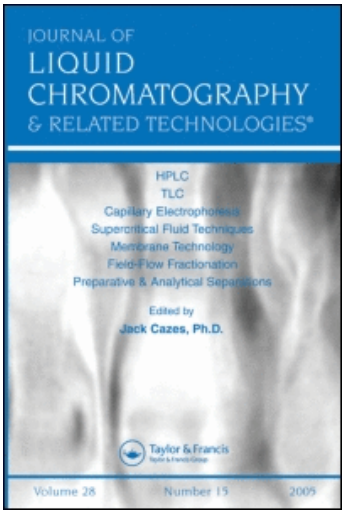

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ANALYSIS OF SYNTHETIC GASTROINTESTINAL DRUGS IN ADULTERATED TRADITIONAL CHINESE MEDICINES BY HPCE

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ABSTRACT

Six synthetic gastrointestinal drugs, cimetidine, homatropine, metoclopramide, pirenzepine, ranitidine, and scopolamine butylbromide, which can be found as adulterants in traditional Chinese medicines were assayed simultaneously, within 6 min., using high performance capillary electrophoresis. The electrolyte was a buffer solution containing 100 mM phosphate buffer (NaH_2PO_4/H_3PO_4, pH 2.5) and 5 % acetonitrile. Applied voltage was 20.5 kV and temperature was 30°C. 3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride was used as an internal standard and the detector was set at 214 nm. The effects of buffer pH and acetonitrile concentration on separation are important. The relative

standard deviations of these gastrointestinal drugs for intra-day and inter-day analyses were 0.78-2.15 % and 1.10-2.75 %, respectively. The recoveries of the synthetic drug adulterants in traditional Chinese medicinal formula ranged from 97.3 to 102.4 %.

INTRODUCTION

The adulteration by synthetic therapeutic substances of traditional Chinese medicines (TCM) has been reported on many occasions and has been a public health concern in Taiwan over the past several years. The term "adulteration" refers to TCM tested and found to contain chemical substances not prescribed or labeled as part of the intended use. The adulteration of synthetic therapeutic substances of TCM has been also reported in several industrialized countries over the past two decades.[1-5] The adulteration by synthetic therapeutic substances of TCM was banned for reasons of public safety by the health authorities in Taiwan. Over the years, TCM have been routinely referred to our laboratories from various sources and some of which have contained adulterants. The results have been reported annually.[6-7] In 1992 an island-wide monitoring of the prohibited adulteration of TCM through hospital pharmacies was initiated. A higher percentage (26.1%) of the TCM without commercial packaging has been reported.[8] Cimetidine (CIM) and ranitidine (RAN) are H_2-receptor antagonists and are used to treat peptic ulcer. These two synthetic drugs have also been detected in TCM.[7]

CIM, RAN, homatropine (HOM), scopolamine butylbromide (SCO), metoclopramide (MET), and pirenzepine (PIR) were chosen for monitoring the adulterants in TCM prescribed for gastrointestinal (GI) discomfort.[9] A number of high performance liquid chromatography and high performance capillary electrophoresis (HPCE) methods have been reported for the determination of GI drugs; all of these methods have been performed to assess one or two of these synthetic drugs in biological fluids.[10-13] However, most formulas of TCM are composed of many crude drugs and their constituents are complicated and therefore, it differs from analysis of biological samples. Our previous studies have established HPCE methods for identification and determination of analgesics,[14] clobenzorex, diazepam,[15] and anorexics.[16] In this study, the simultaneous separation of CIM, HOM, MET, PIR, RAN, and SCO as depicted in Figure 1, by HPCE method for determination in TCM was developed.

