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Short communication

# Analysis of synthetic anti-diabetic drugs in adulterated traditional Chinese medicines by high-performance capillary electrophoresis

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**Abstract**

Four synthetic anti-diabetic drugs, acetohexamide (ACE), chlorpropamide (CHL), glibenclamide (GLI) and tolbutamide (TOL), which can be found as adulterants in traditional Chinese medicines (TCMs) were assayed simultaneously using high-performance capillary electrophoresis (HPCE) in 4 min with UV detection at 200 nm. The electrolyte was a buffer solution containing 100 mM phosphate buffer ($NaH_2PO_4/Na_2B_4O_7$, pH 7.5). Applied voltage was 15.0 kV and temperature was 30 °C. 2-(4-Hydroxyphenyl) ethyl ammonium chloride (HEA) was used as an internal standard. The effects of buffer concentration, pH and supplied voltage on separation were investigated. The relative standard deviations (R.S.D.) of these anti-diabetic drugs for intra-day and inter-day analyses were 0.23–4.27 and 1.23–6.33%, respectively. The recoveries of the synthetic drug adulterants in traditional Chinese medicinal formula ranged from 81.3 to 105.5%. GLI was found and determined in a real sample of TCM.


*Keywords:* Pharmaceutical analysis; Anti-diabetic drugs; Capillary electrophoresis

## 1. Introduction

Many herbal medicines and synthetic drugs are useful at therapeutic doses but toxic at high concentrations. Co-administration of herbs and drugs can enhance or decrease the pharmacological or toxicological effects of drugs. Synergistic therapeutic effects may also complicate long-term medication [1].

The adulteration by synthetic therapeutic substances of traditional Chinese medicines (TCM) was banned for the reason of public safety by the health authorities in Taiwan. Over the past several years, the adulteration by synthetic therapeutic substances of TCM has been reported on many occasions and has been a public health concern in

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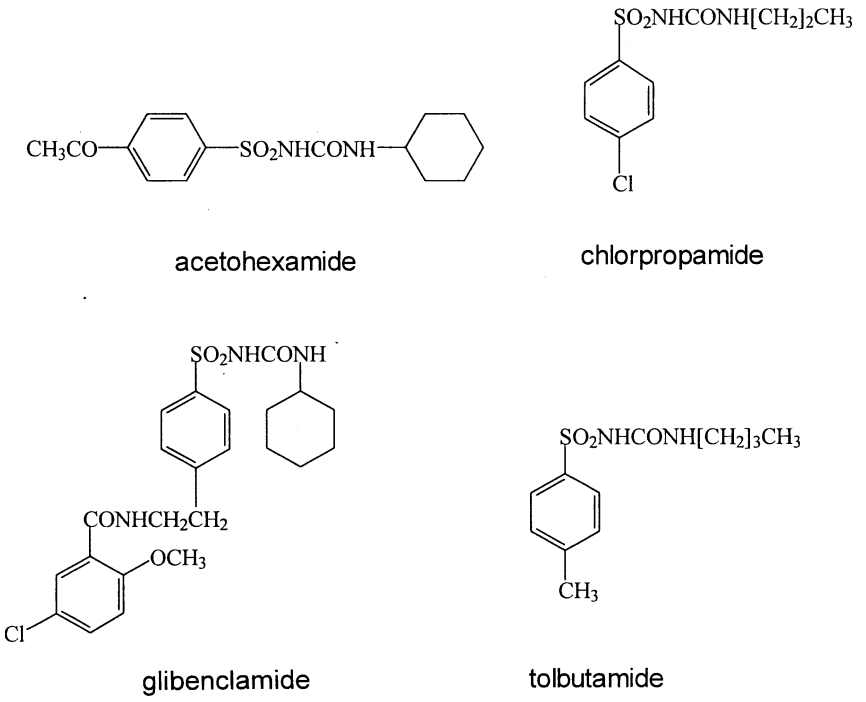


Fig. 1. The structures of four synthetic anti-diabetic drugs.

