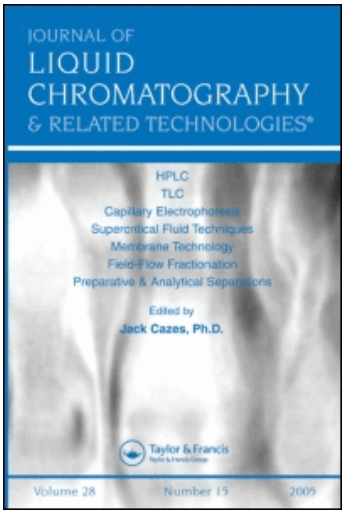

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Fingerprint of Tablet of *Corydalis tuber* for Alleviating Pain by HPLC

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Abstract: An effective analysis method for tablet of *Corydalis tuber* for alleviating pain was developed by high performance liquid chromatography in the presence of solvent mixtures of acetic acid, triethylamine, methanol, and water in gradient elution. It was applied to 12 tablet samples that were collected stochastically from pharmacies and hospitals. An image chromatographic fingerprint was developed and four marker compounds were identified in the fingerprint. A digitized chromatographic fingerprint spectrum was developed based on the seventeen common peaks that were obtained in these samples. Several quantitative parameters including relative retention time, relative standard deviation of relative retention time, relative peak area, relative standard deviation of relative peak area, difference rate, and total difference rate were applied in the digitized fingerprint of tablet of *Corydalis tuber* for alleviating pain to supply an applicable and quantitative quality control method for this kind of traditional Chinese medicine preparation.

Keywords: Image chromatographic fingerprint (ICF), Digitized chromatographic fingerprint spectrum (DCFS), Tablet of *Corydalis tuber* for alleviating pain, Quality control, Pharmaceutical analysis

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INTRODUCTION

Tablet of *Corydalis tuber* for alleviating pain (Yuanhu Zhitong Pian, YZP), a well known Chinese traditional medicine (TCM), is officially listed in the Chinese Pharmacopoeia.[1] As an important over the counter (OTC) gynaecological medicine, YZP was also listed in the first batch Product Catalog of National Non-Prescription Drugs issued by the State Food and Drug Administration (SFDA) of China in 1999. In the TCM practice, YZP has been used for the treatment of gastralgia, hypochondriac pain, migraine, headache and dysmenorrhea.[2,3] The principal bioactive components of YZP are dl-tetrahydropalmatine (dl-THP), berberine, imperatorin, and isoimperatorin, whose structures were shown in Figure 1. Dl-THP has analgesic, sedative, tranquilizing, hypnotic, antihypertensive, and anxiolytic activities.[4,5] Berberine is normally used as an anti-inflammatory.[6] Imperatorin and isoimperatorin have the effects of lipolysis, inhibition of insulin induced lipogenesis.[7]

Active components of TCM are influenced by its growing conditions, the seasons when plants are harvested, process methods, and storage duration, which make quality control essentially necessary for their application. Since there are dozens or hundreds of components in a herb, it is not reasonable to do quality control just by quantifying one or several marker compounds. Fingerprint, normally referring to chemical fingerprint, becomes a popular and authoritative method for the quality control of TCM. It is accepted or recommended by authorities including State Food and Drug Administration (SFDA) of China, Food and Drug Administration (FDA) of United States, the European Agency for the Evaluation of Medicinal Products (EMEA), herbal pharmacopoeia of Britain and India, as well as World Health Organization (WHO).[8] Chromatography, including thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC), and high performance capillary electrophoresis (HPCE), are widely applied[9–11] and recommended for the quality control of TCM by Chinese Pharmacopoeia (2005 edition). High speed countercurrent chromatography (HSCCC) has been developed to be an analytical method for TCM by our research group.[12,13] Among the above techniques, HPLC is the most popular one to develop a fingerprint of TCM due to its high precision, sensitivity, and reproducibility.[14] The whole concentration distribution of components can be expressed effectively by HPLC. The evaluation of fingerprint recognition techniques such as image chromatographic fingerprint (ICF), digitized chromatographic fingerprint spectrum (DCFS), and chemical pattern recognition technique are extensively used. Since the chemical pattern recognition technique is dependent on software, ICF and DCFS were adopted in this study for the quality assessment of YZP because they are easy to be popularized.

