Research Article

ISSN: 2435-1210 | Volume 9

High-Throughput Uplc-G2si-Hdms Based Discovery of the Effective Constituents of Yindan Pinggan Capsule Against Yang Huang Syndrome

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Received: 26 Oct 2022
Accepted: 05 Nov 2022
Published: 11 Nov 2022
J Short Name: JJGH

Citation:

Sun H and Wang X. High-Throughput Uplc-G2si-Hdms Based Discovery of the Effective Constituents of Yindan Pinggan Capsule Against Yang Huang Syndrome. J Gstro Hepato. V9(11): 1-10

Keywords:

Yindan Pinggan Capsule; Yang Huang syndrome; Effective constituents; Metabolomics

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1. Abstract

1.1. Background: Yindan Pinggan Capsule, a patent Chinese medicine, has a definite effect on Yang Huang syndrome. At present, the pharmacological activity and quality control of Yindan Pinggan Capsule have been studied, but its effective constituents on the treatment of Yang Huang syndrome are still unclear.

1.2. Purpose: To discriminately analyze the chemical compositions of Yindan Pinggan Capsule and characterized the ingredients absorbed in blood to confirm the constituents which related with the efficacy of Yindan Pinggan Capsule against Yang Huang syndrome.

1.3. Methods: The chemical compositions and ingredients absorbed in blood have been analyzed by UPLC-G2Si-HDMS and characterized by UNIFI software combined with the pattern recognition method. The ingredients absorbed in blood highly associated with biomarkers of Yang Huang syndrome have been screened as potential effective constituents through correlation analysis.

1.4. Results: 104 chemical compositions and 48 ingredients absorbed in blood of Yindan Pinggan Capsule have been characterized. Finally, 19 components are highly correlated with biomarkers of Yang Huang syndrome, including gallic acid, gentioflavine, swertiamarin, 4'-hydroxyacetophenone, gentiopicringlucuronide metabolites, genipin, gentiopicrin, albiflorin, chrysinglucuronide metabolites, z-ligustilide, baicalein 6-glucoside, geniposide, scoparone, baicalin, baicalein, liquiritigenin, glycyrrhizic acid, capillarisin, and chenodeoxycholic acid.

1.5. Conclusion: Our work clarified the potential effective constituents of Yindan Pinggan Capsule against Yang Huang syndrome and provided a basis for further quality control improvement and innovative drug development.

2. Introduction

Yindan Pinggan Capsule(YDPGC) is the patent Chinese medicine developed by Mr. Huang Guiyuan's experience prescription, which is recorded in the 2020 edition of China Pharmacopoeia and composed of artemisiae scopariae herba, gardeniae fructus, gentianae radix et rhizoma, scutellariae radix, suis fellis pulvis, angelicae sinensis radix, paeoniae radix alba, and glycyrrhizae radix et rhizoma. YDPGC has been used to treat hepatitis B, chronic cholecystitis with the hepatobiliary damp-heat syndrome, alcoholic liver disease, and other diseases with significantly effects [1-4]. Pharmacological studies have shown that YDPGC has a protective effect on acute alcoholic liver

inflammatory injury and oxidative stress [5]. The contents of chlorogenic acid, geniposide, gentiopicroside, ferulic acid, baicalin, and glycyrrhizic acid in YDPGC have been determined simultaneously by HPLC, which provided a reference for quality standard improvement [6]. However, the potential effective constituents of YDPGC are still not clear.

Yang Huang syndrome(YHS) is the common clinical syndrome of traditional Chinese medicine, with eye yellow, body yellow, and urine yellow as its main clinical manifestations, accompanied by different degrees of liver injury and features of cholestasis pathological. In the past, the animal models used to study the drugs for the treatment of Yang jaundice usually focus on the pathological manifestations while ignoring the nature and pathogenesis of the syndrome. Therefore, under the guidance of the theory of traditional Chinese medicine, the animal model of damp-heat jaundice has been established based on the pathological characteristics of cholestasis produced by ANIT and created a damp-heat background by administration of the solution of Rhizoma Zingiberis extracts and ethanol [7, 8]. This model has been successfully applied to the research of Yinchenhao Tang [9, 10], Zhibai Dihuang pill [11], and Jigucao capsule [12].

For the current study, the mice model of YHS has been taken as research objects to analyze the chemical compositions and ingredients absorbed in blood of YDPGC by the high-throughput UPLC-MS technique. The correlation analysis between biomarkers and ingredients absorbed in blood has been used to screen the effective constituents of YDPGC against YHS.

3. Materials and Methods

3.1. Regents

YDPGC is purchased from Zhangzhou Pien Tze Huang Pharmaceutical Co.,Ltd(Zhangzhou, China) and Rhizoma Zingiberis is purchased from Beijing Tong Ren Tang(Harbin, China). Acetonitrile of UPLC grade is obtained from Fisher Scientific Corporation (Loughborough, UK) and methanol of UPLC grade is obtained from Dikma(Beijing, China). Phosphoric acid is bought from Aladdin(Shanghai, China) and formic acid is bought from Kermel Chemical Reagent Company (Tianjin, China). α-Naphthylisothiocyanate (ANIT) is provided by Sigma-Aldrich(MO, USA) and alcohol is supplied by Beijing Reagent Company (Beijing, China). Deionized water is bought from Watson's Food & Beverage Co., Ltd (Guangzhou, China). Olive oil is obtained from Zhongliang Food Marketing Co., Ltd (China).

