Solid-phase extraction for the determination of caffeine in traditional Chinese medicinal prescriptions containing Theae folium by high performance liquid chromatography

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Received 8 September 1998; accepted 5 January 1999

Abstract

A high performance liquid chromatographic method in combination with C-18 reverse phase solid-phase extraction (SPE) was developed for determination of caffeine (CA) in traditional Chinese medicinal prescriptions which contain Theae folium. The frequently used prescriptions include Shin-Yi-San, Chuan-Chyong-Char-Tyau-San, Tsang-Eel-San, San-Hwang-Shyr-Gau-Tang, Tzy-Shenn-Ming-Mu-Tang and Shiang-Chyong-San. The present HPLC system uses a Merck RP-select B column by isocratic elution with methanol and 1% (v/v) acetic acid (1:4) as the mobile phase and detected at UV 270 nm. 8-Chlorotheophylline was used as an internal standard. The extracts of prescriptions were treated by Supelclean LC-18 SPE tube for eliminating interferences. Blank decoctions of each prescription were also examined as a test for interferences. The recoveries of caffeine from the Chinese medicinal prescriptions ranged from 88.5 to 92.3%. The relative standard deviations of caffeine ranged between 0.86 and 1.97% (intraday) and 1.04 and 3.90% (interday). The contents of caffeine in standard decoctions ranged from 13.98 to 19.62 mg g^{-1}.

Keywords: Pharmaceutical analysis; Caffeine; Solid-phase extraction

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1. Introduction

Traditional Chinese medicinal herb prescriptions have been used for over 1500 years, and in recent years concentrated dosage forms have been

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原載於Journal of Pharmaceutical and Biomedical Analysis. 1999.20:351-356

widely adopted for clinical use. Most of them are composed of complex chemical constituents. Proper methods are required for quality control of traditional Chinese medicinal prescriptions, using effective chromatographic methods. In our laboratory, we have developed several HPLC methods for the determination of marker constituents in Chinese medicinal prescriptions [1–8].

The leaves of the tea plant known as Theae folium by its pharmacognosy name are a commonly used Chinese herb. Caffeine (CA) is one of the major constituents in tea. The highest content of CA occurs in tea, but even there it seldom exceeds 5%. Theae folium and/or CA have central nervous effects and diuretic stifmulation, and also causes cardiac stimulation and coronary dilation. It is most commonly employed simply to overcome drowsiness, but in addition, it is used therapeutically as an adjunct in the treatment of headache in traditional Chinese medicinal prescriptions [9,10].

Although many HPLC methods have been developed for the determination of caffeine in tea or preparations [11,12], a good pretreatment procedure, however, still needs to be developed. Solid phase extraction (SPE) is a convenient and time-saving alternative to liquid/liquid partition, the method used to pretreat analytical samples. A number of SPE methods have been developed to measure CA in biological samples [13,14]. However decoction samples of traditional Chinese medicinal prescriptions are decocted with boiling water and are composed of many highly polar complex constituents. Without pretreatment, the HPLC method for determination of CA in prescriptions can only be accomplished under the specific mobile phase condition. But with the above condition [11], the CA contents of San-Hwang-Shyr-Gau-Tang and Tzy-Shenn-Ming-Mu-Tang can not be determined without interferences. Therefore, a general and suitable method for determination of CA in traditional Chinese medicinal prescriptions is required. This prompted us to develop the present method. In this study, we selected six kinds of often used Chinese medicinal prescriptions which contain Theae folium for examination.

2. Experimental

2.1. Materials

The materials used to prepare the traditional Chinese medicine prescriptions were as follows [15]:

Chuan-Chyong-Char-Tyau-San (P1): Theae folium (1.5 g), Cyperi rhizoma (4.0 g), Ligustici rhizoma (3.0 g), Schizonepetae herba, Angelicae dahuricae radix, Menthae folium, Sileris radix,

Table 1
Intraday and interday analytical precisions of three concentrations of CA

Intraday R.S.D.* (%)			Interday R.S.D.* (%)		
4 μg ml^{-1}	40 μg ml^{-1}	200 μg ml^{-1}	4 μg ml^{-1}	40 μg ml^{-1}	200 μg ml^{-1}
1.97	1.41	0.86	3.90	2.29	1.04

* $n = 5$.

