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Qualitative and Quantitative Analysis of Dan-Deng-Tong-Nao Capsules by HPLC-DAD-MS/MS

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Abstract

Dan-Deng-Tong-Nao capsules (DDTNCs), a traditional Chinese medicinal preparation consisting of Radix *Salvia miltiorrhiza*, Radix *Pueraria lobata, Erigeron breviscapus, Rhizoma chuanxiong* has been used for the treatment of apoplexy and the syndrome of apoplexy involving both collaterals and meridians caused by obstruction of collaterals by blood stasis in China. In this paper, a HPLC-DAD-MS/MS method was successfully developed for qualitative and quantitative analysis of major components in DDTNCs for the first time. Twenty-six compounds were identified by comparison of their retention times and MS spectra with those elucidated standards or recorded literatures, and six of them (danshensu, 5-hydroxymethylfurfural, 3,4-dihydroxybenzaldehyde, epicatechin, puerarin and salvianolic acid A) were simultaneously determined by HPLC-DAD quantitatively. The analytical method was fully validated and successfully applied for quantitative and fingerprint analysis of DDTNCs from fifteen different production batches, results indicated that the proposed approach was applicable for the routine analysis and quality control of DDTNCs.

Keywords: Dan-Deng-Tong-Nao capsules; Quantitative analysis; Chemical fingerprint; Quality control; HPLC-DAD-MS/MS

Introduction

"Dan-Deng-Tong-Nao capsules" (DDTNCs) is a commonly used traditional Chinese formula medicine, which is prepared (extracted, purified and combined) from four commonly used Chinese herb drugs, including Radix *Salvia miltiorrhiza*, Radix *Pueraria lobata*, *Erigeron breviscapus* and *Rhizoma chuanxiong*. DDTNCs have the ability of activating blood circulation to dissipate blood stasis, removing obstruction in the meridians and heart-nourishing effects [1,2]. It was usually used for the treatment of apoplexy and the syndrome of apoplexy involving both collaterals and meridians caused by obstruction of collaterals by blood stasis [3,4].

Chemical constituents of the four component herbs of DDTNCs have been intensively studied [5-8]. However, little is known about the chemical composition of DDTNCs, and the current analysis methods could not reflect the quality of it [9,10]. Therefore, practicable and reliable quality evaluation method to monitor the systematic chemical profile changes of DDTNCs in the procedure from preparation and storage to clinic usage is of significant importance.

In this paper, an HPLC-DAD-MS/MS method was established for rapidly identifying and reliably determining multiple components in DDTNCs, and was then successfully applied to quantitative and fingerprint analysis of fifteen batches of DDTNCs samples. This study represents the first detailed investigation of the components of DDTNCs and provides an applicable method for its comprehensive quality evaluation.

Experiment

Apparatus, materials, chemicals and reagents

HPLC-DAD analysis was carried out on an Agilent 1200 Series HPLC with diode-array detector; MS/MS was performed on an Agilent 6410 Triple Quad MS with an electrospray ionization (ESI) interface (Agilent Technologies, Inc., USA). Fifteen batches of DDTNCs were purchased from different pharmacies in China. Standards of danshensu, 5-hydroxymethylfurfural, 3,4-dihydroxybenzaldehyde, epicatechin, puerarin and salvianolic acid A (Structures shown in Figure 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products(Beijing, China), their purities (>98%) were determined by normalization of the peak area detected by HPLC–UV. HPLC grade reagents (methanol, acetonitrile and formic acid) were obtained from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Milford, MA, USA).

Chromatographic and MS conditions

The separation was performed on an Agilent Zorbax SB-Aq column (250 mm × 4.6 mm i.d., 5 μm) with a C_{18} guard column (10 mm × 4.6 mm i.d., 5 μm) at 35°C with a flow rate of 0.8 ml/min. Elution was achieved with a linear gradient solvent program of methanol (A) in water–0.2% acetic acid (B) (v/v): 0–40 min, 20–55% A; 40–41 min, 55–95% A; 41–45 min, 95% A; 45–46 min, 95-20% A. The UV spectra (DAD) were recorded between 190 and 400 nm, and the wavelength of 280 nm was chosen for determination.

The ESI-MS/MS detection was acquired directly after DAD measurement in positive ionization mode, and recorded on a mass range of m/z 100-1000. Drying gas (Nitrogen) temperature was set at 350°C with a flow rate of 10.00 L/min and nebulizer pressure of 45.00 psi. Capillary voltage was 4000 V.