YZP is made of two traditional herbs: *Corydalis yanhusuo* W. T. Wang (*C. yanhusuo*) and *Angelica dahurica*. The fingerprint of *Angelica dahurica* by HPLC has been reported,[15] however, the fingerprint of *C. yanhusuo* and

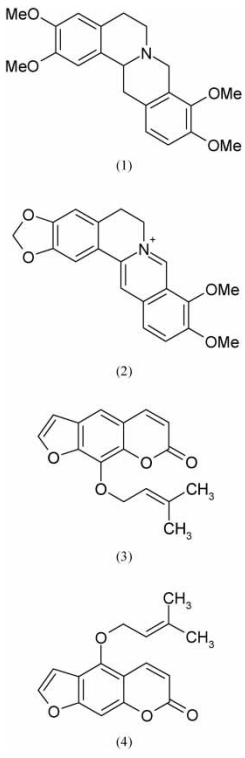

Figure 1. Chemical structures of four marker compounds of tablet of *Corydalis tuber* for alleviating pain (YZP): (1) dl-tetrahydropalmatine; (2) berberine; (3) imperatorin; (4) isoimperatorin.

its preparations have not been published yet. It was investigated on the Internet that there are over 140 Chinese pharmaceutical factories or companies producing YZP with the same prescription, however, no effective quality control method has been published. It is quite necessary to develop an efficient method to do quality control of YZP.

EXPERIMENTAL

Reagents and Materials

Chromatographic grade methanol (Caledon Laboratories, Canada), acetonitrile (Fisher Scientific, UK), acetic acid (Kemiou Chemical, Tianjin, China), and triethylamine (Tedia, USA) were used for preparation of samples and mobile phase. Pure water (18 MΩ, Millipore, Bedford, MA, USA) was used for all the solutions and dilutions.

Dl-THP (110726–200409), berberine hydrochloride (110713–200208), imperatorin (110826–200511), and isoimperatorin (110827–200407) were supplied by the State Food and Drug Administration of China (SFDA). Raw materials *C. yanhusuo* and *Angelica dahurica* were purchased from Tong Ren Tang pharmacy, Beijing, China. Twelve YZP samples were collected stochastically from pharmacies and hospitals in several cities of China (Table 1).

Apparatus

The HPLC system (10 Avp, Shimadzu, Japan) is composed of two pumps, UV detector, oven, system controller, and 20 μL sample loop. The column was Ultrasphere C18 column (250 mm × 4.6 mm i.d., 5 μm, Agilent, USA). An ultrasonic cleaner (Cole-Pharmer, USA) and a centrifuge (Sigma 3K15, Germany) were used in the course of extraction.

Sample Preparation

The sugar coatings of 12 YZP samples were removed completely. The center of each tablet was powdered finely. The accurately weighed 200 mg of each sample powder was dissolved by 1 mL methanol, the mixture extracted in ultrasonic cleaner for 30 min. After centrifugation at 10,000 rpm for 10 min, the supernatant was filtered with a 0.45 μm nylon membrane (Millipore, USA). An aliquot of 20 μL of filtrates was injected for HPLC analysis.

Table 1. A summary of the tablet of *Corydalis tuber* for alleviating pain (YZP) preparations

Sample no.	Production date	Batch no.	Pharmaceutical factory (China)
S1	2006-06-16	060601	Shuzhong, Sichuan
S2	2005-06-13	050610	Guanyuan Rongchong, Sichuan
S3	2006-03-03	060301	Hebang Yangguang, Sichuan
S4	2006-06-09	060601	Yikang, Guangdong
S5	2006-04-04	060401	Tiantianle, Guangxi
S6	2006-06-16	060602	Banzhou Tianlong, Guangxi
S7	2005-09-09	050901	Weiwei, Guangxi
S8	2006-07-20	060701	Xianhe, Shandong
S9	2006-07-15	20060703	Dayv, Shandong
S10	2006-02-09	20060232	Jishi, Henan
S11	2005-04-16	20050402	Fengyuan, Henan
S12	2006-01-03	060101	Longzhong, Hubei

Fine powders of two raw materials, *C. yanhusuo* (13.5 g) and *Angelica dahurica* (6.5 g) were accurately weighed, and 0.5 mL methanol was added, respectively. Each mixture was extracted by ultrasonication at room temperature for 30 min. After centrifugation at 10,000 rpm for 10 min, the supernatant was filtered with a 0.45 μm nylon membrane. An aliquot of 20 μL of filtrates was injected for HPLC analysis.