Table 2
Recoveries of CA after SPE

Added (μg ml^{-1})	Measured (mean, $n = 3$) μg ml^{-1}	Recovery (mean, $n = 3$) (%)	Mean ± S.D. (%)	R.S.D. (%)
20.0	17.7	88.7	–	–
40.0	36.0	90.1	90.1	1.27
80.0	73.2	91.5	–	–

Table 3
Recoveries of CA in six traditional Chinese medicinal prescriptions after SPE

Prescription	Added (μg ml^{-1})	Measured (mean, $n = 3$) μg ml^{-1}	Recovery (mean, $n = 3$) (%)	Mean ± S.D.(%)	R.S.D.(%)
P1	20.0	18.5	92.3		
	40.0	35.0	87.4	89.5	2.29
	80.0	71.1	88.9		
P2	20.0	17.7	88.7		
	40.0	35.8	89.6	89.1	0.42
	80.0	71.2	89.0		
P3	20.0	18.4	91.8		
	40.0	35.4	88.5	90.5	1.60
	80.0	73.0	91.3		
P4	20.0	18.1	90.5		
	40.0	36.8	92.0	91.3	0.68
	80.0	73.1	91.4		
P5	20.0	17.7	88.6		
	40.0	35.6	89.1	89.1	0.46
	80.0	71.7	89.6		
P6	20.0	18.1	90.7		
	40.0	36.8	92.1	91.7	0.74
	80.0	73.8	92.2		

Notopterygii rhizoma (2.0 g each), Glycyrrhizae radix (1.5g).

San-Hwang-Shyr-Gau-Tang (P2): Theae folium (1.0 g), Gypsum Fibrosum (10.0 g), Scutellariae Radix, Ephedrae Herba (3.0 g each), Gardeniae Fructus, Sojae Semen Praeparatum (2.0 g each), Coptidis Rhizoma, Phellodendri Cortex (1.5 g each), Zizyphi Fructus, Zingiberis Recens Rhizoma (1.0 g each).

Tzy-Shenn-Ming-Mu-Tang (P3): Theae Folium (1.5 g), Rehmanniae Radix (6.0 g), Ligustici Rhizoma, Sileris Radix, Paeoniae Radix, Angelicae Radix (3.0 g each), Angelicae Dahuricae Radix, Coptidis Rhizoma, Gardeniae Fructus, Platycodi Radix, Chrysanthemi Flos, Junci Caulis Medulla, Viticis Fructus, Ginseng Radix, Glycyrrhizae Radix (1.5 g each).

Shin-Yi-San (P4): Theae Folium (3.0 g), Ligustici Rhizoma, Angelicae Dahuricae Radix, Glycyrrhizae Radix, Akebiae Caulis, Ligustici Sinensis Rhizoma, Asiasari Radix, Cimicifugae Rhizoma, Magnoliae Rhizoma (3.0g each).

Tsang-Eel-San (P5): Theae Folium (0.5 g), Angelicae Dahuricae Radix (10.0 g), Magnoliae Rhizoma (5.0 g), Xanthii Fructus (2.5 g), Menthae Folium, Allii chinensis Bulbus (0.5g each).

Shiang-Chyong-San (P6): Theae Folium (1.0 g), Ligustici Rhizoma, Menthae Folium, Cyperi Rhizoma, Glycyrrhizae Radix, Angelicae Dahuricae Radix, Gypsum Fibrosum (1.0 g each), Aconiti Tuber (0.5 g).

All materials were obtained from retail outlets in Taipei and cut into pieces. Two commercial brands of concentrated preparations (P2, P4 and P5), three brands of P3 and one brand of P1 were also purchased from retail outlets.