EXPERIMENTAL

Reagents and Materials

CIM, HOM, MET, PIR, RAN, and SCO were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile and methanol were obtained from

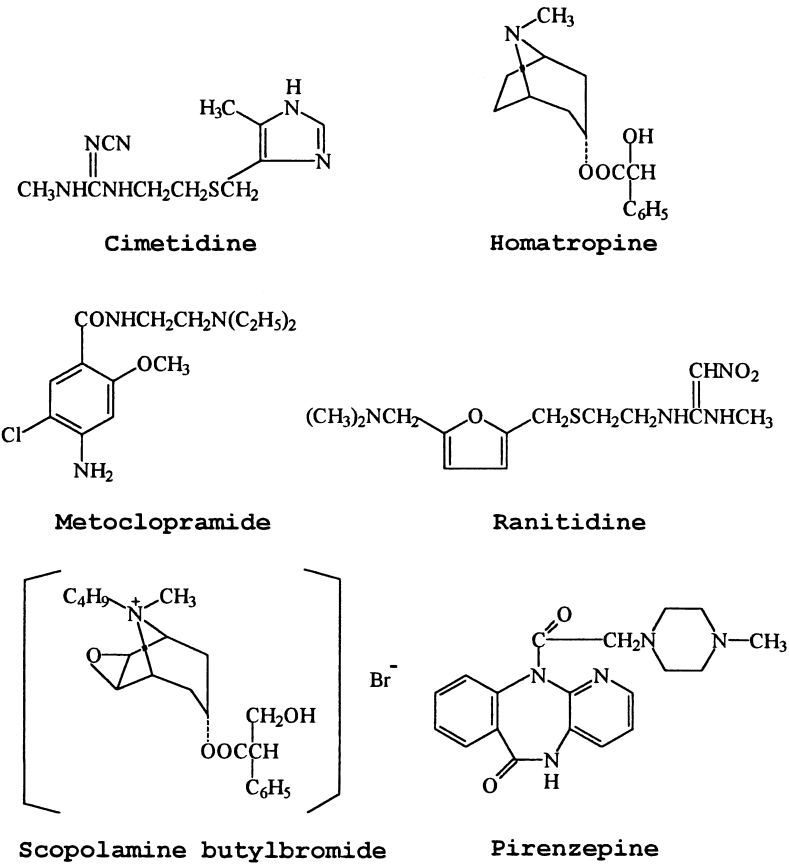

Figure 1. The structures of six synthetic gastrointestinal drugs.

Labscan (Dublin, Ireland). Disodium hydrogenphosphate and sodium dihydrogenphosphate were purchased from Nakalai (Kyoto, Japan). 3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (BHM) was purchased from E. Merck (Darmstadt, Germany). Ortho-phosphoric acid was analytical reagent grade. Ultrapure distilled water with a resistance greater than 18 MΩ was used.

One formula of commercial concentrated herbal preparation, Sheau-Jiann-Jong-Tang, was used as a model preparation. The contents of the crude drug in a daily dose contained Glycyrrhizae Radix (2.0 g), Zingiberis Rhizoma, Cinnamomi Ramulus, Zizyphi Fructus (3.0 g each), Maltosum (4.0 g), Paeoniae Radix (6.0 g). This sample preparation was purchased from retail outlets in Taipei.

Apparatus and Condition

The analysis was carried out on a Beckman P/ACE 5500 CE system equipped with a photodiode array detector. Detector was set at 214 nm and a 47 cm x 75 μm I.D. uncoated capillary (Beckman) with the detection window placed at 40 cm. The conditions were as follows: sampling time, 2 sec, hydrostatic; run time, 7 min; applied voltage, 20.5 kV (constant voltage, positive to negative polarity); and temperature, 30°C. The electrolyte buffer was a solution containing 100 mM sodium phosphate buffer (NaH_2PO_4/H_3PO_4, pH 2.5) and 5% acetonitrile. The electrolyte was filtered through a 0.45 μm syringe filter (Gelman) before use. Between each sample throughout the experiment, the capillary was cleaned with 1% sodium hydroxide, 2 min and water, 2 min, successively. The capillary was rinsed with buffer for 2 min before each experiment. The Gold software (Beckman) for system control and data processing was used.