HPLC Analysis

The mobile phase was solvent A (methanol-water-HAc-TEA = 10:89.1:0.8:0.1) and solvent B (methanol-water-HAc-TEA = 89.1:10:0.8:0.1) in linear gradient mode as follows: 0–10 min: 1–16% B, 10–35 min: 16–35% B, 35–60 min: 35–100% B, 60–70 min: 100% B. The flow rate was 0.9 mL/min. The detection wavelength was set at 280 nm and column temperature was 40°C. The loading sample volume was 20 μL.

Reproducibility, Linearity, and Detection Limit of Four Marker Compounds

The methodology was assessed by three parameters, reproducibility, linearity, and detection limit. Method reproducibility was evaluated by making repetitive injections of a marker compound (0.1 mg/mL for each) six times. The relative standard deviation (RSD) of the relative retention times (RRT) and relative peak areas (RPA) were not exceeding 0.45% and 1%, respectively. To determine the linearity equations and linear scope for the marker compounds, a series of mixed standard solutions ranging from 0.001 to 1.0 mg/mL were tested. The regression equation of dl-THP, berberine, imperatorin, and isoimperatorin were $y = 23726822.16x + 45554.87$, $y = 70133110.34x + 321074.30$, $y = 34394856.11x + 43816.33$ and $y = 25300829.74x + 66852.78$, respectively. Four correlation coefficients (R^2) were all 0.9999. The detection limits of four marker compounds were below 0.001 mg/mL on the basis of a signal-to-noise ratio of 5. This analysis method was suitable for the development of the fingerprint of YZP.

RESULTS AND DISCUSSION

Optimization of HPLC Analysis

Besides the active ingredients in YZP, inactive additives such as starch, lactose, sucrose, dextrin, magnesium stearate, and carboxymethyl starch sodium were added for the preparation of the tablet. It was difficult to remove them completely from the sample solution by extraction, filtration,

or centrifugation. When acetonitrile was used in the mobile phase, these additives caused a strong adsorption peak in the range of retention time of 0–5 min, which was even several times higher than marker compounds; methanol was applied in the mobile phase instead. When a slow gradient mode was used (0–10 min: 1–16% B), the strong adsorption peak was separated effectively and its negative effect on the evaluation of fingerprint was decreased.

The dominant active compounds in YZP are tertiary alkaloids and quaternary ammonium alkaloids.[16] In the process of optimization of HPLC conditions, different compositions with particular functions were added into the mobile phase to get effective separation. It was difficult to separate alkaloids in YZP effectively just by a mobile phase composed of methanol and water even at slow linear gradient, because there were strong interaction between alkaloids and acidic silanol (SiOH) on the surface of the reversed phase silica matrix.[17] Triethylamine (TEA) was an effective competing ion reagent that caused stronger interaction with SiOH than alkaloids.[18] However even at low concentration in the mobile phase, for example 0.1% (v/v), TEA supplied an alkaline environment, which could cause deterioration of the silica gel packed in the reversed phase analytical column. Consequently, superfluous HAc was added to adjust the pH value of mobile phase to acidic condition. Alkaloids were all in salt form in this environment. Moreover, HAc acted as a buffer to control peak tailing to obtain good peak symmetry. HAc and TEA were applied in the mobile phase and HAc was excessive in mole ratio to keep the mobile phase in acidic condition, in which TEA was protonated and triethylammonium acetate (TEAA) was formed. TEAA, as the ion pair reagent, was positively charged and competed for the ionized silanol group with alkaloids. It was the combination of TEA and HAc that played an important role of improving separation of the alkaloids in YZP.

Concentrations of HAc and TEA in mobile phase consisting methanol and water had an evident effect on the resolutions of alkaloids in HPLC analysis. At a settled TEA concentration, resolutions were improved with increasing HAc concentrations from 0.2% (v/v) (34.97 mM) to 0.8% (v/v) (139.88 mM). At a settled HAc concentration, resolutions of alkaloids decreased gradually with increasing concentrations of TEA from 0.1% (v/v) (7.19 mM) to 0.4% (v/v) (28.76 mM). It was indicated by the experiments that 0.8% (v/v) HAc and 0.1% (v/v) TEA was appropriate for YZP analysis. The optimized HPLC elution condition was solvent system A (methanol-water-HAc-TEA = 10:89.1:0.8:0.1) and solvent system B (methanol-water-HAc-TEA = 89.1:10:0.8:0.1) in gradient mode (Figure 2A).