Preparation of standard and sample solutions

Accurately weighed solid portion of each standard was dissolved in methanol to prepare stock solutions: 1.60 mg/mL for danshensu, 2.50 mg/mL for 5-hydroxymethylfurfural, 2.88 mg/mL for 3,4-dihydroxybenzaldehyde, 2.90 mg/mL for puerarin, 2.50 mg/mL for epicatechin and 3.00 mg/mL for salvianolic acid A. A mixture stock solution containing the six standards (300 μg/mL for danshensu, 125 μg/mL for 5-hydroxymethylfurfural, 140 μg/mL for

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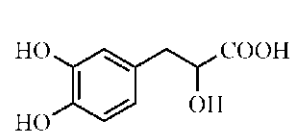
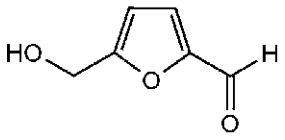
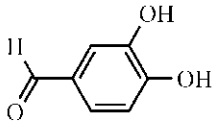


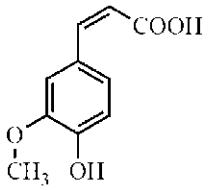
Peak 1: Danshensu m/z=198



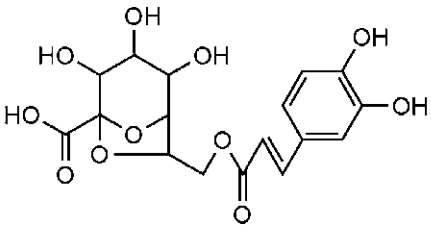
Peak 2: 5- Hydroxymethylfurfural m/z=126



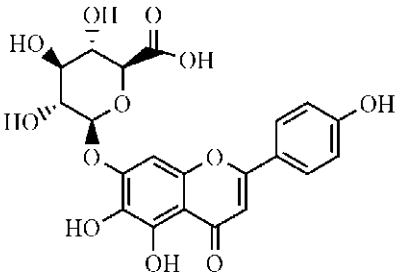
Peak 4: 3,4-Dihydroxybenzaldehyde m/z=138



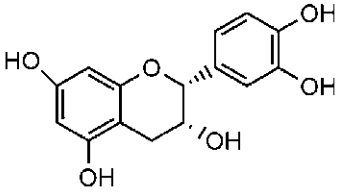
Peak 16: Ferulic acid m/z=194



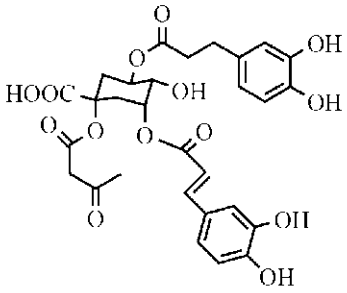
Peak3: 9-COA m/z=398



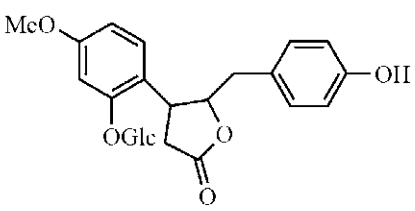
Peak 5: Scutellarin m/z=462



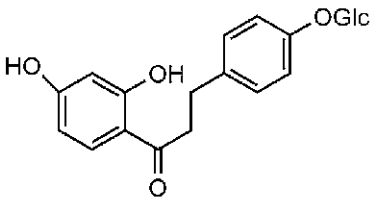
Peak 7: Epicatechin m/z=290



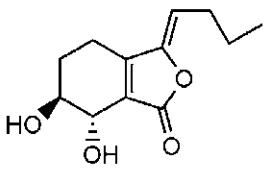
Peak 8: 1-Malonyl-3,5-diCQA m/z 602



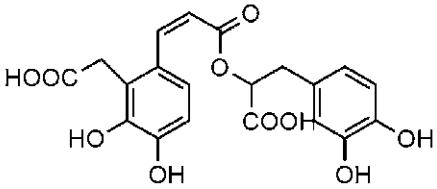
Peak 11: Sophoraside A m/z 474



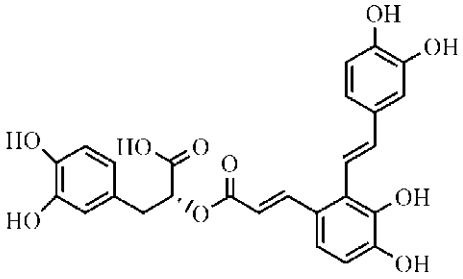
Peak12: Isoliquiritin m/z 416



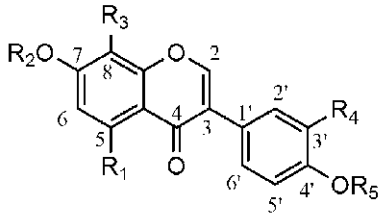
Peak 17: Senkyunolide I m/z=224



Peak 18: Salvianolic acid D m/z=418



Peak 26: Salvianolic acid A m/z=494



Peak No.	Compounds	R1	R2	R3	R4	R5	m/z
6	3'-Hydroxypuerarin	H	H	glc	OH	H	432
9	Puerarin	H	H	glc	H	H	416
10	3'-Methoxydaidzin	H	H	glc	OMe	H	446
13	Genistin	OH	glc	H	H	H	432
14	Daidzein-4'-glucoside	H	OH	H	H	glc	416
15	Sissotorin	H	glc	OH	H	Me	446
19	Ononin	H	glc	H	H	Me	430
20	3'-Methoxypuerarin	H	H	glc	OMe	H	446
21	Daidzin	H	glc	H	H	H	416
22	3'-Hydroxyl-4'methyldaidzin	H	glc	H	OH	Me	446
24	4'-Methoxypuerarin	H	H	glc	H	Me	446
27	Daidzein	H	H	H	H	H	254
28	Biochanin A	OH	H	H	H	Me	284

Figure 1: Structures of the identified compounds from DDTNCs.