3.2. Animal and Treatment

Male Balb/c mice of clean grade with body mass between 18g and 22g are provided by Shanghai Slac Laboratory Animal Co,.Ltd (Shanghai, China) and raised in the GLP center of Heilongjiang University of Chinese Medicine at a temperature of 24±1℃, relative humidity of 60±5%, and a 12 h light/dark cycle environment. They have been randomly divided into five groups with 12 mice in each group, including control group(C), model group(M), high-dosed YDPGC group(YDPGC-H), middle-dosed YDPGC group(YDPGC-M), and low-dosed YDPGC group(YDPGC-L). Except for control group, mice in other groups have been administered with 0.13g·mL–1 Rhizoma Zingiberis solution in the morning and 12.5% (v/v) alcohol in the afternoon at a dose of 0.1mL·10g–1 for 14 consecutive days. On the 15th and 16th days, mice are administered 15mg·kg–1 and 10mg·kg–1 ANIT olive oil solution respectively. From the 17th day to the 30th day, mice of YDPGC-H, YDPGC-M, and YDPGC-L are administered with 1.56g·kg–1, 0.78g·kg–1, and 0.39g·kg–1 YDPGC solution, while mice in control group and model group are given the same dose of water. The investigation procedure is supported by the Animal Care and Ethics Committee of Heilongjiang University of Chinese Medicine and follows the Declaration of Helsinki.

3.3. Biological Sample Collection and Preparation

The blood has been collected by enucleating eyeballs after the last administration for 15 minutes to lay up about 30 minutes and the upper serum has been retained after centrifuged at 4℃ with 4000rpm for 10 minutes. The same volume of 4% phosphoric acid solution has been added into 200 μL serum, and after mixing, it passed slowly through the Waters OASIS solid phase extraction column activated by 1ml methanol and 1ml water. After washing the column with 1ml water, the column is eluted with 1ml methanol and collected the eluent. The eluent has been dried by nitrogen flow and resolute with 100 μL methanol. After suspension and centrifugation, the supernatant is filtered through a 0.22 μm filter membrane for UPLC-HDMS analysis.

3.4. Preparation 0f YDPGC for Chemical Compounds Analysis

200mg powder of YDPGC has been accurately weighed before ultrasonic extraction for 30 minutes and diluted to 50mL with 70% methanol. After mixing and cooling, 70% methanol is used to supplement the lost weight to make a solution with a concentration of 4mg·mL–1. The supernatant is obtained by centrifugation 4℃ with 13000rpm for 10 minutes and filtered through 0.22 μm filter membrane for UPLC-HDMS analysis.

3.5. Analytic Conditions

UPLC-G2Si-HDMS system(Waters Corp.) and ACQUITY UPLC HSS C18 column (100mm×2.1mm i.d., 1.8μm, Waters Corp.) are used to perform chromatographic separation and mass spectrum information collection with A mobile phase of 0.1% formic acid in acetonitrile and B mobile phase of 0.1% formic acid in water at 45℃ column temperature and the injection volume is 4 μL. The gradient elution system with flow rate of 0.4 mL·min–1 is as followed: 0-0.5min, 1%A; 0.5-3min, 1%-26%A; 3-7min, 26%-50%A; 7-9min, 50%-75%A; 9-13min, 75%-100%A.

The mass spectrometry data are collected in MSE mode in full scanning mode with a low collision energy of 6V, high collision energy of 20V~60V under positive ion mode, and 10V~30V under negative ion mode. The mass scanning range is from 50 to 1200 and the parameters are set as follows: the capillary voltage is 3000V under positive ion mode and 2500V under negative ion mode, the sampling

cone voltage is 30 V, the cone extraction voltage is 5V, the source temperature is 110℃, desolvation temperature is 350 °C, and desolvation gas flow is 800 L·h–1.

3.6. Data Processing

Using UNIFI software combined with a traditional Chinese medicine database, the chemical components of YDPGC have been characterized by matching the information of parent ion and fragments ion. Using multivariable data analysis and 3D data processing method in UNIFI software, the analysis of secondary fragments and structure confirmation of ingredients and their metabolites are intelligently carried out through the mass defect filter (MFD) combined with the Metabolynx module. The Pearson correlation coefficients between the relative peak area of ingredients absorbed in blood and biomarkers of YHS are calculated by PCMS software. Finally, the screening condition for effective constituents is correlation coefficients not lower than 0.6 and is extremely correlated with 6 biomarkers in at least two dose groups.

4. Results

4.1. Chemical Compositions of YDPGC

Using UPLC-MS combined with a traditional Chinese Medicine database in UNIFI software, the analysis method of chemical compounds in YDPGC has been established (Figure 1). 104 components have been characterized within 13 minutes by matching the information of parent ion and fragment ion, and then trace back to their original resource. Among them, 13 compounds originate from artemisiae scopariae herba, 10 compounds originate from gardeniae fructus, 6 compounds originate from gentianae radix et rhizoma, 15 compounds originate from scutellariae radix, 3 compounds originate from suis fellis pulvis, 17 compounds originate from angelicae sinensis radix, 24 compounds originate from paeoniae radix alba, and 18 compounds originate from glycyrrhizae radix et rhizoma (Table 1).