Taiwan. Various sources of herbal medicines have been detected by our laboratories to be adulterated with synthetic drugs as previously reported [2,3]. High-performance capillary electrophoresis (HPCE) methods have been developed for measuring five anorexic [4] and four gastrointestinal [5] adulterants in herbal medicines.

Acetohexamide (ACE), chlorpropamide (CHL), glibenclamide (GLI) and tolbutamide (TOL) were chosen for monitoring the adulterants in TCM prescribed for diabetes [6]. A number of high-performance liquid chromatography and (HPCE) methods have been reported for determination of above drugs, all of these methods have been used to assess two or three of these synthetic drugs in biological fluids [7–9]. 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. In this study, the simultaneous separation of ACE, CHL, GLI and TOL as depicted in Fig. 1, by HPCE for determination in TCM was developed.

## 2. Experimental

### 2.1. Reagents and materials

ACE, CHL, GLI and TOL were purchased from Sigma (St. Louis, MO, USA). HPLC grade methanol was obtained from Labscan (Dublin, Ireland). Sodium tetraborate and sodium dihydrogenphosphate were purchased from Nakalai (Kyoto, Japan). 2-(4-Hydroxyphenyl) ethyl ammonium chloride (HEA) was purchased from E. Merck (Darmstadt, Germany). Sodium hydroxide was analytical reagent grade. Ultrapure distilled water with a resistance greater than 18 MΩ was used.

One formula of commercial concentrated herbal preparation, Liow-Wey-Dih-Hwang-Wan, was used as 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.

Sample A was a black pill manufactured by a pharmaceutical plant in mainland China.

### 2.2. Apparatus and condition

The analysis was carried out on a Beckman P/ACE 5500 CE system equipped with a photodiode array detector (set at 200 nm). Separation was performed on a 37 cm × 75 μm I.D. uncoated capillary (Beckman) with the detection window placed at 30 cm. The conditions were as follows: sampling time, 2 s, hydrostatic; run time, 5 min; applied voltage, 15 kV (constant voltage, positive to negative polarity); and temperature, 30 °C. The electrolyte buffer was a solution containing 100 mM sodium phosphate buffer ($NaH_2PO_4$/$Na_2B_4O_7$, pH 7.5). 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, 1.5 min and water, 1.5 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.

### 2.3. Preparation of standard solutions

To prepare a standard solution containing four synthetic drugs, an appropriate amount of internal standard solution (HEA, made to 60.2 μg/ml) was added to an accurately weighed amount (10 mg) of four chemical drug standards dissolved in water (with 5 drops of 1 M NaOH) to make five various concentrations within the range 4–80 μg/ml, respectively. Calibration graphs were plotted subsequently to linear regression analysis of the peak area ratios versus concentrations.

### 2.4. Preparation of sample solution

Sample A (1.0 g) was accurately weighed and extracted with alcohol twice (15 ml each) for 30 min at 40 °C in an ultrasonic bath. The extract was then filtered and concentrated under reduced pressure to dryness. Finally, the residue was dissolved in 30 ml of water (with 5 drops of 1 M NaOH) and made up to 50 ml with water. The sample solution was prepared by mixing 4.5 ml of the above solution and 0.5 ml of IS solution (250 μg/ml) in a 5 ml volumetric flask.

### 2.5. Recovery

Three different concentrations (9.0, 18.0 and 36.0 μg/ml) of the four synthetic anti-diabetic drugs were spiked to the model preparation sample (1.0 g), and then processed as sample solution.

### 2.6. Precision

The intra-day and inter-day variabilities at three typical assay concentrations were evaluated for five replicates within 1 day and over 5 successive days.

## 3. Results and discussion

### 3.1. Analytical conditions

The detection wavelength was chosen at 200 nm because the four synthetic anti-diabetic drugs have high absorptions at this wavelength. For this low wavelength detection phosphate salt was chosen for the buffer solution due to its low absorbance. HEA was used as an internal standard, which migrated out before the analytes. 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 and the real sample was compared with that of standard to facilitate the identification and confirmation of the four synthetic drugs.