3,4-dihydroxybenzaldehyde, 580 μg/mL for puerarin, 125 μg/mL for epicatechin and 300 μg/mL for salvianolic acid A) was prepared in 50% methanol and stored away from light at 4°C until used. Working solutions of the lower concentration were prepared by appropriate dilution of the stock solution with 50% methanol. The chromatogram of the six standards is shown in Figure 2.

Sample solution preparation

The powder of DDTNCs (about 50 mg) was extracted with 20.0 mL solvent composed of 80% methanol in water (v/v) for 20 min in an ultrasonic bath. 1.0 mL of the extract solution was centrifugation at 15,000 rpm for 5 min and 5 μL of the supernatant was injected into the HPLC-DAD-MS/MS system. Content for each component was calculated from its corresponding calibration curve.

Results

Identification of components from DDTNCs

The traditional methods to isolate different standard compounds from herb drugs are restricted and laborious. However, HPLC coupled with MS is a powerful approach to solve this problem. MS, a selective and sensitive detector, allows the detection of minor or even trace amounts of constituents from complex matrix. Furthermore, MS provides abundant structural information and thus, facilitates the structural identification of unknown compounds. In this study, HPLC-DAD-MS/MS was adopted to identify the chemical constituents of DDTNCs.

ESI in both negative and positive mode was tried to detect components in DDTNCs. The results indicated that more peaks could be detected in positive mode than in negative mode. For identification of components contained in the crude extracts of DDTNCs, mass fragmentation with MS^2 spectrometry data provided valuable information. An efficient transmission of molecular ions and fragment ions was obtained by ramping the collision energy from 10 V to 50 V for the rapid identification of trace level components in the crude extracts with large sample throughput.

In the positive ESI-MS spectra of DDTNCs, the quasi-molecular ion peaks of components always appeared as $[M+H]^+$ ion, except for peak 17. Most peaks of components had the fragments of $[M+H-18]^+$,

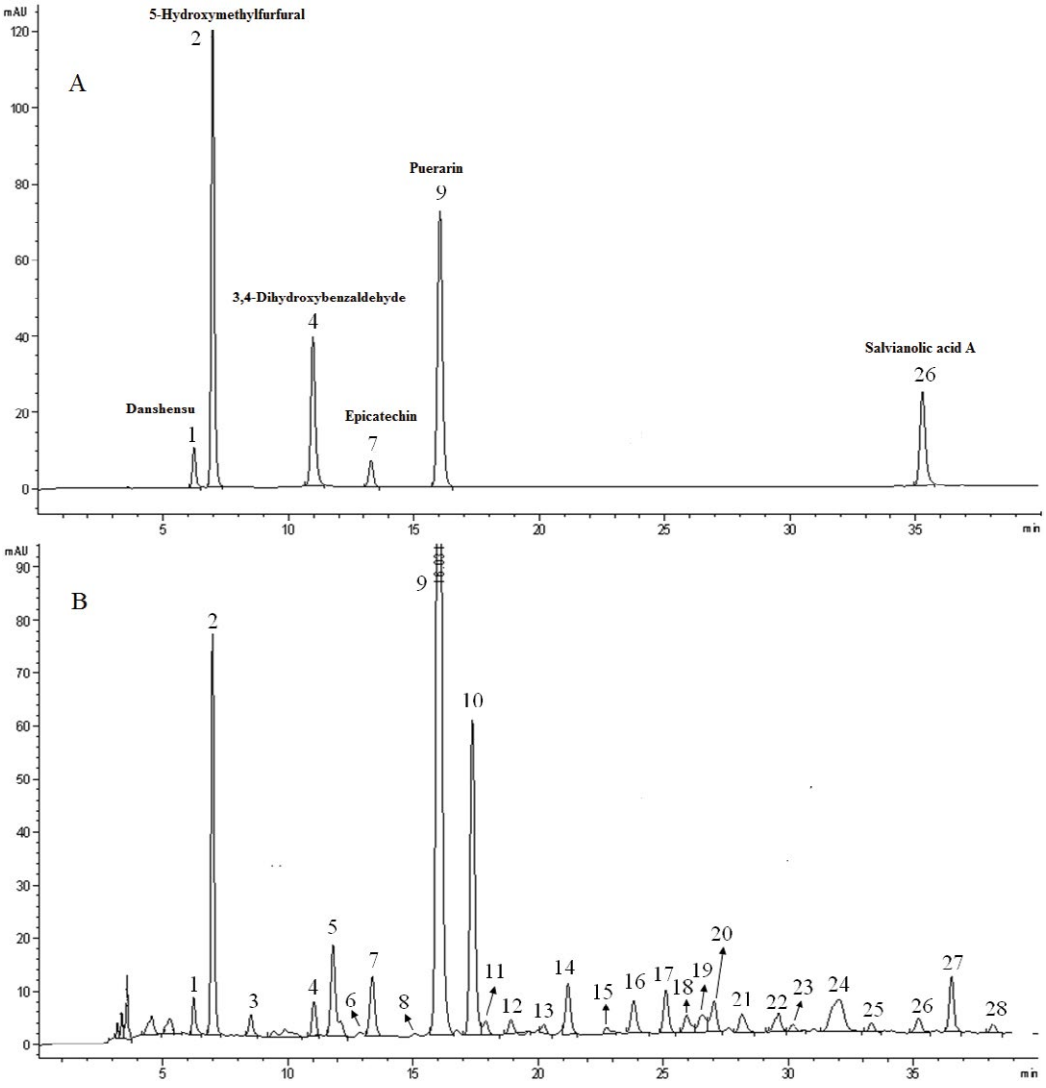


Figure 2: The representative HPLC-DAD chromatogram of DDTNCs samples at 280 nm (A: Standards; B: Sample).

