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Short communication

Analysis and confirmation of synthetic anorexics in adulterated traditional Chinese medicines by high-performance capillary electrophoresis

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Abstract

Six synthetic anorexics, clobenzorex, diethylpropion, fenfluramine, methamphetamine, phenylpropanolamine and phentermine, which can be found as adulterants in traditional Chinese medicines were assayed simultaneously by high-performance capillary electrophoresis. The electrolyte was a buffer solution containing 120 m*M* phosphate buffer (NaH_2PO_4/H_3PO_4, pH 2.0) and 15% acetonitrile. Applied voltage was 16 kV and temperature was 30°C. Fluoren-2,7-diammonium chloride was used as an internal standard and detector set at 200 nm. The recoveries of the synthetic anorexic adulterants in traditional Chinese medicinal formula using C_8-SCX mixed solid-phase extraction were studied. Several traditional Chinese medicinal powders obtained from clinics were also studied by the above HPCE method and confirmed by GC–MS. Clobenzorex, diethylpropion and fenfluramine were found and determined in these samples.

Keywords: Pharmaceutical analysis; Amphetamine-like compounds

1. Introduction

The adulteration by synthetic therapeutic substances of traditional Chinese medicines (TCMs) has been reported on many occasions and has been a public health concern in Taiwan over the past several years [1–7]. The adulteration by synthetic therapeutic substances of TCMs was banned for the reason of public safety by the health authorities in Taiwan. Over the years, TCMs have been routinely referred to our laboratories from various sources and some of which have contained adulterants. In 1992 an island-wide monitoring of the prohibited adulteration of TCMs through hospital pharmacies was initiated. A higher percentage (26.1%) of the TCMs without commercial packaging has been reported [8]. Furthermore, amphetamine-like drugs including methamphetamine and its related substances such as clobenzorex, diethylpropion, phentermine and phenylpropanolamine from TCMs has been reported [9,10].

Although a number of high-performance liquid chromatography (HPLC) and high-performance capillary electrophoresis (HPCE) methods have been

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reported for determination of amphetamine-like drugs, all of these methods were performed to assess biological fluids or seized tablets [11–14]. However, most formulas of TCMs are composed of many crude drugs and their constituents are very complex. There are many differences between analysis of biological samples and TCMs. Therefore, an economical and suitable method for detection and assay of the adulterated TCMs is still needed and thus prompted us to develop this method. To our knowledge, only a few existing methods are dedicated exclusively to the separation of amphetamine-like drugs of interest, especially the drugs adulterating TCMs. After several trials, the simultaneous separation of clobenzorex (CBZ), diethylpropion (DEP), fenfluramine (FEN), methamphetamine (MET), phenylpropanolamine (PPA) and phentermine (PHE) as depicted in Fig. 1, by HPLC was found to be unsuitable. HPCE is a recently developed technique, which requires only a short run time, a small amount of sample, and the capillary column can be easily and thoroughly cleaned. In addition, combined with an autosampler it is convenient for analysis of large amounts of samples. Owing to its speed and high resolving power, HPCE has been shown to be well suited for drug screening [15]. In this study, a HPCE