Image Chromatographic Fingerprint of YZP

The above optimized HPLC condition was applied to 12 batches of YZP (Figure 3). There were more than 40 peaks in each chromatogram. The

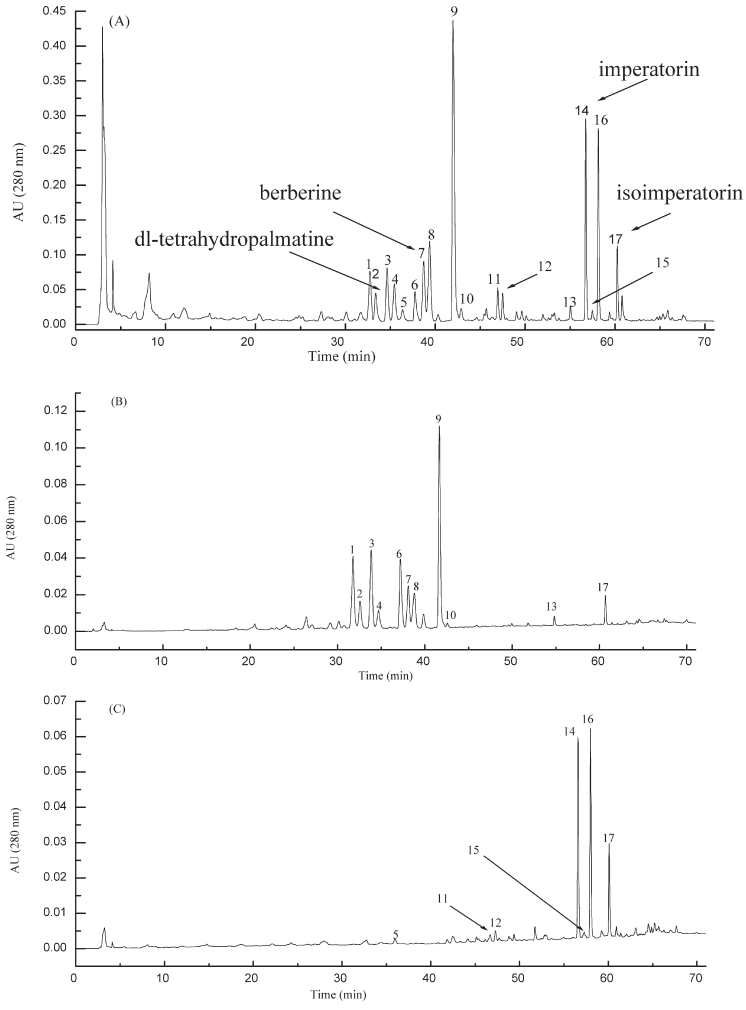

Figure 2. Representative HPLC fingerprint of tablet of *Corydalis tuber* for alleviating pain (YZP) containing 17 common peaks (A), raw material of *Corydalis yanhusuo.* W. T. Wang (B) and raw material of *Angelica dahurica* (C). The HPLC condition was solvent A (methanol-waters-HAc-TEA = 10:89.1:0.8:0.1) and solvent B (methanol-HAc-TEA = 89.1:10:0.8:0.1) in gradient mode as follows: 0–10 min: 1–16% B, 10–35 min: 16–35% B, 35–60 min: 35–100% B, 60–70 min: 100% B. The flow rate was 0.9 mL/min. The column used was Ultrasphere C18 column (250 mm × 4.6 mm i.d., 5 μm, Agilent, USA). The flow-rate was 0.9 mL/min and temperature was 40°C. The effluent was monitored at 280 nm and samples loaded through 20 μL sample loop.

whole concentration distribution of components was obviously shown by ICF. Several peak fractions in the retention time range of 0–5 min were inactive additives that were added for the preparation of tablets. Since they were not