The separation was optimized by adjusting the buffer concentration, pH and the applied voltage. The buffer concentration is one of the most important parameters for improving selectivity and small differences can cause the separation of closely related substances. By keeping the other conditions the same, the buffer concentration was varied from 60 to 120 in steps of 20 mM phosphate

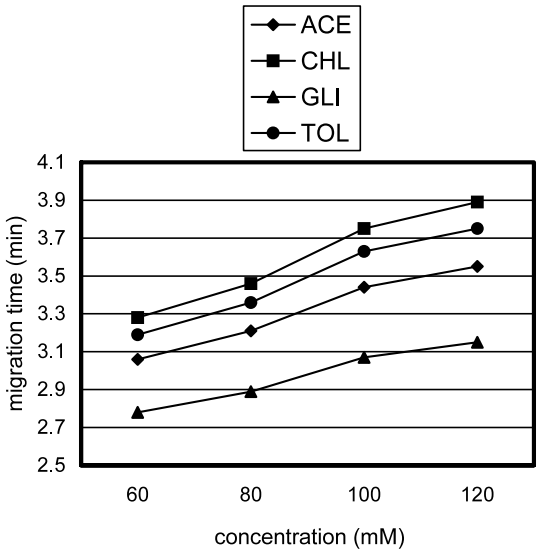


Fig. 2. Effect of buffer concentration on migration time. The carriers were 60–120 mM sodium phosphate buffer ($NaH_2PO_4$/$Na_2B_4O_7$, pH 7.5). All experiments were conducted at a voltage of 15 kV across the 37 cm × 75 μm I.D. uncoated capillary; cartridge temperature, 30 °C; detection wavelength, 200 nm. (◆) ACE, acetohexamide; (■) CHL, chlorpropamide; (▲) GLI, glibenclamide; (●) TOL, tolbutamide.

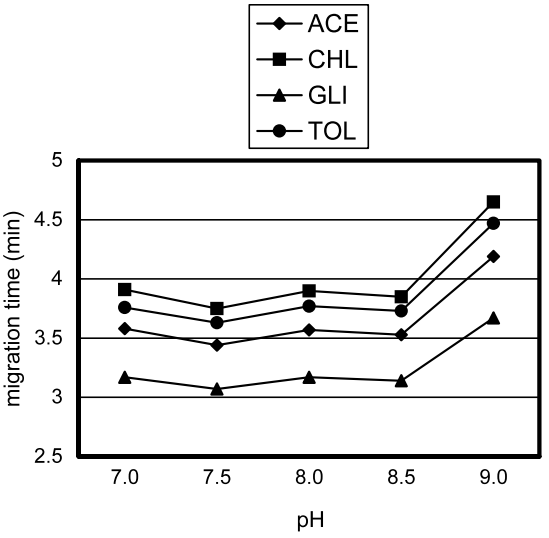


Fig. 3. Effect of pH on migration time. The carriers were pH 7.0–9.0 100 mM phosphate buffer ($NaH_2PO_4$/$Na_2B_4O_7$). Other conditions and symbols as in Fig. 2.