Preparation of Standard Solution

To prepare a standard solution containing six synthetic drugs, an appropriate amount of internal standard solution (BHM, made to 60.2 μg/mL) was added to an accurately weighed amount of six chemical drug standards dissolved in water to give various concentrations within the range 16-260 μg/mL for these six synthetic GI drugs, respectively. Calibration graphs were plotted subsequently to linear regression analysis of the peak area ratios versus concentration.

Preparation of Model Preparation Sample Solution

Model preparation sample of 1.0 g was accurately weighed and extracted with water (50 mL) for 30 min in an ultrasonic bath and then filtered and diluted with water to 50 mL.

Precision

The intra-day and inter-day variabilities at three typical assay concentrations were evaluated for six replicates within one day and over six successive days.

Recovery

Three different concentrations of the six synthetic GI drugs were spiked to the model preparation sample solutions (10 mL). An aliquot of 0.5 mL of BHM

solution (1.2 mg/mL) was added to the above solution and made up to 10 mL with water. These mixtures were analyzed by the procedures mentioned above.

RESULTS AND DISCUSSION

TCM is usually prepared by water decoction, the water soluble constituents are the major part of decoction. Normally, their chemical constituents are complicated in decoction which may interfere in analysis. HPCE is a highly efficient separation method for the analysis of positively charged constituents. Conditions needed to make this analysis successful are described as following:

Analytical Conditions

The detection wavelength was chosen at 214 nm because these six synthetic GI drugs have reasonable absorption at this wavelength. In this low wavelength detection, phosphate salt was chosen for the buffer solution due to its lower absorbance. BHM was used as an internal standard which migrated out before these six synthetic GI drugs. The qualitative characterization of the peak of each drug was carried out using a photodiode array detector. The UV spectrum of each drug examined from the model preparation was the same as that of the standard. Therefore, the photodiode array detector facilitated the identification and confirmation of the six synthetic drugs.

The separation was optimized by adjusting the pH of the buffer and the percentage of modifier (acetonitrile). The pH is one of the most important parameters for improving selectivity in HPCE and small differences can cause the separation of closely related substances. By keeping the other conditions the same, the buffer pH was varied from 2.0 to 3.5 in steps of 0.5 pH units. Results are shown in Figure 2. Only pH 2.5 was considered the best with respect to resolution and migration time.

The content of organic solvent to the buffer can lengthen the separation time and widen the migration window, and alter the selectivity and resolution. Acetonitrile was used in our experiments and Figure 3 shows a graph of the migration time versus acetonitrile concentration for all drugs. The concentration was varied from 0 to 15% (v/v) in steps of 5% (v/v). Although 10% of acetonitrile makes migration shorter, the resolution of CIM and MET is poor. Overall, the 5% acetonitrile solution gave the best results.

The optimal conditions we found comprise an electrolyte containing 100 mM NaH_2PO_4, 5% acetonitrile buffer at pH 2.5 and with the cartridge temperature and voltage setting at 30°C and 20.5 kV. Figure 4 presents an electropherogram showing the separation of the constituents with the migration times of 4.4 min for the internal standard (BHM); 4.6, 5.0, 5.1, 5.2, 5.4, and 5.7 min

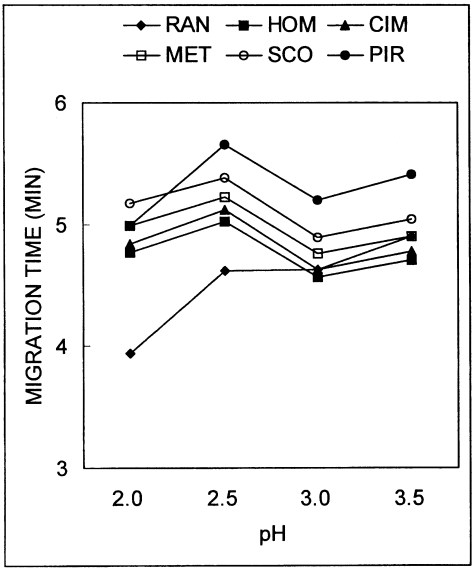

Figure 2. Effect of pH on migration time. All experiments were conducted at a voltage of 20.5 kV across the 47 cm·75 μm I.D. uncoated capillary filled with 100 m*M* sodium phosphate buffer (NaH_2PO_4/H_3PO_4) and 5 % acetonitrile; cartridge temperature, 30°C; detection wavelength, 214 nm.