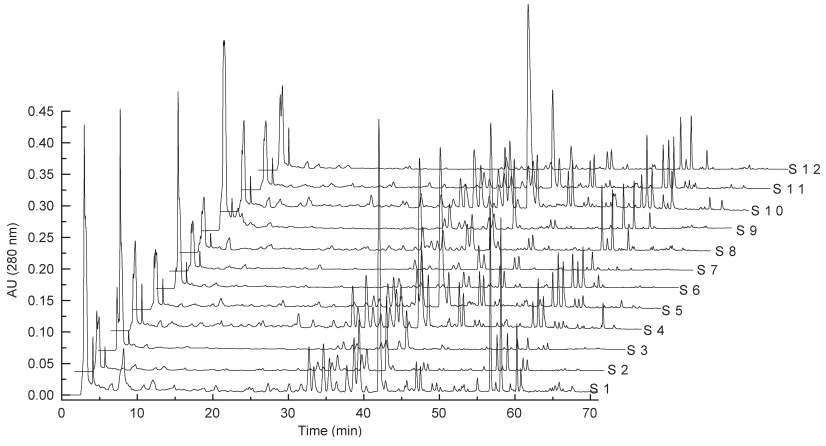

Figure 3. HPLC fingerprint of 12 tablet of *Corydalis tuber* for alleviating pain (YZP) samples. The HPLC condition was solvent A (methanol-waters-HAc-TEA = 10:89.1:0.8:0.1) and solvent B (methanol-HAc-TEA = 89.1:10:0.8:0.1) in gradient mode as follows: 0–10 min: 1–16% B, 10–35 min: 16–35% B, 35–60 min: 35–100% B, 60–70 min: 100% B. The flow rate was 0.9 mL/min. The column used was Ultrasphere C18 column (250 mm × 4.6 mm i.d., 5 μm, Agilent, USA). The flow-rate was 0.9 mL/min and temperature was 40°C. The effluent was monitored at 280 nm and samples loaded through 20 μL sample loop.

components of two raw materials, it was unnecessary to include them in the major part of fingerprint. Seventeen peaks in the retention time range of 30–70 min were common in these chromatograms of 12 samples, which were selected as common peaks.

Two raw materials *C. yanhusuo* and *Angelica dahurica* were analyzed, respectively (Figure 2B, Figure 2C). Seventeen common peaks in ICF of YZP could be found in these two herbs. Peak no. 1, no. 2, no. 3, no. 4, no. 6, no. 7, no. 8, no. 9, no. 10, and no. 13 came from *C. yanhusuo*, which was responsible for promoting blood circulation by removing blood stasis and acting as an analgesic. Peak no. 5, no. 11, no. 12, no. 14, no. 15, no. 16, no. 17 were from *Angelica dahurica*, which had the effects of invigorating the circulation of blood stasis and holding back skin ulcers.[19]

Sample no. 1 (Figure 2A) was selected as a reference sample because of its moderate fraction contents and highest similarity with *C. yanhusuo* and *Angelica dahurica* (Figure 2B, Figure 2C). Seventeen common peaks were marked on sample no. 1. Four marker components of YZP, dl-THP, berberine, imperatorin, and isoimperatorin were identified to be peak fractions no. 2, no. 7, no. 14, and no. 17, respectively (Figure 2A). The contents of them in the reference samples were 0.032 mg/mL, 0.019 mg/mL, 0.079 mg/mL, and 0.037 mg/mL, respectively. The contents of four marker compounds in other samples were also calculated according to the regression equations in the section above (Table 2). It was obvious that

Table 2. Contents of four marker compounds dl-tetrahydropalmatine, berberine, imperatorin and isoimperatorin in tablet of *Corydalis tuber* for alleviating pain (YZP) (mg/mL)

Sample no.	Peak 2	Peak 7	Peak 14	Peak 17
S1	0.032	0.019	0.079	0.037
S2	0.013	0.017	0.051	0.022
S3	0.024	0.005	0.013	0.004
S4	0.097	0.050	0.042	0.009
S5	0.046	0.051	0.052	0.014
S6	0.033	0.00006	0.048	0.021
S7	0.035	0.059	0.003	0.00001
S8	0.028	0.032	0.094	0.040
S9	0.041	0.003	0.026	0.010
S10	0.127	0.087	0.051	0.008
S11	0.036	0.024	0.061	0.008
S12	0.043	0.001	0.073	0.034

Peak 2: dl-tetrahydropalmatine, peak 7: berberine, peak 14: imperatorin and peak 17: isoimperatorin.

contents of the same component in different samples were significantly different. For example, the maximum content of dl-THP was 0.127 mg/mL and the minimum was 0.013 mg/mL. This confirmed the necessity for quality control of TCM and its preparations.

However, it was difficult to compare these 12 chromatograms quantitatively and directly just by ICF. Furthermore, ICF itself was influenced by operation factors, which leads to low integral stability and reproducibility. Digitized chromatographic fingerprint spectrum (DCFS), as an evaluation method, was forwarded.