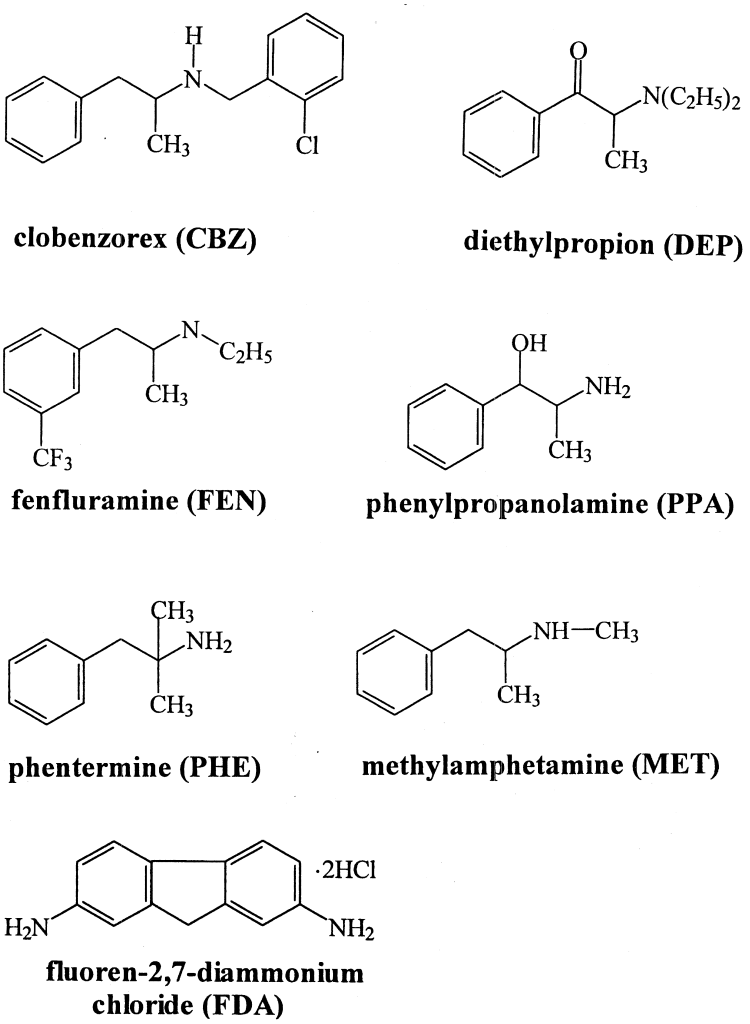

Fig. 1. The structures of six synthetic anorexics and internal standard (FDA).

method for determination of these six synthetic anorexic adulterants in TCMs is assessed. GC–MS was also applied to confirm CE results.

2. Materials and methods

2.1. Reagents and materials

Diethylpropion, fenfluramine, methamphetamine, phenylpropanolamine and phentermine were purchased from Sigma (St. Louis, MO, USA). Clobenzorex was obtained from Roussel Uclaf (Paris, France). HPLC-grade dichloromethane and acetonitrile were obtained from Labscan (Dublin, Ireland). Isopropanol and sodium dihydrogenphosphate were purchased from Nakalai (Kyoto, Japan). Ammonium chloride and fluoren-2,7-diammonium chloride (FDA) were purchased from E. Merck (Darmstadt, Germany). Orthophosphoric acid and ammonia water were analytical-reagent grade. Ultrapure distilled water with a resistance greater than 18 MΩ was used.

One formula of commercial concentrated herbal preparation, Farng-Feng-Tong-Sheng-Saan, was used as model preparation. The contents of the crude drug in a daily dose were Glycyrrhizae Radix, Talcum (2.5 g each), Scutellariae Radix, Platycodi Radix, Gypsum Fibrosum (2.0 g each), Ephedrae Herba, Paeoniae Radix, Ledebouriellae Radix, Forsythiae Fructus, Menthae Herba, Ligustici Rhizoma, Angelicae Radix, Rhei Rhizoma Mirabilitum Depuratum (1.0 g each), Astractylodis Rhizoma, Zingiberis Rhizoma, Schizonepetae Herba (0.5 g each). This sample preparation was purchased from retail outlets in Taipei.

Samples A and B were capsules manufactured by a pharmaceutical plant in mainland China and collected in February 1998. Samples C and D were light brown powders and dispensed by a traditional Chinese physicians clinic and traditional Chinese medicinal store, respectively. Samples C and D were collected from the consumer center of Taipei City Health Department in June 1994 and October 1990, respectively.

2.2. Preparation of standard solution

To prepare a standard solution containing six synthetic drugs, an appropriate amount of internal standard solution was added to an accurately weighed amount of six chemical drug standards dissolved in water to give various concentrations within the range 4–128 μg/ml for these six synthetic anorexics, respectively. Calibration graphs were plotted subsequent to linear regression analysis of the peak area ratios with concentrations.

2.3. Preparation of sample solution

Samples weighing 25 mg each for samples A and B; and 100 mg each for samples C and D, were accurately weighed and extracted with water (25 ml) for 30 min in an ultrasonic bath. Following these steps, each was filtered and diluted with water to 25 ml as sample solutions.

