Chemical Fingerprint of Ginseng Medicinal Fungal Substance by High-performance Liquid Chromatography

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Abstract. To control the quality of Ginseng medicinal fungal substance which is a new bidirectional compound fermentation of Ganoderma strains and Ginseng herbs, a simple and reliable method of high-performance liquid chromatography (HPLC) was developed for fingerprint analysis. The fingerprint of Ginseng medicinal fungal substance was established by using sample chromatography of 10 different sources and 12 common fingerprint peaks were found as well as their retention times and peak area ratioson. HPLC fingerprint can be used as the important parameters of the quality control for Compound Ginseng medicinal fungal substance. 8 peaks in the HPLC fingerprint were identified namely ginsenosides Rg1, Re, Rf, Rh1, Rb1, Ro, Rb2, Rd.

Introduction

Medicinal fungal substance is a new type bi-directional solid fermentation compounded by fungi and traditional Chinese medicine (TCM) [1-2].This kind of new technology is used in fermenting of TCM more and more [2-4]. In the preliminary research process we got a new type bi-directional solid fermentation--Ginseng medicinal fungal substance compounded by Ganoderma strains and Ginseng herbs. And the antitumor activity of these compounds against lung cancer were superior to Ganoderma strains or Ginseng herbs .

HPLC fingerprint has been widely applied to the analysis of Chinese medicinal materials[5-6].In our present study, Gradient elution HPLC was used to analyze methanol extracts from Ginseng medicinal fungal substance, and analyzed the data of their fingerprints by similarity.Under the qualitative conditions, the 12 common peaks in HPLC fingerprint of the effective part from 10 different samples can be used as indexpeaks for qualitative i-dentification.8 peaks in the HPLC fingerprint wer eidentified namely ginsenosides Rg1, Re, Rf, Rh1, Rb1, Ro, Rb2, Rd.

Experimental

Materials and reagents. Ten batches of Ginseng were collected from ten different aera in Fusong County, Jilin provinces,where is kown as “the hometown of Chinese ginseng”. Table 1 reported the sources of samples.

The Strains whose preservation number is CGMCC NO.5.169 were from China General Microbiological Culture Collection Center(CGMCC) .All the raw material samples of Ginseng medicinal fungal substance were processed in the research and development center of Changchun University of Chinese Medicine, changchun 130117,China.

Standard ginsenosides, including Rg1, Re, Rf, Rh1, Rb1, Ro, Rb2, Rd were obtained from National Instistes for Food and Drug Control, China. Chromatogram class acetonitrile and methanol were purchased from SigmaeAldrich. Analytically class methanol and orthophosphoric acid were purchased from Beijing Chemical Works.

Table 1 The source and collection time of Ginseng samples

Sample code	Colection site of samples	Collection time
Sample No.1(S1)	Beigang Town, County, Jilin province	October 4,2012
Sample No.2(S2)	Lushuihe Town, Fusong County, Jilin province	October 4,2012
Sample No.3(S3)	Songjianghe Town, Fusong County, Jilin province	October 4,2012
Sample No.4(S4)	Xianrenqiao Town, Fusong County, Jilin province	October 4,2012
Sample No.5(S5)	Changbai mountain, Jilin province	October 4,2012
Sample No.6(S6)	Quanyang Town, Fusong County, Jilin province	October 4,2012
Sample No.7(S7)	Donggang Town, Fusong County, Jilin province	October 4,2012
Sample No.8(S8)	Manjiang Town, Fusong County, Jilin province	October 4,2012
Sample No.9(S9)	Xingshen Town, Fusong County, Jilin province	October 4,2012
Sample No.10(S10)	Wanliang Town, Fusong County, Jilin province	October 4,2012

Sample preparation. All the samples were milled into powder and oven-dried at 50℃ until constantweight was reached. 2.5 g powder of each dried sample was extracted with 50ml methanol in an Ultrasonic bath for 0.5h and then cooled at room temperature.The extract was added to the original weight with methanol .The resulting supernatant liquid was filtered through a 0.45μm membrane filter prior to HPLC analysis after centrifuge and the injection volume was 20μl.