$[M+H-28]^+$ and $[M+H-15]^+$ corresponding to the loss of "H_2O", "CO" and "CH_3", respectively. Furthermore, there were some fragments of $[M+H-176]^+$ which corresponded to the loss of "Glu" and indicated the structure belonged to glycosides.

Through on-line DAD and ESI–MS spectra, six major peaks: 1, 2, 4, 7, 9 and 26 in the chromatogram of DDTNCs (Figure 2) were identified by comparing their retention time, ultraviolet absorption and MS/MS spectra with standard compounds. Finally, 26 compounds were characterized by comparing their retention times, UV spectra and MS data with those of reference compounds or literature data [11-16], and most of them belonged to flavonoids, the representative cleavage pathway of flavonoid is shown in Figure 3. The results of the MS/MS and UV data are listed in Table 1, and the chemical structures of the identified compounds are shown in Figure 1.

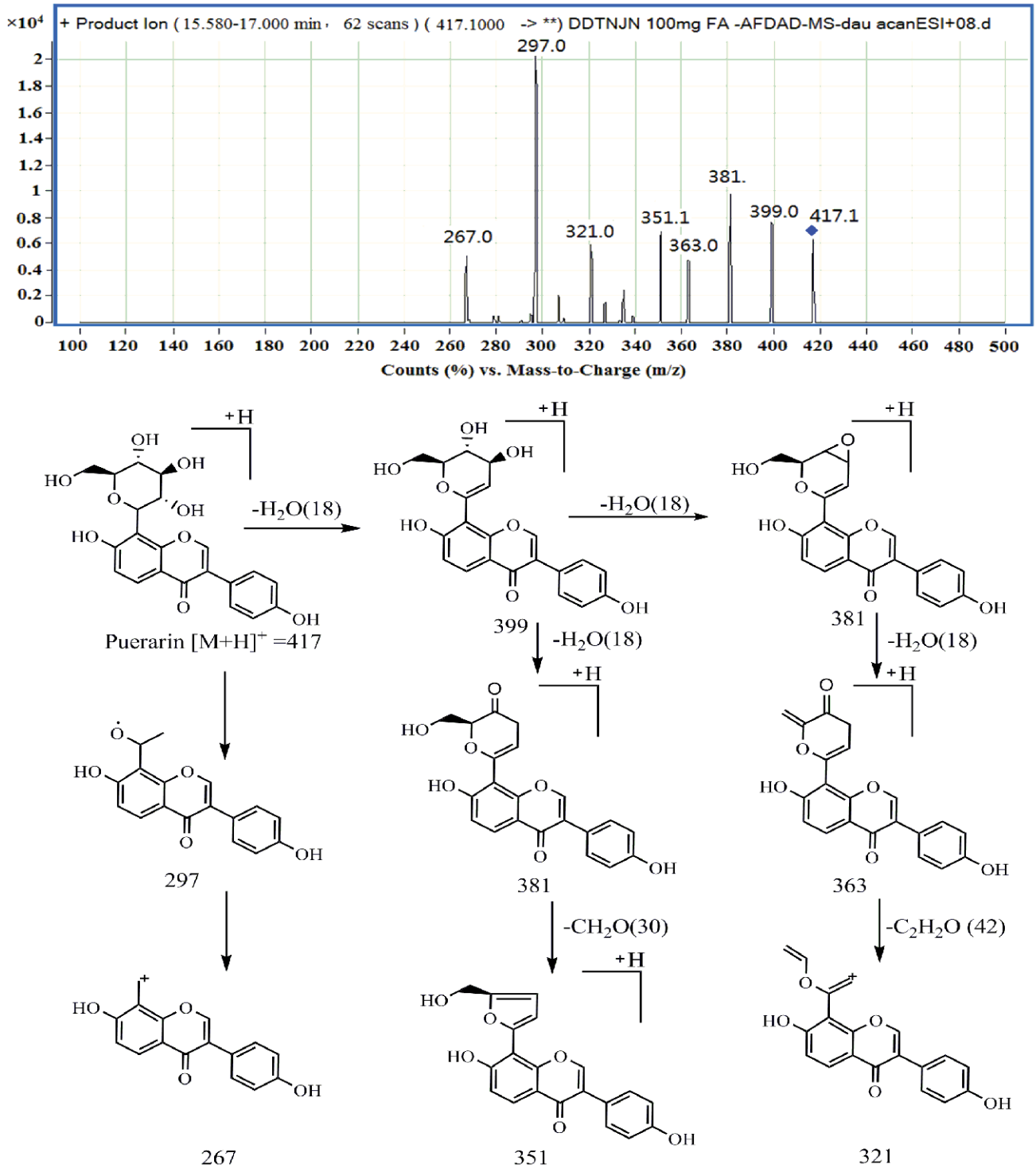


Figure 3: The representative MS/MS cleavage pathway of flavonoids in DDTNCs.