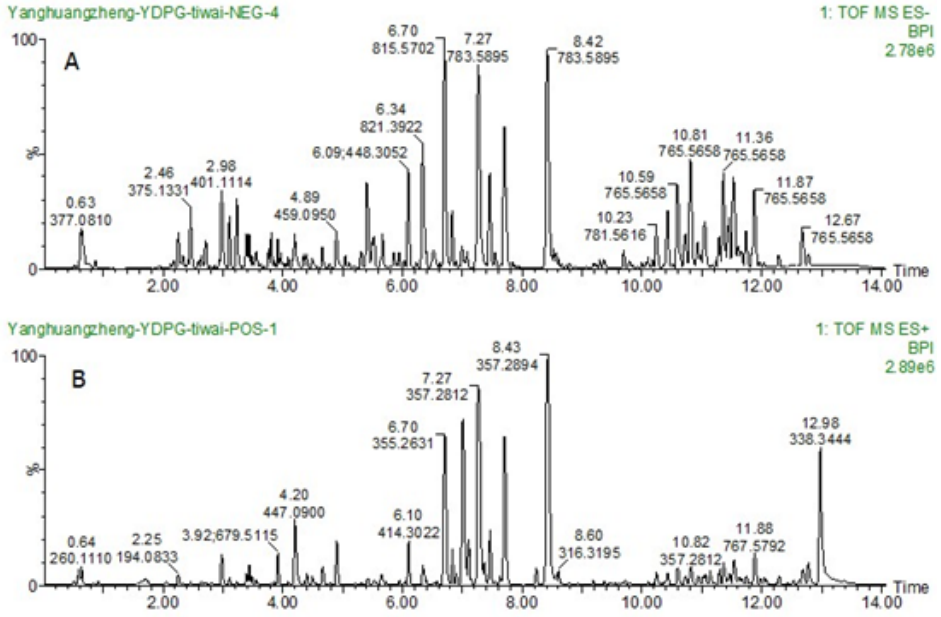

Figure 1: UPLC-HDMS BPI chromatogram of chemical compounds in YDPGC.
A. Chromatogram in positive ion mode; B. Chromatogram in negative ion mode.

Table 1: Characterization of chemical compounds in YDPGC extract by UPLC-Q-TOF/MS

			Positive (m/z)		Negativeion (m/z)					
No.	**Rt(min)**	**Compound Name**	**Indicated**	**ppm**	**Indicated**	**ppm**	**Formular**	**MW(Da)**	**Fragment**	**Origin**
1	0.57	Arcapillin	361.0983	7.9	—	—	C18H16O8	360.0845	361,346,221	a
2	0.59	BrefeldinA	303.1602, [M+Na]+	1.6	—	—	C16H24O4	280.1675	303,285,247	f
3	0.61	Phenol	95.0483	-8.9	—	—	C6H6O	94.0418	95,53,51	g
4	0.63	Nicotinic acid	124.0389	-3.1	—	—	C6H5NO2	123.0316	124,80,51	f
5	0.64	Succinic Acid	119.0347	7.1	117	2.5	C4H6O4	118.0266	119,101,73	f
6	0.64	Adenine	136.0617	-0.6			C5H5N5	135.0545	136,119,109	f
7	0.65	Salicylic acid	139.0373	-2.2	137	4.2	C7H6O3	138.0317	139,77	a
8	0.67	Uracil	113.034	-4.6	—	—	C4H4N2O2	112.0273	113,96,87	f
9	2.11	Gallic acid	171.0286	-1.2	169	4	C7H6O5	170.0215	125,107	g

10	2.26	Gentianine	176.07	-3.3	—	—	C10H9NO2	175.0633	176,146	c
11	2.36	Gentioflavine	194.0809	-1.2	—	—	C10H11NO3	193.0738	194,166	c
12	2.46	Loganate	—	—	375.1	2.5	C16H24O10	376.1369	375,211,169,116, 151,133,89	c
13	2.46	Paeonilactone B	197.0803	-2.5	—	—	C10H12O4	196.0736	197,180,169	g
14	2.47	Isoeugenol	187.0772, [M+Na]+	2.9	163.1	7.8	C10H12O2	164.0837	163,147,137	f
15	2.49	Furfural	97.0281	-3.6	—	—	C5H4O2	96.02113	97,79,67	g
16	2.64	Chlorogenic Acid	355.1021	-0.8	353.1	0.8	C16H18O9	354.095	353,191,179,135	a b f
17	2.69	Benzoic acid	123.0438	-2.1	121	3.5	C7H6O2	122.0367	123,105,79	g
18	2.8	Swertiamarin	375.1103	-1.2	—	—	C16H22O10	374.1213	375,213,195,151	c
19	2.81	Gardenoside	—	—	403.1	0.7	C17H24O11	404.1319	403,345,241,165	b
20	2.85	4'-Hydroxyacetophenone	137.0593	-3	—	—	C8H8O2	136.0524	353,170,135	a
21	2.9	Paeoniflorin	481.1731	5.6	—	—	C23H28O11	480.1632	451,359,329,167	g
22	2.96	Umbelliferone	163.0384	-3.8	161	-6.4	C9H6O3	162.0317	163,145,119	h
23	2.97	Genipin	227.0907	-3.3	225.1	-3.1	C11H14O5	226.0841	227,209,195,177	b
24	2.98	Gentiopicrin	357.1179	-0.3	—	—	C16H20O9	356.1107	357,195,177,151	c
25	2.99	Ferulic Acid	195.0649	-1.6	193.1	3	C10H10O4	194.0579	195,177,133,89	h
26	3.01	Phenylacetic acid	159.0437, [M+Na]+	2.8	—	—	C8H8O2	136.0524	159,141,113	d
27	3.04	Guaiacol	125.0437	3.9	123	5	C7H8O2	124.0524	125,107	f
28	3.05	Ethyl salicylate	167.0694	-5.4	165.1	2.7	C9H10O3	166.0629	167,139,121	g
29	3.07	Benzaldehyde	107.0492	0.2	—	—	C7H6O	106.0419	107,77	g
30	3.09	Herniarin	177.0546	0	175	1.7	C10H8O3	176.0473	177,147,133	h
31	3.11	p-Anisic acid	175.0382, [M+Na]+	9.6	151	1.7	C8H8O3	152.0473	153,109,123	f
32	3.12	Albiflorin	481.1703	-0.4	479.2	0.9	C23H28O11	480.1632	319,301,197,105	g
33	3.13	Paeonilactone C	319.1175	-0.3	—	—	C17H18O6	318.1103	319,197,179	g
34	3.19	Sweroside	359.141	2.4	—	—	C16H22O9	358.1264	359,197,179,151	c
35	3.22	Viscidulin I	303.0493	-2	—	—	C15H10O7	302.0427	303,285,181	d
36	3.23	Salidroside	323.1113, [M+Na]+	3.8	—	—	C14H20O7	300.1209	323,305,179	d
37	3.25	Z-ligustilide	191.1059	-4	—	—	C12H14O2	190.0994	191,173,155,105	f
38	3.36	Baicalein 6-glucoside	433.1124	-1.3	—	—	C21H20O10	432.1057	433,270	d
39	3.41	3,5,7,2',6'-Pentahydroxyflavone 2'-glucoside	465.1086	2.6	—	—	C21H20O12	464.0955	465,303	d
40	3.45	5-Hydroxymethyl-2-furaldehyde	127.0385	-3.8	125	5.9	C6H6O3	126.0317	127,109,97	g
41	3.47	Geniposide	389.1368	-9	387.1	4.2	C17H24O10	388.1369	387,225,207	b
42	3.5	Paeonol	167.0697	-3.2	—	—	C9H10O3	166.0629	167,135,105	g
43	3.55	5,2',6'-Trihydroxy-6,7-dimethoxyflavone 2'-glucoside	493.133	-2.2	—	—	C23H24O12	492.1268	493,475,331	d
44	3.56	Liquiritin	441.1164, [M+Na]+	1.9	417.1	3.2	C21H22O9	418.1264	417,255,135	h
45	3.82	4-O-beta-D-Glucosyl-4-hydroxycinnamate	327.1124	5.2	—	—	C15H18O8	326.1002	327,309,165	d
46	3.86	Scoparone	207.065	-1	—	—	C11H10O4	206.0579	207,176	a
47	3.87	Vanillin	175.0386, [M+Na]+	1.5	—	—	C8H8O3	152.0474	175,145	f
48	3.91	Safflor yellow A	595.1677	2.4	—	—	C27H30O15		593,473	a
49	3.92	Paeonilactone A	221.0796, [M+Na]+	5.2	—	—	C10H14O4	198.0892	221,191	g
50	3.95	Nonanedioic acid	—	—	187.1	5.2	C9H16O4	188.1048	187,170	a
51	4.09	Isoliquiritin	—	—	417.1	2.7	C21H22O9	418.1264	417,255,135	h
52	4.19	Kaempferol	287.0551	-1.7	—	—	C15H10O6		287,245,195,153	a