Digitized Chromatographic Fingerprint Spectrum of YZP

By DCFS, a complicated chromatogram of ICF was converted into intuitive numbers. A reference peak was chosen in common peaks of chromatograms to introduce relative values, including RRT and RPA, which eliminated the negative effects of operation factors on integral stability and reproducibility of fingerprint so as to facilitate quantitative and quick comparison of different samples. A series of parameters, rules, and formulae were employed in DCFS to form a whole methodology. The key part of the original DCFS was a table including RRT and RPA of characteristic peaks of different samples. The original DCFS method was improved in our research by introducing RSD of RRT, RSD of RPA, and DR of RPA into one table to make it more intuitive for quantitative comparison of any part of the samples.

Dl-THP was chosen as the reference peak in the respect that it was a major active component of YZP, located in the center part of the chromatogram and obtained good resolution with the neighboring peaks. RRT and RPA of the reference peak were set to 1.00. Retention time and peak area of other characteristic peaks were divided respectively by those of the reference peak to get their RRT and RPA (Table 3). RSD of RRT of the 17 corresponding common peaks in these 12 samples was calculated and the results were in the range of 0.42–0.65%, within the error band of the HPLC method, of 1%. It meant that the corresponding peaks in the 12 samples were the same components. RSD of RPA of the 17 corresponding common peaks in these 12 samples ranged from 44.52% to 91.07%. It indicated that qualities of YZP samples from different producers were a lot different, which confirmed the necessity of quality control of TCM and its preparations.

DR was the integrative parameter, which could reflect the difference of each corresponding characteristic peak of test samples and reference sample. The total difference rate (TDR) indicated the total difference of the test sample and reference sample. The smaller TDR indicated the similarity between test sample and reference sample. It was helpful for the producer to compare the quality of different batches of samples or the difference between their own samples with the reference sample. DR and TDR of test samples were calculated according to the following equations:[20]

$$DR = \frac{|A_I^S - A_I^T|}{A_I^S} \tag{1}$$

A_I^S: relative peak area of reference sample, A_I^T: relative peak area of test sample

$$TDR = \frac{\sum (|A_I^S - A_I^T|/A_I^S)}{n} \tag{2}$$

where A_I^S: relative peak area of reference sample, A_I^T: relative peak area of test sample, n: number of common peaks.

DR values of 11 test samples were shown in Table 3. From the table we could check subtle differences of each component; they ranged from 0.00 to 9.14. For example, peak fraction no. 3 of S2, the DR value was 0.20 which indicated the difference between S2 and S1 was very small, while DR of peak fraction no. 4 was 2.14 which denoted that there was remarkable difference in this component.

TDR values of the 11 test samples were S2 (0.91), S3 (0.53), S4 (0.71), S5 (1.49), S6 (1.02), S7 (0.89), S8 (0.80), S9 (0.64), S10 (0.67), S11 (1.27), and S12 (0.65), respectively. Batch differences were evident by these quantitative data. From the DCFS table, one could be easily deduced the reason which caused the high TDR value. For example, TDR of S5 was 1.49, because there were 13 peaks' RPA of 17 common peaks were higher than 1.00 which caused 5 DR values of S5 higher than 1.00. According to the TDR

Table 3. Digitized chromatographic fingerprint spectrum (DCFS) of common peaks of tablet of *Corydalis tuber* for alleviating pain (YZP) by HPLC