Preparation sample (Farng-Feng-Tong-Sheng-Saan) 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.

2.4. Sample clean-up by solid-phase extraction (SPE)

Sample solutions (10 ml) were loaded onto a SPE column (mixed absorbent C_8-SCX, Evidex, J&W Scientific, 400 mg, 6 ml column volume), which was conditioned with methanol (5 ml), water (5 ml) and NH_4Cl/NH_4OH buffer at pH 6.0 (10 ml). The SPE column was eluted with mixed solvent CH_2Cl_2–isopropanol–NH_4OH (78:20:2, v/v/v, 2×10 ml) and the vacuum set at 2.5 mmHg (1 mmHg= 133.322 Pa). The eluent was collected and then evaporated to dryness with a rotary vacuum evaporator. Finally, the residue was dissolved in 8 ml of 50% methanol and transferred to a 10 ml volumetric flask. An aliquot of 0.5 ml of FDA solution (0.5 mg/ml) was placed into this flask and made up to 10 ml with water. This solution was filtered through a 0.45 μm syringe filter before analysis.

2.5. Apparatus and condition

2.5.1. CE system

The analysis was carried out on a Beckman P/ACE 2200 CE system equipped with a photodiode array detector. The detector was set at 200 nm and a 57 cm×75 μm I.D. uncoated capillary (Beckman)

with the detection window placed at 50 cm. The conditions were as following: sampling time, 2 s, hydrostatic; run time, 15 min; applied voltage, 16 kV (constant voltage, positive to negative polarity); and temperature, 30°C. The electrolyte buffer was a solution containing 120 m*M* sodium phosphate buffer (NaH_2PO_4/H_3PO_4, pH 2.0) and 15% acetonitrile. The electrolyte was filtered through a 0.45 μm syringe filter (Gelman) before use. Between every two samples throughout the experiment, the column was cleaned with 1% sodium hydroxide, 2 min and water, 3 min, successively. The capillary was rinsed with buffer for 3 min before each experiment. The Gold software (Beckman) for system control and data processing was used.

2.5.2. GC–MS system

The samples were confirmed with a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a HP 6890 series injector and equipped with a HP 5973 mass-selective detector. The analytes were separated with a HP-5MS capillary column (fused-silica coated with 5% phenylmethylsilicone phase, 0.25 μm film thickness, 30 m×0.25 mm I.D.). Helium was used as the carrier gas and the column head pressure was set at 10.5 p.s.i. (1 p.s.i.=6894.76 Pa). A 1-μl volume of the sample was injected using the splitless mode. Temperature conditions were as follows: the initial temperature was 65°C for 1 min, then increased at 10°C/min to 280°C, and held for 5 min. Full-scan mass spectra were collected between 40 and 550 amu at 2.89 scan/s. The MS was operated in the electron impact (EI) ionization mode with an electron energy of 70 eV. The ion source and quadrupole temperatures were maintained at 230 and 150°C, respectively.

2.6. Precision

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

2.7. Recovery

Three different concentrations of the six synthetic anorexics were spiked to water (10 ml, used as blank) and the model preparation sample solutions (10 ml), respectively. These mixtures were extracted and analyzed by the procedures mentioned above.

3. Results and discussion

3.1. Analytical conditions

The detection wavelength was chosen at 200 nm because these six synthetic anorexics have high absorption at this wavelength. In this low wavelength detection, phosphate salt was chosen for the buffer solution due to its lower absorbance. Diammonium salt, FDA, was used as an internal standard which migrated out before these six synthetic anorexics.

In our previous study [10], a micellar electrokinetic chromatography method was successfully used to determine CBZ and diazepam in TCMs. The same method used for this analysis however failed because of PHE and MET overlap.

Experiments were initially conducted at pH 1.5, 2.0, 2.5 and 3.0 (120 m*M* NaH_2PO_4) without acetonitrile in the electrophoretic medium. In all instances, MET, DEP, FEN and CBZ and internal standard were successfully separated, but PHE and PPA overlapped, which indicated the mass to charge ratios of PHE and PPA in the run buffer are similar. However, in the presence of acetonitrile, these components in the mixture can be separated. Therefore, a buffer system was chosen with suitable amounts of acetonitrile.