53	4.2	Viscidulin III	347.076	-0.5	—	—	C17H14O8	346.0689	347,329	d
54	4.21	Baicalin	447.0923	0.3	445.1	1.3	C21H18O11	446.0849	447,271,253	d
55	4.31	Neoliquiritin	441.124, [M+Na]+	19	417.1	2.3	C21H22O9	418.1264	417,399,255	h
56	4.52	Baicalein	271.06	-0.3	269	3.4	C15H10O5	270.0528	271,253,241	d
57	4.58	Bergapten	217.0472	-1.9	215	-6.5	C12H8O4	216.0422	217,199,187	f
58	4.62	Licoflavone A	323.1242	-1.2	—	—	C10H18O4	322.1205	323,305,145	h
59	4.7	Chrysin	255.0648	-1.6	253.1	1.7	C15H10O4	254.0579	253,237,153	d
60	4.78	D-catechin	313.069, [M+Na]+	2.4	289.1	-6.3	C15H14O6	290.079	289,271,191,151	g
61	4.8	Skullcapflavone II	375.1076	0.3	—	—	C19H18O8	374.1001	375,360,345,327	d
62	4.9	Oroxylin A	285.0758	0.1	283.1	3.2	C16H12O5	284.0685	285,270	d
63	4.95	Licoricone	383.1508	4.9	381.1	-2.5	C22H22O6	382.1416	383,365,339	h
64	5.33	(all-E)-Crocetin	329.1743	-1.4	327.2	-0.5	C20H24O4	328.1675	329,284	b
65	5.69	Liquiritigenin	257.0804	-1.7	255.1	4.2	C15H12O4	256.0736	255,153,135,119	h
66	5.74	Isoliquiritigenin	257.0808	-0.1	255.1	2.7	C15H12O4	256.0736	255,153,135,119	h
67	5.94	Formononetin	269.0802	-2.3	267.1	5.5	C16H12O4	268.0736	269,251	h
68	5.95	Glabrolide	469.3302	-2.2	—	—	C30H44O4	468.3239	469,451,433	h
69	6.17	Glycyrrhizic acid	823.4087	-2.9	821.4	-0.2	C42H62O16	822.4038	821,645,351,193	h
70	6.28	Licochalcone A	361.1436, [M+Na]+	-2.7	337.2	7.1	C21H22O4	338.1518	337,305,297	h
71	6.35	Enoxolone	471.3464	-1	—	—	C30H46O4	470.3396	427,281,257,121	h
72	6.36	Capillartemisin B	317.1823	3.9	—	—	C19H24O4	316.1675	317,163	a
73	6.93	Campesterol	423.3614, [M+Na]+	4	—	—	C28H48O	400.3705	423,405,319	d
74	7	Capillarisin	317.0676	8.5	—	—	C16H12O7	316.0583	206,300,315	a
75	7.04	Licochalcone B	287.0894	-6.9	—	—	C16H14O5	286.0841	287,269,121	h
76	7.06	(2S)5,7,2',6'-Tetrahydroxyflavanone	311.0565, [M+Na]+	2.6	—	—	C15H12O6	288.0634	311,293,167	d
77	7.09	Hyodesoxycholic Acid	393.2995	-1.1	—	—	C24H40O4	392.2927	393,375,357	e
78	7.11	4-Ethylphenol	123.0805	0.2	121.1	-1.7	C8H10O	122.0732	123,107,105	f
79	7.13	n-Nonaldehyde	165.1263, [M+Na]+	8	—	—	C9H18O	142.1358	165,147	g
80	7.17	Perillyl alcohol	175.1112, [M+Na]+	1.6	—	—	C10H16O	152.1201	175,157,131	g
81	7.27	Benzyl alcohol	109.0651	2.7	—	—	C7H8O	108.0575	109,91	g
82	7.32	Oleanolic acid	479.3487, [M+Na]+	-1.8	—	—	C30H48O3	456.3604	479,461	g
83	7.36	Cirsimaritin	315.0863	-0.2	—	—	C17H14O6	314.079	315,300,282,254	a
84	7.43	Isoglabrolide	469.3311	-0.4	—	—	C30H44O4	468.3239	469,451,433	h
85	7.46	Furfuryl alcohol	99.0436	-4.4	—	—	C5H6O2	98.0367	99,83	g
86	7.47	Sebacic Acid	225.1097, [M+Na]+	-0.3	201.1	2.1	C10H18O4	202.1205	201,183,157,	f
87	7.71	Pectolinarigenin	315.0858	-1.6	—	—	C7H14O6	314.079	315,285,284	a
88	8.31	Nerolidol	245.1866, [M+Na]+	-3.9	—	—	C15H26O	222.1983	245,227	b
89	8.34	Allo-ocimene	159.1166, [M+Na]+	3.6	—	—	C10H16	136.1252	159,105,81	f
90	8.37	Neocnidilide	195.1375	-2.5	—	—	C12H18O2	194.1306	195,135,105	f
91	8.42	Thymol	151.1113	-3	—	—	C10H14O	150.1044	151,133,109	g
92	8.45	BHT	243.1712, [M+Na]+	-2.9	—	—	C15H24O	220.1827	243,225	g
93	9.71	Linoleic acid	303.2313, [M+Na]+	6	—	—	C18H32O2	280.2402	303,258	b
94	9.73	Neoligustilide	191.1063	-1.9	—	—	C12H14O2	190.0993	191,173,163,145	f