2.2. Chemicals and reagents

CA and 8-chlorotheophylline (CT) were purchased from Sigma (St. Louis, MO, USA). Methanol from Labscan (Dublin, Ireland) was LC grade. Acetic acid and dichloromethane were analytical reagent grade. Ultrapure distilled water with a resistance greater than 18 MΩ was used.

2.3. Sample clean-up by solid-phase extraction

Sample solution (5 ml) was loaded onto a SPE column (Supelclean C-18, 500 mg, 3 ml column volume), which had been conditioned with methanol (5 ml) and water (5 ml). The SPE column was washed with water (2 × 5 ml), and then dichloromethane (10 ml) was used to elute the drugs. The eluate was collected and concentrated under reduced pressure to dryness. Finally, the residue was dissolved in 2 ml of 50% methanol and transferred to a 5 ml volumetric flask. An aliquot of 1 ml of 8-chlorotheophylline solution (0.404 mg ml^{-1}) was placed into this flask and adjusted to 5 ml with 50% methanol. This solution was filtered through a 0.45 μm syringe filter before use.

2.4. Apparatus and condition

HPLC was conducted with a HITACHI L-6200 intelligent pump with HITACHI L-3000 photodiode array detector and Shimadzu SIL-9A auto injector. Merck RP-select B 250 × 4.6 mm I.D. reversed phase column was used. The mobile phase was carried out using isocratic elution with methanol and 1% (v/v) acetic acid (1:4, v/v). The flow rate was 1.0 ml min^{-1} and detection wavelength was 270 nm. Peak areas were calculated with a Shiunn Haw computing integrator (Taipei, Taiwan).

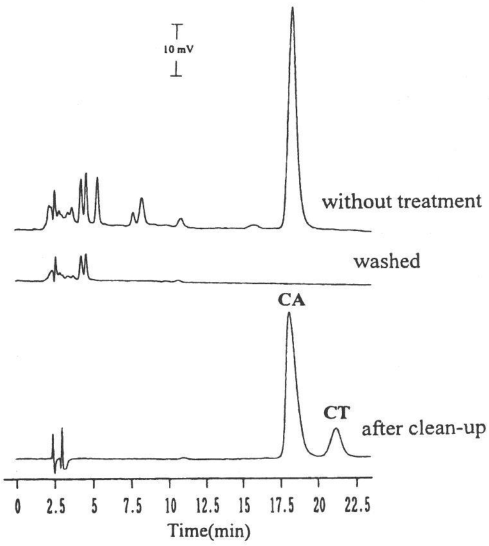

Fig. 1. HPLC chromatograms of solutions on the process of clean-up with Supelclean C-18 for Theae folium extract. CA, caffeine; CT, 8-chlorotheophylline; HPLC conditions, column: Merck RP-select B 250 × 4.6 mm I.D.; mobile phase: methanol and 1% (v/v) acetic acid (1:4, v/v); flow rate: 1.0 ml min^{-1}; detection wavelength: 270 nm.

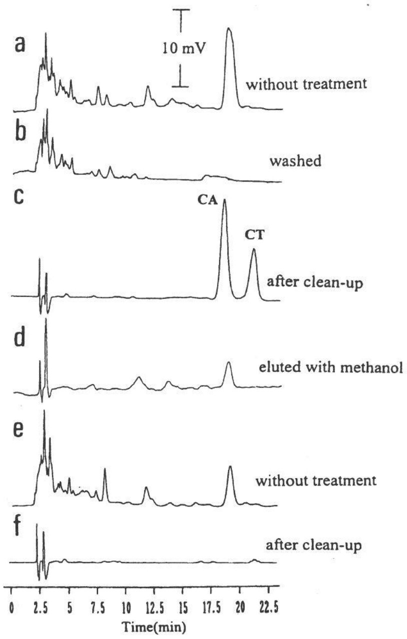

Fig. 2. Comparison of HPLC chromatograms of standard decoction of P2 (a–c) and blank decoction (prescription without Theae folium); (d) before and after clean-up with Supelclean C-18.

