24,732 of SB, which was the results analyzed by the procedures mentioned above.

Precision

The intra-day and inter-day variabilities, through determining the standard solutions approximately equal to the typical assay concentration of the sample, were evaluated for five replicates within one day and over five successive days. The precision RSDs of the newly developed method, in terms of peak-area ratios for five replicate injections, were 0.7–1.5% for intra-day and 1.1–3.2% for inter-day. So the precisions are acceptable.

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Method Reproducibility

Five replicate samples were prepared (manufactured in Shenyang Pharmaceutical Factory, P.R. China) to inject and determine the contents of the four analytes in one tablet. The RSDs of contents of the four constituents were between 1.7–2.2%, which denoted that the method reproducibility was good.

Recoveries

Two tablets of known contents of CLTs were added to 50 mL (50 mM sodium borate solution containing 10% (v/v) methanol as solvent) of mixed solution containing suitable amounts of the four standards (1 mL containing 0.298 mg of GHIA, 0.085 mg of GHEA, 0.015 mg of MP, 0.043 mg of SB for one solution; 0.559 mg of GHIA, 0.126 mg of GHEA, 0.020 mg of MP, 0.087 mg of SB for another) and the same concentration of internal standard. The solutions, so made, were extracted for 30 min in an ultrasonic bath and then filtered to obtain sample solutions. The sample solutions were analyzed by the newly established method. The average recoveries were: 98.2%, 97.3%, 97.1%, and 97.5% for GHIA, GHEA, MP, and SB, respectively, indicating that the method accuracy was pretty good.

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Application to CLTs Manufactured in Different Factories

The CLTs samples manufactured in different factories were analyzed to obtain the results listed in Table 2. The CLTs made in Shenyang Pharmaceutical Factory are the best of the four kinds of CLTs. The main

Table 2. The contents of the four constituents in CLTs made in the different factories ($n = 4$).

	X (R.S.D.%)			
Pharm. factory lot no.	Qinghai 20010209	Huhehaote 20011024	Beijing 20011104	Shenyang 20011106
GHIA (mg/tablet)	14.33 (2.2)	13.65 (2.4)	13.65 (2.3)	16.16 (2.6)
GHEA (mg/tablet)	5.34 (2.3)	5.51 (2.6)	4.93 (2.5)	6.55 (2.5)
MP (mg/tablet)	0.45 (2.7)	0.43 (2.5)	0.46 (2.0)	0.49 (2.3)
SB (mg/tablet)	2.10 (1.9)	2.12 (1.8)	2.13 (1.7)	2.20 (2.1)

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objective of this study is to develop a new method to assay the important constituents in CLTs. The separations presented above, were reproducible over extended periods of time. We routinely determined the four constituents in CLTs for months with no observable changes in contents. Finally, we believe that this approach is potentially useful for CLTs analyses.

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