95	9.76	Hederagenin	—	—	471.3	-5.5	C30H48O4	472.3552	471,441,426	g
96	10.57	Rhamnocitrin	301.0705	-2.3	—	—	C16H12O6	300.0634	301,286,258	a
97	10.58	Betulinic acid	457.3654	-4.8	—	—	C30H48O3	456.3603	457,439,391	g
98	10.59	1-O-Galloyl-beta-D-glucose	355.0692, [M+Na]+	5.8	—	—	C13H16O10	332.0744	355,207,153,146	g
99	10.61	4-O-Methyl-gallate	207.0316, [M+Na]+	5.2	—	—	C8H8O5	184.0372	207,126	g
100	11.84	Chenodeoxycholic acid	393.3093	3.7	391.3	4.3	C24H40O4	392.2927	393,375,357	e
101	12.06	Desoxoglabrolide	477.3267, [M+Na]+	-15	—	—	C30H46O3	454.3447	477,449,403	h
102	12.56	Bilirubin	585.2706	-0.4	—	—	C33H36N4O6	584.2639	585,567,539	e
103	12.79	Stearic acid	307.2618, [M+Na]+	3.4	283.3	3.3	C18H36O2	284.2715	307,289,262,	b
104	12.98	2-Ethyl-2-hexena	127.1117	-0.2	—	—	C8H14O	126.1045	127,81	b

NOTE: a: artemisiae scopariae herba ; b: gardeniae fructus ; c: gentianae radix et rhizoma ; d: scutellariae radix ; e: suis fellis pulvis ; f: angelicae sinensis radix ; g: paeoniae radix alba ; h: glycyrrhizae radix et rhizoma.

4.2. Ingredients Absorbed in Blood of YDPGC

The differential exogenous components in the blood between YHS mice and YHS mice treated with YDPGC have been found by multivariate data analysis. The secondary fragments of metabolites were intelligently analyzed by mass defect filter(MFD) combined with the Metabolynx module, and the possible mode of biological metabolic transformation is given (Figure 2). Based on the chemical constituents of YDPGC in vitro, it is confirmed that 48 ingredients are absorbed into the blood after YHS mice oral administration of YDPGC, of which 36 ingredients are the prototype ingredients of YDPGC, and the other 12 are drug metabolites (Table 2).

4.3. Biomarkers of YDPGC Against YHS

The animal model of YHS has been successfully replicated and evaluated by biochemical indexes and histopathological results in preliminary studies [13, 14]. The effectiveness of YDPGC against YHS has been evaluated by the method of metabolomics and the biomarkers have been analyzed. The results showed that YDPGC has a curative effect on the animal model of YHS. High-dosed YDPGC could regulate 17 YHS biomarkers, middle-dosed YDPGC could regulate 12 YHS biomarkers and low-dosed YDPGC could regulate 10 YHS biomarkers (Table 3).

4.4. Confirmation of Effective Constituents in YDPGC by Correlation Analysis

Through the analysis of PCMS software, the ingredients absorbed in blood of YDPGC have been associated with the biomarkers of YHS (Figure 3). The result shows that 19 ingredients are extremely related to biomarkers to be potentially effective constituents of YDPGC against YHS, including gallic acid, gentiopicrin, swertiamarin, 4'-hydroxyacetophenone, gentiopicrin+C6H8O6, genipin, gentiopicrin, albiflorin, chrysin+C6H8O6, Z-ligustilide, baicalein 6-glucoside, geniposide, scoparone, baicalin, baicalein, liquiritigenin, glycyrrhizic acid, capillarisin, Chenodeoxycholic acid.