Validation of the method

Calibration curve, limits of detection and quantification: Solutions containing the six standards at six different concentrations were analyzed. The calibration curve of each analyte was constructed by plotting the peak area versus the corresponding concentration. The mixed standards solution was further diluted to a certain concentration to explore the limits of detection (LOD) and quantification (LOQ). The LOD and LOQ were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively. The satisfactory results regarding regression equations, correlation coefficients, linear ranges, LODs and LOQs were listed in Table 2. The high correlation coefficient values (R^2>0.9992) indicated good linearity between their peak areas (y) and investigated compound concentration (x, μg/ml) in relatively wide concentration ranges.

Precision, stability and accuracy of the method: The intra- and inter-day precisions were determined by continuously injecting the sample solution for six replicates on the same day and by measuring it once a day for three consecutive days, respectively. Stability was assessed by analyzing the same sample solution at different time within 24 h (0, 8, 12, 24 h) at room temperature.

Recoveries test that reflecting method's accuracy was performed employing the method of standard addition. Three different quantities (low, medium and high) of the authentic standards were added into the known real sample. The recovery was figured out according to the following formula: Recovery (%)=(amount detected−original amount)/amount spiked × 100%, and RSD (%)=(SD/mean) × 100%. All the results were estimated on the ground of relative standard deviation (RSD%).

Tables 3 and 4 list the results of precision, stability and recovery test, which suggested that the RSDs for the six compounds are less than 5.0%, the average recoveries of the six compounds is 94.6–104.8%. These data indicated that the established HPLC-DAD method was precise, accurate and sensitive enough for simultaneously quantitative determination of these six compounds in DDTNCs.

Fingerprint analysis of DDTNCs: The chromatograms of different samples have to be standardized, so as to perform fingerprint analysis. The process of standardization included the selection of "common peaks" in chromatograms and the normalization of retention times of all the common peaks [17]. In this study, the extracts of fifteen samples of DDTNCs were analyzed. More than thirty peaks appeared in the HPLC-DAD chromatographic fingerprints as shown in Figure 2. The peak of puerarin (peak 9) was selected as a reference peak because it is an intense peak situated nearly in the middle of chromatogram. Among all the peaks observed, fifteen of them (1-5, 7, 9, 10, 14, 16, 17, 21, 22, 26 and 27) were defined as common peaks.

Similarity analysis was performed by using the software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicines" (Version 2004A), which used to generate the mean chromatogram, and calculate the similarities of the sample chromatograms with mean chromatogram with cosine method. The average chromatogram of the fifteen batches of commercial samples was taken as the standard characteristic fingerprint of DDTNCs. It was subsequently compared with the chromatograms from fifteen batches of DDTNCs to generate the similarity values which were in the range of 0.671~0.991 (Table 5). It was found that correlation coefficients

Peak No.	Rt (min)	UV	MS [M+H]$^+$	MS/MS	Identify
1	6.24	282, 288	199	181, 137, 125, 75	Danshensu
2	6.99	285	127	109, 99, 81,69,53	5 - Hydroxymethylfurfural
3	8.52	242, 295, 325	399	355, 337, 295, 283, 237, 181, 163	9-C^{O}Aa
4	10.97	220, 280, 309	139	121, 93, 67	Protocatechualdehyde
5	11.80	250, 315	463	287, 17,159, 115	Scutellarin
6	12.56	198, 249, 310	433	397, 367, 313, 283	3'-Hydroxypuerarin
7	13.26	/	291	247, 205, 181, 127, 111	Epicatechin
8	15.36	240, 300, 340	603	559, 517, 441, 397, 379, 235	1-Malonyl-3,5-diCQA[b]
9	16.03	198, 250, 304	417	399, 381, 363, 351, 321, 297, 267	Puerarin
10	17.37	225, 260	447	432, 411, 327, 285, 270, 253	3'-Methoxydaidzin
11	17.90	215	475	299	Sophoraside A
12	18.91	235, 368	419	257, 137	Isoliquiritin
13	20.22	260, 325	433	415, 397, 313, 273, 257	Genistin
14	21.19	250, 300	417	255	Daidzein-4'-glucoside
15	22.74	280	447	285	Sissotorin
16	24.04	238, 290, 320	195	151, 119, 85, 57	Ferulic acid
17	25.10	250	471 [2M+Na]$^+$	247 [M+Na]$^+$	Senkyunolide I
18	25.93	250, 290, 325	419	/	Salvianolic acid D
19	26.55	195, 238	431	416, 365, 311, 255	Ononin
20	27.02	250	447	429, 411, 393, 351, 327, 297	3-Methoxypuerarin
21	28.14	248, 270, 306	417	399, 381, 351, 297, 255	Daidzin
22	29.58	215, 240	447	414, 383, 337, 297	3'-Hydroxyl-4'methyldaidzin
23	30.16	275	247	188, 170, 146, 118	Unknown
24	32.02	210,280	447	429, 414, 393, 337, 297,	4'-Methoxypuerarin
25	33.32	/	431	269	Unknown
26	35.20	230, 285, 340	495	385, 297, 187, 137	Salvianolic acid A
27	36.51	249, 310	255	227, 199, 173, 153, 137	Daidzein
28	38.17	250	285	270, 252, 225, 270, 213, 185	Biochanin A

[a]COA: caffeoyl-2,7-anhydro-2-octulopyranosonic acid; [b]CQA: caffeoylquinic acid

Table 1: The on-line detected UV and MS spectrometric data of the identified and deduced compounds.

of DDTNCs samples varied considerably, indicating the quality of DDTNCs was inconsistent.