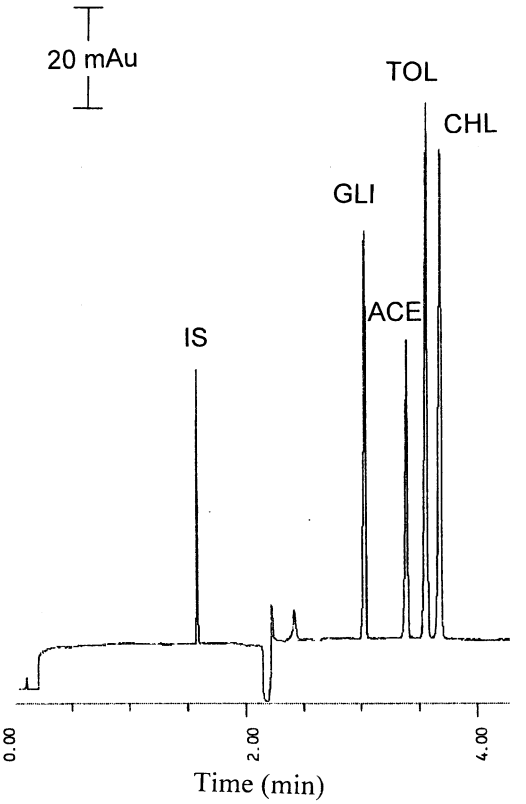


Fig. 4. Capillary electropherogram of a mixture of the four synthetic anti-diabetic drugs. ACE, acetohexamide; CHL, chlorpropamide; GLI, glibenclamide; TOL, tolbutamide. IS, 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride. HPCE conditions; capillary, 37 cm × 75 μm I.D.; buffer, 100 mM phosphate buffer ($NaH_2PO_4$/$Na_2B_4O_7$, pH 7.5); voltage, 15 kV; temperature, 30 °C; detection wavelength, 200 nm.

buffer ($NaH_2PO_4$/$Na_2B_4O_7$, pH 7.5). The results are shown in Fig. 2. In all instances, GLI and ACE were successfully separated, but CHL and TOL had poor resolution ($Rs < 1.5$) in 60 and 80 mM buffer. This indicated the mass to charge ratios of CHL and TOL in the buffer are similar. Using more than 100 mM phosphate buffer for CHL and TOL gave better resolution.

The effect of buffer pH (7.0, 7.5, 8.0, 8.5 and 9.0) was tested but only pH 7.5 was considered satisfactory with respect to resolution and migration time (Fig. 3). Different voltages (7.5, 10.0, 12.5 and 15.0 kV) were also used to study the effect of applied voltage on the selectively of the separa-

Table 1
Intra-day and inter-day analytical precisions of three concentrations of four synthetic drugs

| Synthetic drugs | Concentration (μg/ml) | Intra-day (R.S.D., %)* | Inter-day (R.S.D., %)* |
|---|---|---|---|
| ACE | 4.0 | 4.11 | 6.33 |
| | 16.0 | 1.17 | 3.65 |
| | 80.0 | 0.47 | 1.23 |
| CHL | 4.0 | 2.61 | 4.29 |
| | 15.8 | 0.92 | 3.47 |
| | 79.2 | 0.66 | 1.60 |
| GLI | 4.0 | 4.27 | 3.06 |
| | 16.0 | 1.37 | 2.61 |
| | 80.0 | 0.33 | 2.02 |
| TOL | 4.1 | 3.38 | 4.33 |
| | 16.3 | 0.71 | 2.69 |
| | 81.6 | 0.23 | 1.39 |

$n = 6$.

Table 2
Recoveries of four synthetic drugs in three spiked TCM preparation

| Synthetic drugs | Added (μg/ml) | Measured (mean, $n = 3$, μg/ml) | Recovery (mean, $n = 3$, %) | Mean ± S.D. (%) | R.S.D. (%) |
|---|---|---|---|---|---|
| ACE | 9.0 | 9.7 | 107.3 | 100.6 ± 0.7 | 0.7 |
| | 18.0 | 19.6 | 108.8 | | |
| | 36.0 | 36.1 | 100.3 | | |
| CHL | 9.0 | 8.3 | 92.3 | 99.7 ± 1.9 | 1.9 |
| | 18.0 | 17.6 | 98.0 | | |
| | 36.0 | 33.7 | 93.7 | | |
| GLI | 9.0 | 7.5 | 83.8 | 100.0 ± 1.6 | 1.6 |
| | 18.0 | 13.0 | 72.4 | | |
| | 36.0 | 31.5 | 87.5 | | |
| TOL | 9.0 | 7.2 | 80.0 | 99.5 ± 1.5 | 1.5 |
| | 18.0 | 15.8 | 87.8 | | |
| | 36.0 | 30.1 | 83.6 | | |

tion. In this investigation 7.5 kV gave the best resolution and showed shortest migration time.