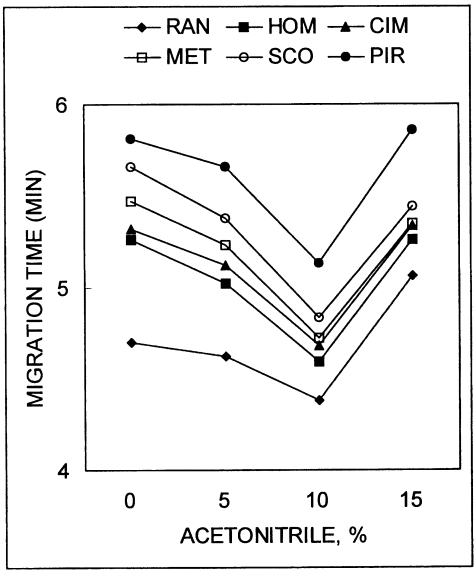

Figure 3. Effect of acetonitrile concentration on migration time. The carriers were 100 m*M* sodium phosphate buffer (NaH_2PO_4/H_3PO_4 pH 2.5) and 0-15 % acetonitrile. Other conditions and symbols as in Figure 2.

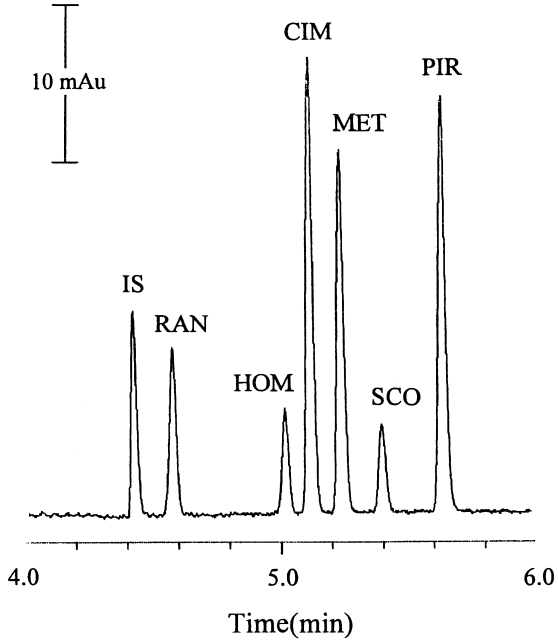

Figure 4. Capillary electropherogram of a mixture of the six synthetic gastrointestinal drugs. RAN = Ranitidine; HOM = Homatropine; CIM = Cimetidine; MET = metoclopramide; SCO = Scopolamine Butylbromide; PIR = Pirenzepine. IS= 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride. HPCE conditions, capillary: 47 cm x 75 μm I.D.; buffer: 100 m*M* phosphate buffer (NaH_2PO_4/H_3PO_4 pH 2.5) and 5 % acetonitrile; voltage: 20.5 kV; temperature: 30°C; detection wavelength: 214 nm.

for RAN, HOM, CIM, MET, SCO, and PIR, respectively. The complete separation was done within 6 min.