Apparatus and chromatographic conditions. Chromatographic analysis was performed on a Shimadzu LC-15C liquid chromatography system, equipped with a Dual high pressure gradient pump， a SPD-15C UV detector working in the range of 190－800 nm, a AT-330 column temperature controller and an autosampler .The chromatographic data was recorded and processed with LC-Solution15C chromatography workstation Software. Analysis was carried out at 30℃ on a Agilent Zorbax SB-C18 column (250 mm×4.6 mm, 5μm). A linear gradient elution of eluent A (acetonitrile) and B(0.4%, v/v orthophosphoric acid) was used for the separation. Table 2 reported the conditions of gradient elution

Table 2 Conditions of gradient elution

Time//min	A acetonitrile //%	B 0.4%, v/v orthophosphoric acid//%
0-35	19	81
35-55	19→29	81→71
55-70	29	71
70-110	29→40	71→60

Data analysis. The spectra were compared with a software "Similarity Evaluation System for Chromatographic Fingerprint of TCM" published by recommended by State Food and Drug Administration (Version 2004A).

Results and discussion

Establishment of fingerprint. A total of 10 batches of samples were detected under the chromatographic conditions in “2.3”.The optimized fingerprints were established. Chromatographic peaks at corresponding time sites in fingerprints were compared, as well as their retention time. It could be concluded that peaks No.1, 2, 5, 7, 8, 10, 11, and 12 were ginsenosides Rg1, Re, Rf, Rh1, Rb1, Ro, Rb2, Rd.(Fig.1and 2)

Note: 1-2, 5, 7, 8, 10, 11,and 12 were ginsenosides 1-Rg1, 2-Re, 5-Rf, 7-Rh1, 8-Rb1, 10-Ro, 11-Rb2, 12-Rd.

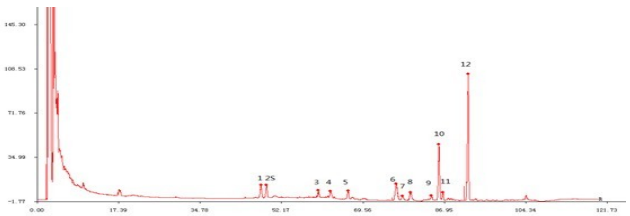

Fig.1 HPLC fingerprint of Ginseng medicinal fungal substance extracts

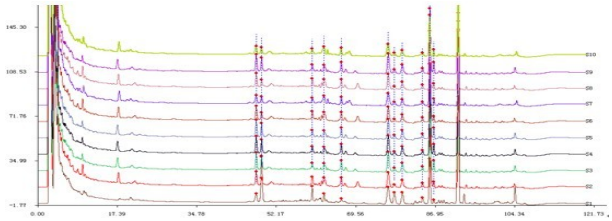

Fig.2 The common modes of HPLC fingerprints of total ginsenosides extracts from 10 batches of Ginseng medicinal fungal substance

Analyses and evaluation of fingerprints. The HPLC fingerprint profiles of Ginseng medicinal fungal substance contained

12 common peaks, which were of high resolution and were selected as the

characteristic peaks of fingerprints. The spectra were compared with a software

"Similarity Evaluation System for Chromatographic Fingerprint of TCM" published by recommended by State Food and Drug Administration (Version 2004A).Peak 2(ginsenosides Re,) which is one of the most important active constituents in Ginseng was chosen to calculate the relative retention time and relative peak area. The relative retention time characteristic peaks in 10 samples was shown in Table 3. And the relative peak area characteristic peaks in 10 samples was shown in Table 4.The similarities of chromatograms of 10 samples comparing with the reference fingerprint, which was developed with the median of all chromatograms, were shown in Table 5.