The optimal conditions comprised an electrolyte containing 100 mM phosphate buffer ($NaH_2PO_4$/$Na_2B_4O_7$) at pH 7.5 and with the cartridge temperature and voltage setting at 30 °C and 15 kV. Fig. 4 presents an electropherogram showing the separation of the constituents with the migration times of 1.6 min for the internal standard (HEA); 3.4, 3.8, 3.1 and 3.6 min for ACE, CHL, GLI and TOL, respectively. The complete separation was done within 4 min.

### *3.2. Calibration graphs for synthetic anti-diabetic drugs and detection limits of synthetic anti-diabetic drugs*

Calibration graphs: peak area ratio, $y$, versus concentration, $x$, μg/ml were obtained over the range of 4.0–80.0, 4.0–79.2, 4.0–80.0 and 4.1–81.6 μg/ml for ACE, CHL, GLI and TOL, respectively. The regression equations of the four curves and their correlation coefficients were calculated as: ACE, $y = 3.81\text{E}-2x-7.23\text{E}-3$ ($r = 0.9998$); CHL, $y = 6.97\text{E}-2x-1.67\text{E}-2$

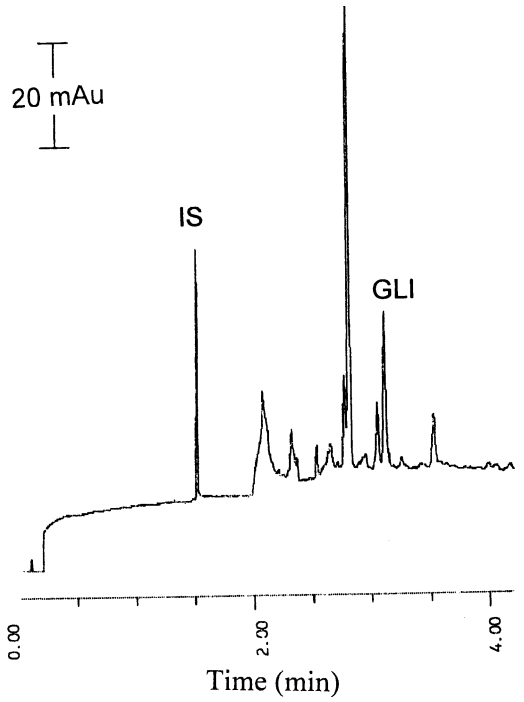


Fig. 5. Capillary electropherogram of a real sample.

($r = 0.9998$); GLI, $y = 4.81\text{E}-2x-8.65\text{E}-3$ ($r = 0.9997$) and TOL, $y = 7.42\text{E}-2x-2.30\text{E}-2$ ($r = 0.9999$). A signal three times higher than the peak noise height was regarded as the detection limit. The detection limits of the four synthetic anti-diabetic drugs were: 0.4, 0.2, 0.4 and 0.2 μg/ml for ACE, CHL, GLI and TOL, respectively.

### 3.3. 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 four synthetic anti-diabetic drugs, on the basis of peak area ratios for five replicate analyses were 0.23–4.27% for intra-day and 1.23–6.33% for inter-day, respectively, (Table 1). All of these data indicated that precision was acceptable.

### 3.4. Application to adulterated TCM

One kind of formula of a commercial concentrated herbal preparation, Liow-Wey-Dih-Hwang-Wan, is traditionally prescribed for diabetes use [10]. This preparation was used as a model sample for assessing recovery. The recovery studies of four 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. Fig. 5 shows an electropherogram of a real sample. The content of GLI in this sample was 0.19% (0.50 mg/pill).

In conclusion, we have developed a fast and efficient method to detect these four synthetic anti-diabetic adulterants in TCM. An HPCE method analyzed the adulterants of ACE, CHL, GLI and TOL in TCM within 4 min. The recoveries were 99.0, 101.2, 100.0 and 99.5% for ACE, CHL, GLI and TOL, respectively.

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