Calibration Graphs for Synthetic GI Drugs and Detection Limits of Synthetic GI Drugs

Calibration graphs: peak-area ratio, *y*, vs. concentration, *x*, μg/mL were obtained over the range of 16.4-262.4 μg/mL, 16.2-258.6 μg/mL, 16.4-262.4 μg/mL, 16.3-261.1 μg/mL, 16.2-259.8 μg/mL and 16.4-262.4 μg/mL for CIM, HOM, MET, PIR, RAN, and SCO, respectively. The regression equations of the six curves and their correlation coefficients were calculated as: CIM, $y = 23.29x + 1.93$ (r = 0.9997); HOM, $y = 101.77x + 1.18$ (r = 0.9996); MET, $y = 26.59x +$

3.78 (r = 0.9997); PIR, y *F*= 23.22x + 3.26 (r = 0.9993); RAN, y = 58.96x + 1.30 (r = 0.9997) and SCO, y = 111.96x + 5.00 (r = 0.9990). A signal three times higher than the peak noise height was regarded as the detection limit. The detection limits of the six synthetic anorexics were: 1.0, 4.0, 1.0, 1.0, 2.0, and 4.0 μg/mL for CIM, HOM, MET, PIR, RAN, and SCO, respectively.

Suitability Tests

The precision of the electrophoretic assay method was evaluated by measuring the reproducibility [relative standard deviation (R.S.D.)] while the accuracy was determined by recovery tests. The precision R.S.D.s of the proposed method of the six synthetic GI drugs, on the basis of peak-area ratios for six replicate analyses were 0.78-2.15% for intraday and 1.10-2.75% for inter-day, respectively (Table 1). All of these data indicated that the precisions are acceptable.

Table 1

Intra-Day and Inter-Day Analytical Precisions of Three Concentrations of Six Synthetic Drugs

Synthetic Drugs	Concentration (μg/mL)	Intra-Day (R.S.D., %)*	Inter-Day (R.S.D., %)*
CIM	16.4	1.07	1.46
	65.6	1.27	1.24
	262.4	0.93	1.27
HOM	16.2	1.10	1.33
	64.6	0.78	1.31
	258.6	1.15	1.75
MET	16.4	1.16	1.86
	65.6	0.84	1.10
	262.4	1.11	1.76
PIR	16.3	1.33	1.90
	65.3	1.05	1.38
	261.1	1.71	1.92
RAN	16.2	1.43	1.96
	65.0	1.25	1.85
	259.8	1.22	1.72
SCO	16.4	2.15	2.75
	65.6	1.99	2.67
	262.4	2.03	2.40

* n = 6.

Table 2

Recoveries of Six Synthetic Drugs in Three Spiked TCM Preparations

Synthetic Drugs	Added (μg/mL)	Measured (mean, n=3, (μg/mL)	Recovery (mean, n=3 μg/mL)	Mean±SD (%)	RSD (%)
CIM	32.8	32.7	99.8	100.6±0.7	0.7
	65.6	66.5	101.4		
	131.2	131.9	100.5		
HOM	32.4	32.0	98.7	99.7±1.9	1.9
	64.6	63.3	98.0		
	129.2	132.3	102.4		
MET	32.8	32.1	97.8	99.0±1.0	1.0
	65.6	64.9	98.9		
	131.2	131.6	100.3		
PIR	32.6	32.7	100.4	101.2±0.6	0.5
	65.3	66.2	101.4		
	130.6	132.8	101.7		
RAN	32.4	33.1	102.3	100.0±1.6	1.6
	65.0	64.4	99.1		
	130.0	128.2	98.6		
SCO	32.8	31.9	97.3	99.5±1.5	1.5
	65.6	65.9	100.5		
	131.2	132.0	100.6		

One kind of formula of a commercial concentrated herbal preparation, Sheau-Jiann-Jong-Tang, is traditionally prescribed for GI use.[17] This preparation was used as a model sample for assessing recovery. The recovery studies of six adulterants were conducted by model preparation samples with known spiked concentrations of the synthetic drugs. The results are given in Table 2. The R.S.D.s (n=3) of recoveries were lower than 2%. These assessments indicate good accuracy for this method.

In conclusion, we have developed a fast and efficient method to detect these six synthetic GI adulterants in TCM. An HPCE method analyzed the adulterants of CIM, HOM, MET, PIR, RAN, and SCO in TCM within 6 min. The recoveries were 100.6, 99.7, 99.0, 101.2, 100.0, and 99.5% for CIM, HOM, MET, PIR, RAN, and SCO, respectively.

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