	Peak																
Sample no.	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
RRT																	
S1	0.98	1.00	1.04	1.06	1.09	1.13	1.16	1.18	1.26	1.29	1.41	1.42	1.65	1.70	1.72	1.74	1.81
S2	0.98	1.00	1.04	1.06	1.09	1.13	1.16	1.18	1.26	1.28	1.40	1.42	1.65	1.70	1.72	1.74	1.80
S3	0.98	1.00	1.04	1.06	1.08	1.13	1.15	1.17	1.26	1.28	1.40	1.43	1.65	1.71	1.73	1.75	1.81
S4	0.98	1.00	1.03	1.06	1.09	1.13	1.16	1.18	1.25	1.28	1.41	1.43	1.66	1.71	1.73	1.75	1.81
S5	0.98	1.00	1.04	1.06	1.09	1.13	1.15	1.17	1.25	1.28	1.41	1.42	1.65	1.70	1.73	1.75	1.81
S6	0.98	1.00	1.04	1.06	1.09	1.14	1.16	1.18	1.27	1.29	1.42	1.43	1.66	1.72	1.74	1.76	1.82
S7	0.98	1.00	1.04	1.06	1.09	1.14	1.16	1.18	1.26	1.28	1.41	1.42	1.65	1.70	1.72	1.74	1.81
S8	0.97	1.00	1.02	1.05	1.08	1.12	1.14	1.16	1.24	1.26	1.39	1.40	1.63	1.68	1.70	1.72	1.78
S9	0.98	1.00	1.04	1.06	1.09	1.13	1.16	1.18	1.26	1.28	1.41	1.43	1.65	1.70	1.72	1.74	1.81
S10	0.97	1.00	1.03	1.05	1.09	1.13	1.15	1.17	1.25	1.28	1.41	1.43	1.65	1.70	1.73	1.75	1.81
S11	0.98	1.00	1.04	1.06	1.09	1.13	1.16	1.18	1.26	1.28	1.41	1.42	1.65	1.70	1.72	1.74	1.81
S12	0.98	1.00	1.04	1.06	1.09	1.14	1.16	1.18	1.26	1.28	1.41	1.42	1.65	1.70	1.73	1.75	1.81
Average	0.98	1.00	1.04	1.06	1.09	1.13	1.16	1.18	1.26	1.28	1.41	1.42	1.65	1.70	1.72	1.74	1.81
RSD of RRT (%)	0.45	0.00	0.48	0.43	0.42	0.43	0.42	0.45	0.65	0.45	0.49	0.49	0.52	0.52	0.52	0.52	0.52
RPA																	
S1	1.85	1.00	1.74	1.38	0.52	1.04	2.07	2.95	9.39	0.47	0.80	0.64	0.45	3.48	0.27	3.44	1.27
S2	2.73	1.00	2.08	4.33	0.84	0.78	4.30	6.47	19.98	0.84	1.59	1.22	0.88	5.02	0.90	5.42	1.72
S3	0.61	1.00	0.60	0.94	0.13	0.23	1.05	1.55	5.34	0.12	0.71	0.21	0.19	0.81	0.11	0.86	0.29
S4	1.79	1.00	2.44	0.93	0.70	0.97	1.64	2.16	10.03	2.12	1.31	0.94	0.18	0.64	1.21	0.96	0.13
S5	1.01	1.00	1.95	2.68	1.03	0.37	3.47	5.71	19.75	3.12	2.13	1.76	0.69	1.63	2.66	2.49	0.36

S6	0.14	1.00	0.16	0.31	0.77	0.05	0.39	0.47	1.53	1.27	1.67	1.30	0.14	2.06	1.68	2.83	0.74
S7	0.19	1.00	0.17	0.48	0.18	0.07	5.05	0.82	2.19	1.41	0.92	0.93	0.06	0.18	1.03	0.15	0.05
S8	3.39	1.00	1.38	3.01	0.49	0.65	3.66	5.76	21.66	0.74	1.11	1.80	0.39	4.65	1.13	4.63	1.54
S9	0.47	1.00	0.28	0.41	0.13	0.13	0.49	0.71	2.30	0.18	0.34	0.34	0.11	0.93	0.13	1.00	0.33
S10	0.91	1.00	2.31	1.43	0.40	0.98	2.10	2.74	10.63	1.88	0.85	1.15	0.16	0.60	1.34	0.83	0.09
S11	1.67	1.00	1.83	1.63	1.34	0.78	2.25	3.16	10.04	2.95	1.93	2.17	0.32	2.41	2.76	3.21	0.32
S12	0.17	1.00	0.14	0.27	0.23	0.07	0.36	0.70	1.71	0.35	1.06	1.22	0.08	2.40	0.51	2.49	0.86
RSD of RPA (%)	85.09	0.00	73.00	85.09	68.33	77.23	70.75	77.75	78.13	80.54	44.52	51.31	84.56	77.76	77.15	69.93	91.07
DR																	
S2	0.47	0.00	0.20	2.14	0.61	0.26	1.08	1.19	1.13	0.80	0.99	0.90	0.96	0.44	2.31	0.57	1.35
S3	0.67	0.00	0.65	0.32	0.76	0.78	0.49	0.47	0.43	0.74	0.11	0.68	0.58	0.77	0.58	0.75	0.23
S4	0.03	0.00	0.40	0.33	0.34	0.07	0.21	0.27	0.07	3.52	0.63	0.46	0.59	0.82	3.44	0.72	0.10
S5	0.45	0.00	0.12	0.94	0.97	0.64	0.67	0.94	1.10	5.66	1.66	1.74	0.54	0.53	8.78	0.28	0.28
S6	0.93	0.00	0.91	0.77	0.47	0.95	0.81	0.84	0.84	1.71	1.09	1.02	0.68	0.41	5.18	0.18	0.58
S7	0.90	0.00	0.91	0.65	0.65	0.93	1.44	0.72	0.77	2.02	0.15	0.45	0.86	0.95	2.78	0.96	0.04
S8	0.83	0.00	0.21	1.18	0.07	0.38	0.77	0.95	1.31	0.58	0.39	1.81	0.13	0.34	3.15	0.35	1.21
S9	0.75	0.00	0.84	0.70	0.76	0.87	0.76	0.76	0.75	0.62	0.58	0.47	0.76	0.73	0.53	0.71	0.26
S10	0.51	0.00	0.33	0.03	0.23	0.06	0.01	0.07	0.13	3.02	0.06	0.79	0.65	0.83	3.91	0.76	0.07
S11	0.10	0.00	0.05	0.18	1.55	0.26	0.08	0.07	0.07	5.30	1.41	2.38	0.29	0.31	9.14	0.07	0.25
S12	0.91	0.00	0.92	0.81	0.57	0.93	0.83	0.76	0.82	0.26	0.32	0.91	0.81	0.31	0.87	0.28	0.68