Quantitative analysis of DDTNCs: The proposed HPLC method was applied to analyze six components in fifteen batches of DDTNC. The quantitative analyses were performed by means of the external standard method. Samples were prepared as described in section "2.3". The typical chromatogram is given in Figure 2. The results, shown in Table 5, indicate that puerarin showed the highest amount (43.60-72.70 μg/mg), followed by 5-hydroxymethylfurfural (1.24-7.96 μg/mg), danshensu (1.48-5.04 μg/mg) and salvianolic acid A (0.85-4.33 μg/mg). Our results showed that the contents of the six analytes in different batches are noticeably different, which may occurred due to various factors such as cultivation, harvest, storage and so on.

Discussion

Optimization of HPLC conditions

The selection of the HPLC conditions was guided by the requirement for obtaining chromatograms with better resolution of adjacent peaks. The optimization of HPLC conditions was performed using standard mixture and sample extract. Different HPLC parameters including mobile phase (acetonitrile–water, methanol–water, acetonitrile–acid aqueous solution, methanol–acid aqueous solution), category of column (Agilent Zorbax SB-C_{18} 250 mm × 4.6 mm i.d., 5 μm, Agilent Zorbax Extended-C_{18} 250 mm × 4.6 mm i.d., 5 μm, Agilent Zorbax SB-Aq column 250 mm × 4.6 mm i.d., 5 μm) and column temperature (25, 35, 45°C) were examined and compared.

Analytes	Regressive equation	R^2	Test range (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)
Danshensu	y=3.333x-5.215	0.9992	1.60~160	0.45	1.00
5-Hydroxymethylfurfural	y=43.555x-17.189	1.0000	1.25~125	0.10	0.41
3,4-Dihydroxybenzaldehyde	y=18.995x-28.514	0.9996	0.35~70	0.11	0.32
Epicatechin	y=3.798x-2.690	0.9994	1.25~125	0.12	0.40
Puerarin	y=9.008x-19.794	0.9999	5.80~580	0.60	1.50
Salvianolic acid A	y=7.392x-29.247	0.9995	3.00~300	0.45	1.30

y, peak area; *x*, concentration of compound (μg/ml); limit of detection (LOD), S/N=3; limit of quantification (LOQ), S/N=10

Table 2: Linear regression data, LOD and LOQ of the six investigated components from DDTNC.

Analytes	Intra-day precision (n=6)		Inter-day precision (n=3 days)		Stability (24 h)
	Mean ± SD (μg/ml)	RSD[a] (%)	Mean ± SD (μg/ml)	RSD (%)	RSD (%)
Danshensu	7.16 ± 0.12	1.68	7.12 ± 0.29	4.03	1.00
5-Hydroxymethylfurfural	7.56 ± 0.29	3.84	7.62 ± 0.33	4.33	1.06
3,4-Dihydroxybenzaldehyde	0.48 ± 0.008	1.68	0.49 ± 0.02	4.49	1.91
Epicatechin	3.81 ± 0.11	2.89	3.91 ± 0.15	3.84	0.57
Puerarin	157.6 ± 4.77	3.03	159.3 ± 5.1	3.20	1.11
Salvianolic acid A	3.25 ± 0.07	2.15	3.27 ± 0.11	3.36	0.83

[a]RSD (%)=(SD/mean) × 100%.

Table 3: Intra-day and inter-day precisions of the method and the stability of the extract samples.

Analytes	Initial (μg/25 mg)	Added (μg)	Found (μg)	Recovery[a] (%)	RSD (%)
Danshensu	71.6	55.0	128.4	103.3	3.24
		70.0	141.1	99.3	1.44
		80.0	155.4	104.8	2.32
5-Hydroxymethylfurfural	75.6	60.0	137.1	102.5	3.34
		75.0	150.1	99.3	4.31
		90.0	165.3	99.7	3.45
3,4-Dihydroxybenzaldehyde	4.8	4.0	8.8	101.0	4.32
		4.8	9.5	97.9	2.57
		5.6	10.2	97.1	3.12
Epicatechin	38.1	30.0	68.4	101.0	1.87
		37.5	75.2	98.9	2.33
		45.0	83	99.8	2.36
Puerarin	1575.5	1250.0	2806.8	98.5	3.32
		1550.0	3119.2	99.6	1.36
		1900.0	3461.8	99.3	3.91
Salvianolic acid A	32.5	26.0	58.1	98.5	3.35
		35.0	65.6	94.6	1.34
		40.0	72.5	100.0	2.76

Table 4: Recovery of analytes from DDTNC through the current HPLC–DAD method.