Table 4
The contents of CA in Theae folium and the standard decoction of six prescriptions

Sample	Content (mg g^{-1}, mean ± S.D., $n = 3$)
Theae folium	18.96 ± 0.41
P1	15.55 ± 0.31
P2	18.42 ± 0.25
P3	13.98 ± 0.47
P4	17.90 ± 0.47
P5	14.75 ± 0.35
P6	19.62 ± 0.13

2.5. Preparation of standard solution

To prepare a standard solution containing caffeine, an appropriate amount of internal standard solution was added to an accurately weighed amount of CA standard dissolved in water to give various concentrations within the range of 4–200 μg ml^{-1}. Calibration curves were plotted based on linear regression analysis of the peak-area ratios versus concentrations.

2.6. Preparation of sample solution

2.6.1. Standard decoction

Amounts of individual crude drugs equivalent to a daily dose of six kinds of Chinese medicinal prescriptions were weighed and pulverized, a 20-fold mass of water was added, and the mixture of each prescription was boiled, respectively, for more than 30 min to halve the original volume. After filtration while hot, the filtrate was diluted to the ten-fold volume with water. This solution was used as a sample solution of standard decoction.

2.6.2. Blank decoction

Amounts of individual crude drugs equivalent to a daily dose of six kinds of Chinese medicinal prescriptions without Theae folium were treated according to the method described above for the preparation of standard decoction.

2.6.3. Commercial tea and concentrated preparations

One gram of the concentrated preparation was weighed accurately and extracted with 20 ml of water at 40°C for 30 min in an ultrasonic bath. After filtration, the filtrate was diluted to 25 ml with water.

2.7. Precision

The intraday and interday variability at three assay concentrations of CA, 4, 40 and 200 μg ml^{-1}, were evaluated for five replicates over 5 successive days.

2.8. Recovery

Three different concentrations, 20.0, 40.0 and 80.0 μg ml^{-1}, of CA were added to water (5 ml) and the six blank decoction solutions (5 ml). These mixtures were extracted and analyzed using the procedure mentioned above.

3. Results and discussion

Traditional Chinese medicinal prescription is usually prepared by decoction, with the high polarity constituents being a major part of the decoction. The chemical constituents in the decoction are very complex, and thus analysis is not easy. In this study, more than six crude drugs were combined in each prescription. The kinds of composed crude drugs and weight ratios of Theae folium were 9 and 7.5%, 10 and 3.8%, 15 and 4.5%, 9 and 11.1%, 6 and 2.7%, and 8 and 13.3% in these six prescriptions (P1–P6), respectively. All possess very complex chemical compositions and without passing through SPE extraction, analyses of these co-existing constituents can be very difficult.

Photodiode array detection was used in this experiment, so that UV spectra of CA could be compared with the reference standard.

Calibration graph: peak-area ratio, y, versus concentration, x, μg ml^{-1} was obtained over the range of 4–200 μg ml^{-1} for CA. The regression equation and its correlation coefficient was calculated as $y = 37.55x + 0.60$ and 0.9999, respectively. A signal three times higher than the peak noise height was regarded as the detection limit. The detection limit of CA in this method was 0.25 μg ml^{-1}.

The precision of the present chromatographic method was evaluated by measuring the reproducibility [relative standard deviation (R.S.D.)] and the accuracy was determined by recovery tests. The precision R.S.D.s of the proposed method of caffeine, on the basis of peak-area ratios for five replicate injections were 0.86–1.97% for intra- and 1.04–3.90% for inter-day, respectively (Table 1).

The recovery studies of SPE of CA standard solutions ranged from 88.7 to 91.5% (Table 2). The recovery studies of SPE of CA were conducted by blank decoction samples with known concentrations of CA. Results are given in Table 3. The R.S.D.s ranged from 0.42 to 2.29%.