Table.3 Relative retention time of common peaks for 10 samples of Ginseng medicinal fungal substance

Peak No.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Mean value	RSD//%
1	0.9712	0.9739	0.9577	0.9685	0.9773	0.9510	0.9527	0.9779	0.9502	0.9725	0.9651	1.1
2 S	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0
3	1.2535	1.2477	1.2557	1.2564	1.2571	1.2612	1.2637	1.2566	1.2674	1.2632	1.2589	0.46
4	1.3413	1.3428	1.3387	1.3406	1.3389	1.3446	1.3442	1.3426	1.3478	1.3366	1.3412	0.28
5	1.4118	1.4132	1.4078	1.4116	1.4145	1.4143	1.4142	1.4156	1.4142	1.4161	1.4133	0.16
6	1.6124	1.6135	1.6127	1.6121	1.6133	1.6139	1.6164	1.6158	1.6107	1.6144	1.6139	0.13
7	1.6443	1.6404	1.6417	1.6455	1.6483	1.6421	1.6446	1.6421	1.6481	1.6461	1.6448	0.19
8	1.6849	1.6859	1.6820	1.6857	1.6808	1.6806	1.6847	1.6855	1.6869	1.6833	1.6840	0.13
9	1.7955	1.7975	1.7923	1.7927	1.7989	1.7936	1.7962	1.7924	1.7990	1.7983	1.7957	0.15
10	1.8343	1.8338	1.8310	1.8326	1.8347	1.8397	1.8399	1.8366	1.8380	1.8331	1.8355	0.16
11	1.8594	1.8592	1.8518	1.8613	1.8679	1.8570	1.8583	1.8499	1.8532	1.8512	1.8566	0.29
12	1.9366	1.9404	1.9359	1.9426	1.9443	1.9354	1.9447	1.9575	1.9400	1.9451	1.9420	0.32

Table.4 Relative peak area of common peaks for 10samples of Ginseng medicinal fungal substance

Peak No.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Mean value	RSD//%
1	0.2232	1.7592	1.3626	0.7756	0.8611	2.1113	1.2760	1.1053	1.0685	1.0964	1.1639	0.4481
2 S	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0
3	0.2088	1.0749	0.5570	1.0348	0.6855	0.5989	0.5056	0.9072	0.6819	0.3894	0.6644	0.4166
4	0.1295	1.6683	0.6482	0.6354	0.3755	0.9891	1.2341	1.2249	1.1103	0.4980	0.8513	0.5537
5	0.0926	1.311	0.9943	0.6409	0.5202	1.3217	0.9477	1.0413	0.6369	0.8419	0.8349	0.4502
6	0.5694	2.8538	2.3108	1.3009	1.8361	2.3476	2.7779	2.2979	1.6734	1.7731	1.9741	0.3523
7	0.3424	0.5538	0.4754	0.5350	0.7553	0.4983	0.8471	0.5818	0.6069	0.3940	0.5590	0.2731
8	1.3256	1.3744	0.9971	0.7776	0.8807	1.5497	1.3115	1.3842	0.8724	0.8889	1.1362	0.2454
9	0.1492	1.0382	0.5381	0.3277	0.3286	0.3649	0.7424	0.4662	0.4299	0.3892	0.4774	0.5241
10	0.8212	6.7175	4.4638	2.0989	2.8158	2.6017	4.7382	2.9648	3.2847	2.5853	3.3092	0.4938
11	0.0887	0.7585	0.7309	0.2557	0.4331	0.4695	0.8428	0.8017	0.4538	0.8155	0.5650	0.4654
12	4.7909	8.8651	9.1721	5.3111	6.8969	10.686	8.1402	9.9969	6.2161	8.0046	7.8080	0.2522

Table.5 The similarities of chromatograms of 10 samples

Sample No.	similarities
S1	1.000
S2	0.999
S3	0.996
S4	1.000
S5	0.999
S6	0.999
S7	0.999
S8	0.999
S9	1.000
S10	0.988

Conclusions

12 common featured peaks were found on HPLC fingerprint, their retention time and area ratio can be used as important parameters for the quality control of Ginseng medicinal fungal substance.

It would show a important effect in impersonally and effectively controlling the quality of other related traditional Chinese medicinal fermentation preparations else.

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