The results showed that the best resolution and retention behavior were achieved when the methanol–water/ acetic acid (100/0.2, v/v) system and the column (Agilent Zorbax SB-Aq column 250 mm × 4.6 mm i.d., 5 μm) were used. Finally, an optimized HPLC condition was developed by comprehensively evaluating the resolution, baseline, elution time. Meanwhile, linear gradient was applied in HPLC procedure to obtain a perfect resolution, which could be examined by DAD and ESI-MS spectrometer. DAD detection was employed at wavelength range of 190–400 nm to investigate the UV spectra of the compounds in DDTNCs. It was found that UV at 280 nm was the best wavelength for the detection, because the most peaks can be detected under 280 nm. The optimal HPLC condition was shown in section "2.2", and the representative HPLC-DAD chromatograms of DDTNCs samples are shown in Figure 2.

Optimization of extraction conditions

In order to obtain as many peaks as possible, related extraction conditions were examined and evaluated. Firstly, different extraction solvents (water and aqueous methanol of 20%, 50%, 70%, 80%, 90% and methanol (v/v)) were compared, the total number of peaks and the content of the six quantitative indicators were used as response to evaluate the extraction solvents. The results showed that the peak intensities of middle eluted constituents increased with the increase of methanol concentration in the extraction solution, while the peak intensities of early eluted constituents decreased with the increase of methanol concentration. The sample extracted with 80% methanol exhibited higher contents of the indicators than those with other tested solvents, which was further confirmed by the HPLC-DAD quantitative analysis method (Figure 4). Therefore, 80% methanol was selected as

Batch no.	Content of investigated components (μg/mg)						Similarity[a]
	Danshensu	5 –Hydroxyl-methylfurfural	3,4-Dihydroxy-benzaldehyde	Epicatechin	Puerarin	Salvianolic acid A	
C1710002	3.15	2.39	1.20	0.87	59.30	2.42	0.986
C1710011	2.73	1.33	0.21	0.80	65.60	1.76	0.945
C1710013	5.04	6.35	0.20	1.69	64.35	1.27	0.752
C1710017	2.48	1.24	1.19	0.89	58.35	3.06	0.983
C1710025	2.80	7.96	0.89	1.03	72.70	1.29	0.947
C1711006	3.00	3.33	0.20	2.07	63.80	1.27	0.976
C1711017	3.14	1.39	0.20	3.93	59.45	1.12	0.957
C1711011	3.00	3.16	0.19	1.66	65.55	4.33	0.991
C1711021	3.23	2.34	0.23	0.79	58.32	1.27	0.909
C1712001	4.15	3.26	0.29	1.54	54.23	2.10	0.866
C1712005	3.45	3.19	0.43	2.43	65.24	2.41	0.721
C1712012	5.28	2.35	0.32	1.34	61.26	3.09	0.671
C1713005	4.21	4.27	0.87	0.98	71.21	1.35	0.947
C1713014	3.68	1.96	0.24	2.31	49.38	1.49	0.952
C1713021	3.46	3.8	1.09	1.24	60.12	1.27	0.982

[a]The similarity of DDTNCs was calculated professional software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A).

Table 5: Contents of the six investigated components and similarity of DDTNCs from fifteen different batches.

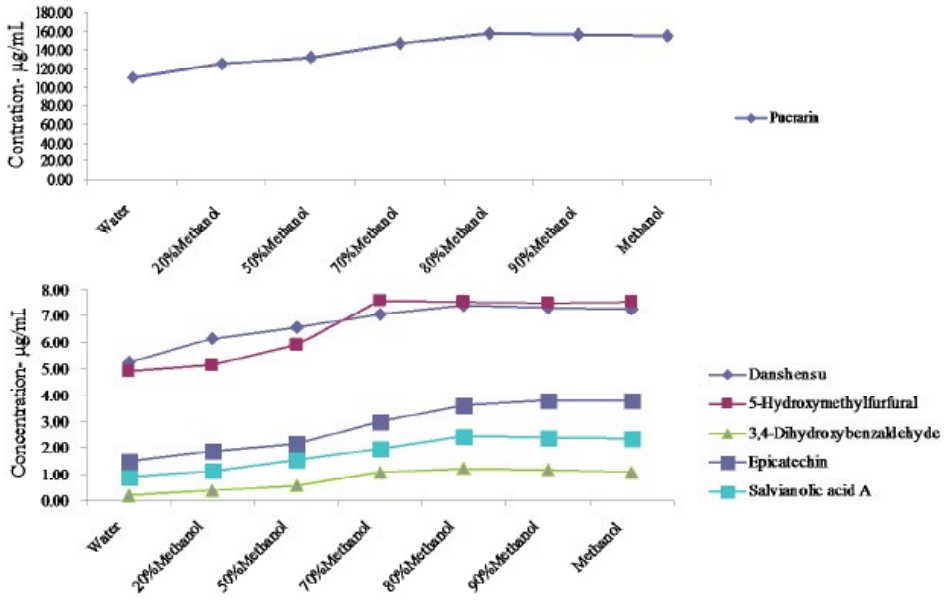


Figure 4: Comparison of the extraction efficiencies of different solvents.

the extraction solvent for both identification and quantitative analysis of DDTNCs. Then extraction repetitions (1, 2, 3 times) and extraction time (10 min, 20 min, and 30 min) were also evaluated. Finally, by comparing the areas of characteristic peaks in chromatograms obtained under different conditions, the optimal condition for extraction of DDTNCs was selected and presented in detail in section "2.3".