Table 2: The ingredients of serum after oral administration of YDPGC

No	Rt/min	Compounds	m/z	ppm	Adducts	Formula	MW(Da)	Fragment	Origin
1	0.7	Salicylic acid	139.039	0.7	[M+H]+	C7H6O3	138.0317	139,77	a
2	2.12	Gallic acid	169.013	-4.8	[M-H]-	C7H6O5	170.0215	125,107	g
3*	2.12	Ferulic Acid+H2O	213.06	1.12	[M+H]+	C10H12O5	212.0579	213,169,151,125,107	h
4	2.26	Gentioflavine	194.081	-3.2	[M+H]+	C10H11NO3	193.0738	194,166	c
5*	2.45	Paeonol+O	181.05	1.5	[M-H]-	C9H10O4	182.0629	181,163,135	g
6	2.46	loganate	375.129	-2.4	[M-H]-	C16H24O10	376.1369	375,211,169,116,151,133	c
7	2.46	Paeonilactone B	197.081	-0.4	[M+H]+	C10H12O4	196.0736	197,180,169	g
8	2.48	Isoeugenol	163.078	7.8	[M-H]-	C10H12O2	164.0837	163,147,137	f
9	2.69	Benzoic acid	121.028	-8.9	[M-H]-	C7H6O2	122.0367	123,105,79	g
10*	2.76	Chlorogenic Acid+H2O	371.097	1.2	[M-H]-	C16H20O10	372.0958	371,255,162	a b f
11	2.8	Swertiamarin	375.191	-3.5	[M+H]+	C16H22O10	374.1213	375,213,195,151,179	c
12	2.85	4'-Hydroxyacetophenone	137.059	-3.1	[M+H]+	C8H8O2	136.0524	135,170,353	a
13	2.97	Genipin	227.091	-3.5	[M+H]+	C11H14O5	226.0841	227,209,195,177,149	b
14	2.98	Gentiopicrin	357.118	0.1	[M+H]+	C16H20O9	356.1107	357,195,177,151,121	c
15*	2.98	Genipin+C6H8O6	401.109	2.4	[M-H]-	C17H22O11	402.0941	401,225,207	b
16	2.99	Ferulic Acid	195.065	-0.7	[M+H]+	C10H10O4	194.0579	195,177,133	h

17*	2.99	Geniposide+C6H8O6	565.162	2.6	[M+H]+	C23H32O16	564.1369	565,389,227,125	b
18*	3.02	Gentiopicrin+C6H8O6	531.135	1.6	[M-H]-	C22H28O15	532.1107	531,355,193,	c
19	3.05	Ethyl salicylate	167.07	-2.7	[M+H]+	C9H10O3	166.0629	167,139,121	g
20	3.07	Benzaldehyde	107.049	1	[M+H]+	C7H6O	106.0419	107,77	g
21	3.09	Herniarin	177.055	0.1	[M+H]+	C10H8O3	176.0473	177,147,133	h
22	3.11	p-Anisic acid	175.039	9.7	[M+Na]+	C8H8O3	152.0473	153,109,123	f
23*	3.11	Chrysin+C6H8O6	429.082	0.7	[M-H]-	C21H18O10	430.0579	429,253,153	d
24	3.14	Albiflorin	479.155	-1.3	[M-H]-	C23H28O11	480.1632	319,301,197	g
25*	3.15	Geniposide-O	371.133	1.2	[M-H]-	C17H24O9	372.1369	371,314,255	b
26	3.25	Z-ligustilide	191.106	-1.8	[M+H]+	C12H14O2	190.0994	191,173,163,155,149,117	f
27	3.37	Baicalein 6-glucoside	433.115	4.8	[M+H]+	C21H20O10	432.1057	433,270	d
28	3.42	3,5,7,2',6'-Pentahydroxyflavone 2'-glucoside	465.094	1.6	[M+H]+	C21H20O12	464.0955	465,303	d
29	3.46	5-Hydroxymethyl-2-furaldehyde	127.039	-2.5	[M+H]+	C6H6O3	126.0317	127,109,97	g
30	3.47	geniposide	387.129	-1.2	[M-H]-	C17H24O10	388.1369	207,225,387	b
31	3.55	Liquiritin	417.118	-3.9	[M-H]-	C21H22O9	418.1264	417,255,135	h
32	3.86	Scoparone	207.065	-1	[M+H]+	C11H10O4	206.0579	207,176	a
33	4.21	Baicalin	445.078	-0.1	[M-H]-	C21H18O11	446.0849	447,271,253	d
34	4.52	Baicalein	271.06	-0.1	[M+H]+	C15H10O5	270.0528	271,253,241	d
35	4.92	Oroxylin A	285.075	-1.3	[M+H]+	C16H12O5	284.0685	285,270	d
36*	5.23	skullcapflavone II+C6H8O6	549.123	2.2	[M-H]-	C25H26O14	550.1001	549,373,327	d
37	5.67	Liquiritigenin	255.065	-4	[M-H]-	C15H12O4	256.0736	255,153,135	h
38*	5.74	Hyodesoxycholic Acid+C6H8O6	567.317	1.6	[M-H]-	C30H48O10	568.2927	567,391,357	e
39	6.17	Glycyrrhizic acid	821.396	-0.2	[M-H]-	C42H62O16	822.4038	821,645,351	h
40	6.28	Licochalcone A	337.15	7.1	[M-H]-	C21H22O4	338.1518	337,305,297	h
41*	6.36	Chenodeoxycholic acid+O	407.279	1.1	[M-H]-	C24H40O5	408.2927	407,391,357	e
42	7	Capillarisin	317.067	8.5	[M+H]+	C16H12O7	316.05811	206,300,315	a
43	8.31	Nerolidol	245.189	5.7	[M+Na]+	C15H26O	222.1983	245,227	b
44	9.07	Allo-ocimene	159.117	2.6	[M-H]-	C10H16	136.1252	159,105,81	f
45*	9.46	Linoleic acid+H2O	297.243	2.3	[M-H]-	C18H34O3	298.2402	297,279	b
46	9.5	Linoleic acid	303.231	6.4	[M+Na]+	C18H32O2	280.2402	303,258	b
47	11.83	Chenodeoxycholic acid	393.298	-6	[M+H]+	C24H40O4	392.2927	393,375,357	e
48	12.81	Stearic acid	283.263	-5.1	[M-H]-	C18H36O2	284.2715	283,265,238	b

NOTE: a: artemisiae scopariae herba; b: gardeniae fructus; c: gentianae radix et rhizoma; d: scutellariae radix; e: suis fellis pulvis; f: angelicae sinensis radix; g: paeoniae radix alba; h: glycyrrhizae radix et rhizoma. "*" means the metabolic ingredients of YDPGC.