SPE was used for the preparation of the six Theae folium in traditional Chinese medicinal prescriptions. Fig. 1 shows chromatograms of Theae folium extract solutions on the process of clean-up with SPE. The retention times for CA and internal standard, CT, were 18.2 and 21.4 min, respectively. The results were as accurate as those obtained with pure chemical drugs without interferences from other peaks. Comparison of HPLC chromatograms of standard and blank decoctions of P2 before and after clean-up with SPE are shown in Fig. 2. No peak appeared on the retention time of CA, 18.2 min. It showed that CA cannot be eluted by the washing procedure. But CA was still not free of interference from co-existing constituents unless cleaned up by SPE. The chromatogram of a (standard decoction without SPE pretreatment) showed a broad peak on CA position. In blank decoction, another component was also appeared at the same position of CA (chromatogram e). This component was disappeared after clean-up with SPE and trapped in SPE tube (chromatogram f). The SPE trapped component could be washed out by methanol after clean-up procedure (chromatogram d). This method also can be applicable for the other prescriptions in this study.

The contents of CA in commercial Theae folium and standard decoctions are shown in Table 4. The contents of CA in commercial preparations also can be analyzed with the same method. The turnover ratio of CA was defined as the percentage yields of this constituent detected in the Chinese medicinal preparation, calculated on the basis of its contents in the individual crude drug. The turnover ratios of CA in P1–P6 were 82.0, 97.2, 73.7, 94.4, 77.8 and 103.5%, respectively. The reason for the various turnover ratios caused by molecular interactions or insufficient extraction remains to be clarified.

In conclusion, we have demonstrated the importance and accuracy in using the combination of SPE and HPLC methods to analyze the content of CA in traditional Chinese medicinal herbal prescriptions.

References

[1] K.-C. Wen, C.-Y. Huang, F.-S. Liu, J. Chromatogr. 593 (1992) 191–199.

[2] K.-C. Wen, C.-Y. Huang, F.-L. Lu, J. Chromatogr. 631 (1993) 241–250.

[3] Y.-C. Lee, C.-Y. Huang, K.-C. Wen, T.-T. Suen, J. Chromatogr. A 660 (1994) 299–306.

[4] Y.-C. Lee, C.-Y. Huang, K.-C. Wen, T.-T. Suen, J. Chromatogr. A 692 (1995) 137–145.

[5] S.-J. Lin, H.-H. Tseng, M.K.-C. Wen, T.-T. Suen, J. Chromatogr. A 730 (1996) 17–23.

[6] K.-C. Wen, C.-Y. Huang, F.-S. Liu, K.-S. Huang, F.-L. Lu, L. Chin, W.-H. Tsai, S.-J. Lin, Determination of Marker Constituents in Traditional Chinese Medicinal Prescriptions, 1996, pp. 1–454 National Laboratories of Foods and Drugs, Taipei.

[7] Y.-R. Ku, Y.-T. Lin, K.-C. Wen, J.-H. Lin, C.-H. Liao, J. Liq. Chrom. Rel. Technol. 19 (1996) 3265–3277.

[8] J.-H. Lin, Y.-R. Ku, Y.-S. Huang, K.-C. Wen, C.-H. Liao, J. Liq. Chrom. Rel. Technol. 20 (1997) 1617–1632.

[9] V.E. Tyler, Herbs of Choice—The Therapeutic Use of Phytomedicinals, Pharmaceutical Products Press, New York, 1994, p. 87.

[10] J.D. Keys, Chinese Herbs—Their Botany, Chemistry, and Pharmacodynamics, Charles E. Tuttle, Tokyo, 1976, p. 189.

[11] M. Dulitzky, E.D. L. Teja, H.F. Lewis, J. Chromatogr. 317 (1984) 403–405.

[12] A. Finger, S. Kuhr, U.H. Engelhardt, J. Chromatogr. 624 (1992) 293–315.

[13] Z.-Y. Zhang, M.J. Fasco, L.S. Kaminsky, J. Chromatogr. B 665 (1995) 201–208.

[14] I.N. Papadoyannis, V.F. Samanidou, K.A. Georga, J. Liq. Chrom. Rel. Technol. 19 (1996) 2559–2578.

[15] H.-Y. Hsu, C.-S. Hsu, Commonly Used Chinese Herb Formulas with Illustrations, 1980, pp. 26–490 Oriental Health Arts Institute, Taipei.