Conclusion

In the last decades, only one or two markers or pharmacologically active components in TCMs were usually employed for evaluating the quality of them, which could not give a complete picture of TCMs, because multiple constituents are usually responsible for its therapeutic effects. The proposed HPLC-DAD-MS/MS method makes it possible to comprehensively evaluate the quality of the commonly used TCM (DDTNCs) through a simultaneous qualitative and quantitative analysis of multi-components.

This method has been applied successfully to simultaneously identify and quantify the main components in different batches of DDTNCs. Results indicated: (1) the established method is rapid, simple and accuracy; (2) most ingredients of DDTNCs belong to flavonoids and phenolic acids; (3) the quality of the collected samples was inconsistent, which could be easily affected by the exterior factors, not only the processing methods, but also the harvested time and habitats of the four raw herbs.

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References

1. Wei WH, Bai L, Si X (2012) Clinical observation of Dan-Deng-Tong-Nao Capsule for the treatment of sequelae of cerebral infarction. Chinese Journal of Practical Nervous Diseases 15: 51-52.
2. Liu XD, Zhong WJ (2012) The impact of Dan-Deng-Tong-Nao Capsule on blood rheology and ECG of patients with coronary heart disease. China Medicine 7: 260-261.
3. Jing SG, Hou PH (2011) Treatment of 42 cases of vertigo after lacunar infarction with Dan-Deng-Tong-Nao Capsule. Jilin Journal of Traditional Chinese Medicine 31: 1079-1080.
4. Liu YM (2008) Treatment of 60 cases of vertebrobasilar insufficiency with Dan-Deng-Tong-Nao Capsule. Asia-Pacific Traditional Medicine 4: 119-120.
5. Chinese Materia Medica (1999) Editorial Board organized by State Administration of TCM. Shanghai Science and Technology Press, Beijing 5: 977-980.
6. China Pharmacopoeia Committee (2010) Pharmacopoeia of the People's Republic of China. China Chemical Industry Press, Beijing 1: 38-71.
7. Dua G, Zhao HY, Zhang QW, Li GH, Yang FQ, et al. (2010) A rapid method for simultaneous determination of 14 phenolic compounds in Radix Puerariae using microwave-assisted extraction and ultra high performance liquid chromatography coupled with diode array detection and time-of-flight mass spectrometry. Journal of Chromatography A 1217: 705-714.
8. Lv SH, Xie JB, Lv GB (2003) Advances in studies on Erigeron breviscapus. Tianjin Pharmacy 15: 46-48.
9. He Y, Wang DM, Dong ZM, Shen T, Luan S, et al. (2007) Simultaneous determination of scutellarin and salvianolic acid B in Dan-deng-tong-nao capsules by HPLC. Journal of Shenyang Pharmaceutical Univiversity 24: 491-494.
10. Wu BH, Zhang XG, Hong DS, Ma KF, Jiang SP (2014) Simultaneous, determination of eight active components in the traditional Chinese medicine Dan Deng Tong Nao Capsules by HPLC-MS/MS. Planta Medicine 80: 517-522.
11. Liu AH, Lin YH, Yang M, Guo H, Guan SH, et al. (2007) Development of the fingerprints for the quality of the roots of Salvia miltiorrhiza and its related preparations by HPLC-DAD and LC-MS(n). J Chromatogr B Analyt Technol Biomed Life Sci 846: 32-41.
12. Liao SG, Zhang LJ, Li CB, Lan YY, Wang AM, et al. (2010) Rapid screening and identification of caffeic acid and its esters in Erigeron breviscapus by ultra-performance liquid chromatography/tandem mass spectrometry. Rapid Communication Mass Spectrometry 24: 2533-2541.
13. Zhang YF, Shi PY, Qu HB, Cheng YY (2007) Characterization of phenolic compounds in Erigeron breviscapus by liquid chromatography coupled to electrospray ionization mass spectrometry. Rapid Communication Mass Spectrometry 21: 2971-2984.
14. Ruan M, Li Y, Li X, Luo J, Kong L (2012) Qualitative and quantitative analysis of the major constituents in Chinese medicinal preparation Guan-Xin-Ning injection by HPLC-DAD-ESI-MS(n). J Pharm Biomed Anal 59: 184-189.
15. Du G, Zhao HY, Zhang QW, Li GH, Yang FQ, et al. (2010) A rapid method for simultaneous determination of 14 phenolic compounds in Radix Puerariae using microwave-assisted extraction and ultra high performance liquid chromatography coupled with diode array detection and time-of-flight mass spectrometry. J Chromatogr A 1217: 705-714.
16. Yan Y, Chai CZ, Wang DW, Yue XY, Zhu DN, et al. (2013) HPLC-DAD-Q-TOF-MS/MS analysis and HPLC quantitation of chemical constituents in traditional Chinese medicinal formula Ge-Gen Decoction. J Pharm Biomed Anal 80: 192-202.
17. Li J, Li WZM, Huang W, Cheung AWH, Bi CWC, et al. (2009) Quality evaluation of Rhizoma Belamcandae (Belamcanda chinensis (L.) DC.) by using high-performance liquid chromatography coupled with diode array detector and mass spectrometry. Journal of Chromatography A 1216: 2071-2078.