equation, TDR was the average value of DR, so the TDR value of S5 was consequently high. It is clear that a qualified TDR value range could be set in the quality control standard by the government branches or producers.

CONCLUSIONS

The analysis method of alkaloids in tablet of *Corydalis tuber* for alleviating pain was established effectively with TEA and HAc added in the mobile phase. Image chromatographic fingerprint and digitized chromatographic fingerprint spectrum of 12 batches of tablet of *Corydalis tuber* for alleviating pain were developed based on it. This fingerprint study gave an applicable qualitative and quantitative quality control method for alkaloids related TCM and its preparations.

REFERENCES

1. Committee of National Pharmacopoeia. In *Pharmacopoeia of P. R.China*; Press of Chemical Industry: Beijing, 2005.
2. Mao, Y.Q.; Lu, C.L. Chinese J. Compos. Clin. Hyg.**2005**, *7*, 82.
3. Chen, G.H.; Sun, K.; Zhang, S.Q. J. Tradit. Chin. Med. **2003**, *18*, 46.
4. Lin, M.T.; Chueh, F.Y.; Hsieh, MT. Clin. Exper. Pharm. Physiol. **1996**, *23*, 738.
5. Leung, W.C.; Zheng, H.; Huen, M. Prog. Neuro-Psychoph. **2003**, *27*, 775.
6. Kuo, C.L.; Chi, C.W.; Liu, T.Y. Cancer Lett. **2004**, *203*, 127.
7. Kimura, Y.; Ohminami, H.; Arichi, H.; Okuda, H. Planta. Med. **1982**, *45*, 183.
8. Dniel, K.W.M.; Chau, F. T. Chemometer. Intell. Lab. **2006**, *82*, 210.
9. Chen, S.B.; Liu, H.P.; Tian, R.T. J. Chromatogr. A **2006**, *1121*, 114.
10. Yan, S.K.; Xin, W.F.; Luo, G.A. J. Chromatogr. A **2005**, *1090*, 90.
11. Lu, H.M.; Liang, Y.Z.; Chen, Y.Z. J. Ethnopharmacol. **2006**, *105*, 436.
12. Gu, M.; Wang, X.L.; Su, Z.G.; Ouyang, F. J. Chromatogr. A **2007**, *1140*, 107.
13. Gao, M.; Gu, M.; Liu, C.Z. J. Chromatogr. A **2006**, *838*, 139.
14. Gu, M.; Ouyang, F.; Su, Z.G. J. Chromatogr. A **2004**, *1022*, 139.
15. Deng, J.Y.; X, G.H.; Sun, J. Pharm. Care. & Res. **2004**, 335.
16. Xiao, C.H.; Yang, S.S.; Hong, X.K. *Tradit. Chin. Med.l Chem. (*in Chinese*)*; Shanghai Scientific and Technical Publishers: Shanghai, 1997; 147.
17. Wu, A.B.; Huang, M.C.; Ho, H.-O. Biomed. Chromatogr. **2004**, *18*, 443.
18. Tesarova, E.; Bosáková, Z.; Zusková, I. J. Chromatogr. A **2000**, *879*, 147.
19. Chen, Q. *Research Methodology of Chinese Drugs Pharmacology*; The People's Medical Publishing House: (in Chinese), Beijing, 2000; 564.
20. Hong, X.K.; Wang, Z.H. *Digital Fingerprint of Chinese Traditional Medicine (*in Chinese*);* Shanghai Scientific and Technical Publishers: Shanghai, 2003; 83.

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