In order to study the influence between of pH, NaH_2PO_4 concentration, acetonitrile concentration, temperature and voltage, serial experiments were carried out. The migration times at pH 2.5 and 3.0 were short, but the separation on PHE and PPA was poor. The six synthetic anorexics can be completely separated at 90, 120, 150 and 180 m*M* of NaH_2PO_4 concentration and 120 m*M* NaH_2PO_4 has a shorter run time than others. Then 120 m*M* NaH_2PO_4 buffer was used with different acetonitrile concentrations (0, 10, 15, 20 and 25%) to study the effect of organic solvent on the separation. The 15% acetonitrile solution gave the best resolution and showed shorter migration time. The studies also indicated that these six synthetic anorexics under the condition of 16 kV

voltage and 30°C temperature yielded the best resolution.

The optimal condition comprises an electrolyte containing 120 m*M* NaH_2PO_4 at pH 2.0 and with the cartridge temperature and voltage setting at 30°C and 16 kV. Fig. 2A presents an electropherogram showing the separation of these anorexics with the internal standard (FDA). The complete separation was done within 13 min.

3.2. Calibration graphs for synthetic anorexics and detection limits of synthetic anorexics

Calibration graphs: peak-area ratio, *y*, vs. con-

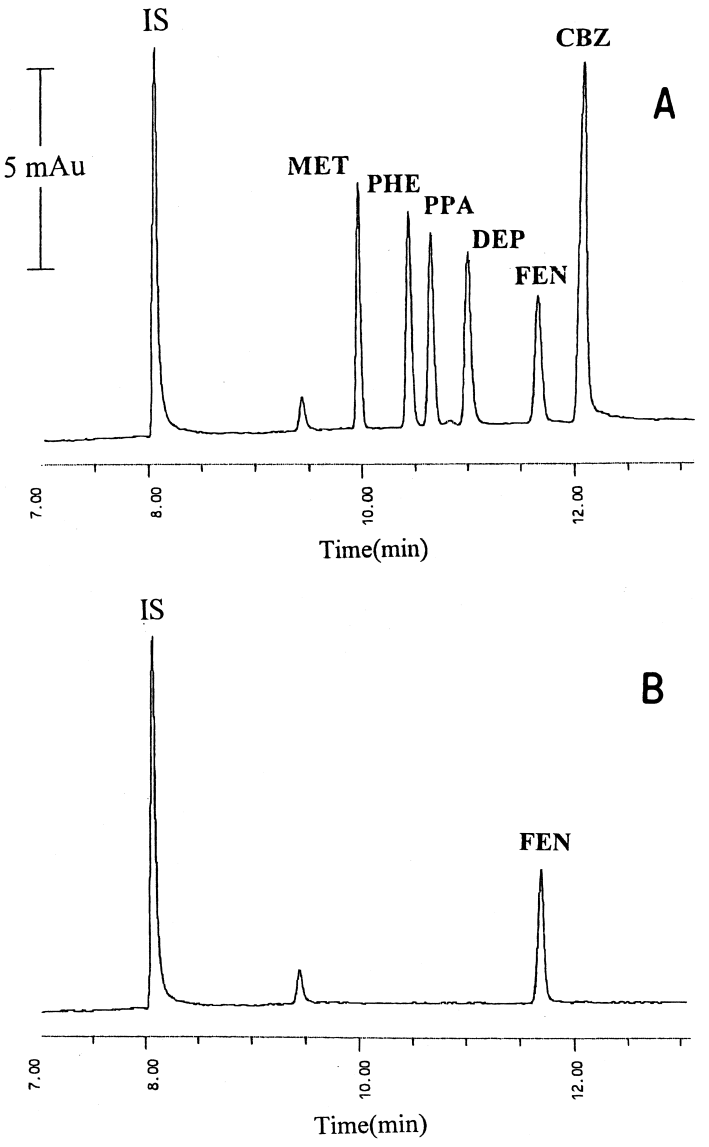

Fig. 2. (A) Capillary electropherogram of a mixture of the six synthetic anorexics. (B) electropherogram of real sample A after SPE treatment. I.S.=fluoren-2,7-diammonium chloride.