Table 3: Trend of biomarkers of YHS by different dose of YDPGC

NO	Rt min	M/Z	Mode	Formula	Name	Trend	YDPGC-H	YDPGC -M	YDPGC -L
1	1.92	209.0929	ESI+	C10H12N2O3	Formyl-5-hydroxykynurenamine	↑	√	√	√
2	0.65	130.0501	ESI+	C5H7NO3	Pyroglutamic acid	↓	√	√	√
3	2.65	185.0818	ESI+	C9H12O4	Vanylglycol	↑	-	-	-
4	5.64	224.0561	ESI+	C10H9NO5	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	↑	√	√	√
5	7.44	546.3539	ESI+	C28H52NO7P	LysoPC(20:3(5Z,8Z,11Z))	↓	-	-	-
6	8.18	556.53	ESI+	C34H69NO4	Cer(t18:0/16:0)	↑	√	√	√
7	8.62	772.5931	ESI+	C43H82NO8P	PC(15:0/20:2(11Z,14Z))	↓	√	-	-
8	0.64	118.0864	ESI+	C5H11NO2	Betaine	↑	-	-	-
9	3.34	542.3236	ESI+	C28H48NO7P	LysoPC(20:5(5Z,8Z,11Z,14Z,17Z))	↓	-	√	√
10	3.49	544.3388	ESI+	C28H50NO7P	LysoPC(20:4(5Z,8Z,11Z,14Z))	↓	√	-	-
11	4.11	317.2111	ESI+	C20H28O3	15-Deoxy-d-12,14-PGJ2	↑	√	√	√

12	4.38	315.1957	ESI+	C20H26O3	4-oxo-Retinoic acid	↑	√	-	-
13	2.53	363.2158	ESI+	C21H30O5	Cortisol	↓	√	√	√
14	3.11	518.3235	ESI+	C26H48NO7P	LysoPC(18:3(6Z,9Z,12Z))	↓	√	-	-
15	5.27	329.2481	ESI+	C22H32O2	Docosahexaenoic acid	↓	√	-	-
16	2.63	345.2053	ESI+	C21H28O4	11-Dehydrocorticosterone	↓	-	√	√
17	3.76	301.2164	ESI+	C20H28O2	All-trans-retinoic acid	↑	√	-	-
18	4.21	317.2107	ESI+	C20H28O3	4/-Hydroxyretinoic acid	↑	√	-	-
19	2.45	349.2359	ESI+	C21H32O4	3a,21-Dihydroxy-5b-pregnane-11,20-dione	↓	-	-	√
20	2.7	500.2811	ESI-	C32H39NO4	Fexofenadine	↑	-	√	
21	1.69	308.0792	ESI-	C14H15NO7	Inodxyl glucuronide	↑	√	√	√
22	2.61	134.06	ESI-	C8H9NO	2-Phenylacetamide	↓	√	√	-
23	4.45	337.2357	ESI-	C20H34O4	14,15-DiHETrE	↓	√	-	-
24	1.9	163.0385	ESI-	C9H8O3	Phenylpyruvic acid	↑	-	-	-
25	1.81	193.035	ESI-	C6H10O7	D-Glucuronic acid	↓	√	√	-
26	6.15	480.3093	ESI-	C23H48NO7P	LysoPC(15:0)	↓	-	-	-

Note: "↑↓" presents the level of biomarkers increase or decrease in the blood of mice with YHS. YDPGC-H: High-dosed YDPGCl; YDPGC–M: Middle-dosed YDPGC; YDPGC–L: Low-dosed YDPGC. "√" presents YDPGC has callback effect; "—" presents YDPGC has no callback effect.