centration, x, μg/ml were obtained over the range of 4.0–128.5, 4.0–129.0, 4.0–128.0, 4.1–130.6, 4.0–127.5 and 4.0–128.5 μg/ml for CBZ, DEP, FEN, MET, PPA and PHE, respectively. The regression equations of the six curves and their correlation coefficients were calculated as: CBZ, $y=14.46x+2.00$ ($r=0.9998$); DEP, $y=30.56x+0.71$ ($r=0.9999$); FEN, $y=40.75x+0.33$ ($r=0.9999$); MET, $y=33.02x+1.92E-03$ ($r=0.9999$); PPA, $y=31.78x+0.93$ ($r=0.9999$) and PHE, $y=30.96x+0.44$ ($r=0.9999$), respectively. A signal three times higher than the peak noise height was regarded as the detection limit. The detection limits of the six synthetic anorexics are: 0.2 μg/ml for CBZ and 0.4 μg/ml for DEP, FEN, MET, PPA and PHE, respectively.

3.3. Method suitability tests

The precision of the electrophoretic assay method was evaluated by measuring the reproducibility [relative standard deviation (RSD)] and the accuracy was determined by recovery tests. The precision RSDs of the proposed method of the six synthetic anorexics, on the basis of peak-area ratios for five replicate injections were 0.49–2.71% for intra-day and 1.05–2.90% for inter-day, respectively. All of these data indicated that precisions are acceptable.

One kind of formula of a commercial concentrated herbal preparation, Farng-Feng-Tong-Sheng-Saan, is traditionally prescribed as an anorexic [16]. This preparation was used as a model sample for assessing interference and recovery. The recovery studies of SPE of six synthetic anorexics were conducted by model preparation samples with spiked known concentrations of the synthetic drugs. The recoveries ranged from 81.9 to 90.1%. The recovery of MET, 84.3% was near the report of Lee [11]. The RSDs ($n=3$) of recoveries of six synthetic anorexics in spiked water and TCMs treated with SPE were lower than 2 and 4%, respectively. These assessments indicate good accuracy for this method.

Comparison of HPCE electropherograms of the model preparation before and after clean-up with SPE showed that it is interference-free after SPE treatment. The results of recoveries were as accurate as those obtained with pure synthetic anorexics without interference from other peaks.

Table 1
The retention times (by GC–MS) and the fragment ions of six synthetic anorexics

Synthetic anorexics	Retention time (min)	Fragment ions (m/z)
CBZ	17.9	168, 125, 91, 170, 127, 77
DEP	12.7	100, 72, 79, 56
FEN	9.1	72, 159, 109, 216
MET	8.3	58, 91, 65, 134, 77
PHE	8.0	58, 91, 134, 65
PPA	10.4	77, 105, 51, 132, 91, 117

3.4. Application to adulterated traditional Chinese medicines

Four suspected TCM samples of synthetic therapeutic substances determined by HPCE were confirmed by GC–MS. The EI mass spectra of six synthetic anorexics were measured. The retention times and the fragment ions by GC–MS are shown in Table 1. Fig. 2B showed an electropherogram of real sample A. The electropherogram did not exhibit interference after SPE treatment. The contents of FEN in samples A and B were 2.9 and 4.1 mg/capsule, respectively, CDZ in sample C was 16.5 mg/pack and DEP in sample D was 7.1 mg/pack.

In conclusion, we have demonstrated the importance and accuracy in using the combination of SPE and HPCE methods to analyze the contents of CBZ, DEP, FEN, MET, PPA and PHE in TCM. Qualitative results were also confirmed by GC–MS analysis. The precisions were lower than 3.0% using mixed adsorbent (C_8-SCX) and mixed elution solvent for SPE extraction. The recoveries were greater than 89, 88, 88, 84, 84 and 84% for CBZ, DEP, FEN, MET, PPA and PHE, respectively.

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