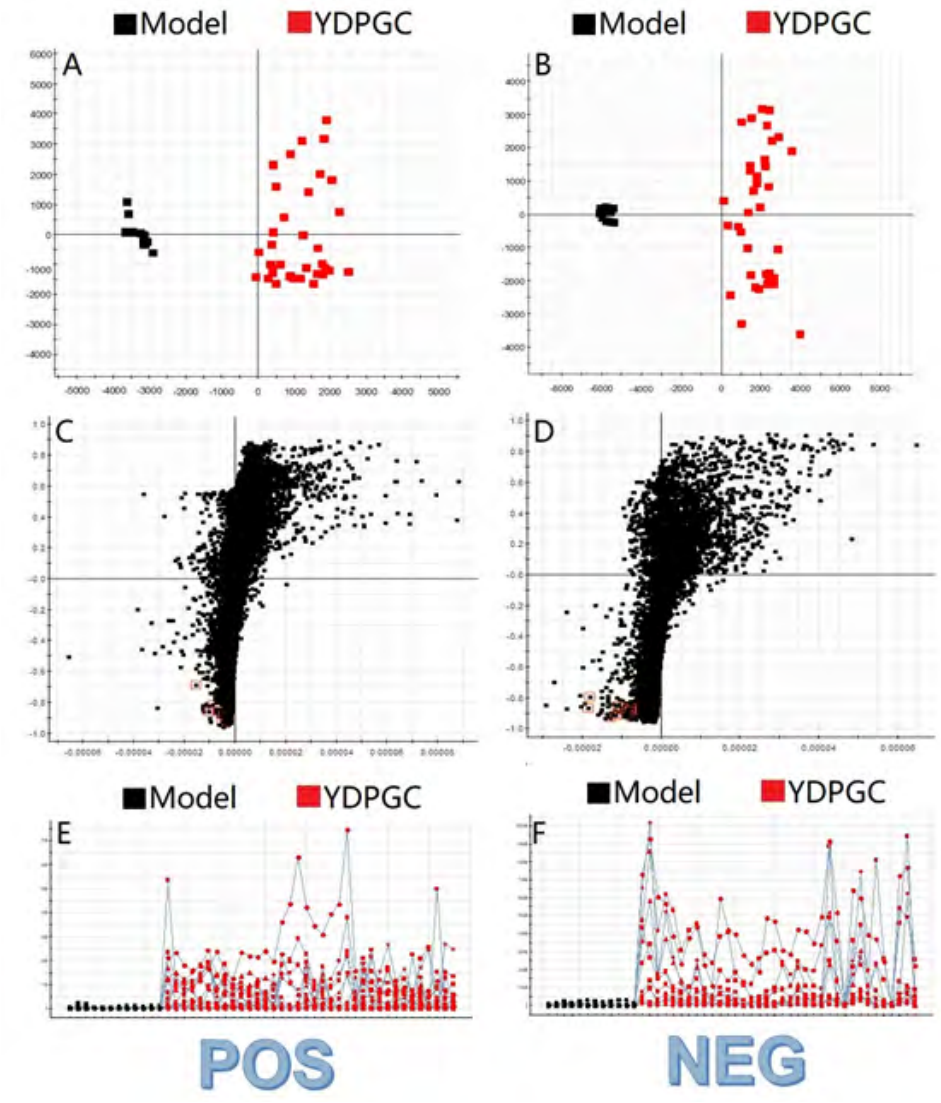

Figure 2: Multivariate data analysis of ingredients absorbed in blood of YDPGC

A.OPLS-DA score plot in positive ion mode; B. OPLS-DA score plot in negative ion mode; C. S-plot in positive ion mode; D. S-plot in negative ion mode E. Ion variables trend plot in positive ion mode; F. Ion variables trend plot in negative ion mode.

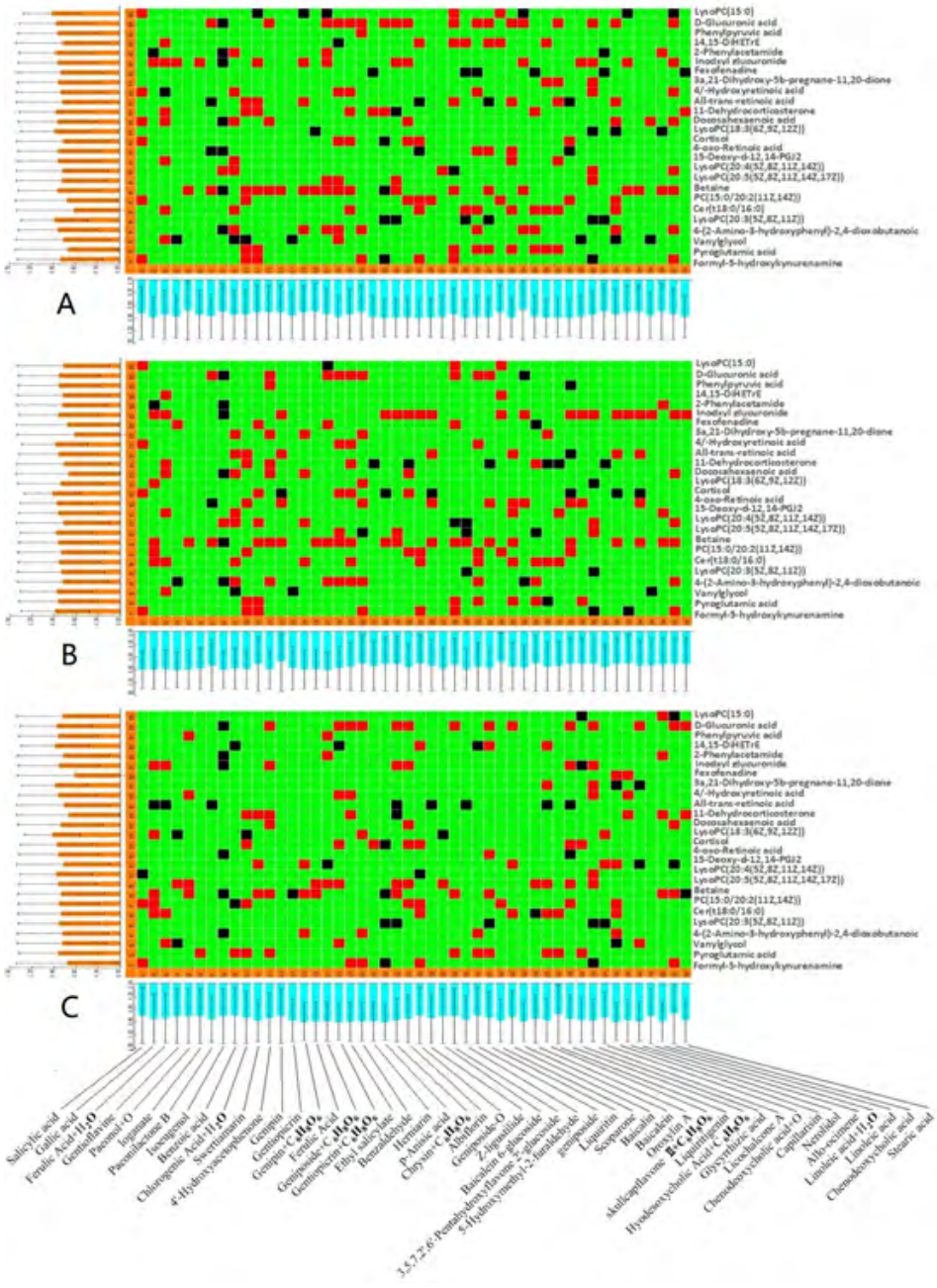

Figure 3: Plotting of correlation between serum biomarker and serum constituents

5. Discussion

Jaundice is a common syndrome in a variety of liver and biliary diseases, in which YHS is based on damp-heat background. An in-depth study on the pathogenesis of YHS is helpful to clarify the mechanism and effective constituents of drugs with definite efficacy. To better explain the essence of YHS, the animal model of liver injury induced by ANIT under the background of damp-heat was established, and guided by the theory of traditional Chinese medicine. The biomarkers of YHS have been analyzed and characterized by high-throughput metabolomics. In this study, 26 biomarkers of YHS are reproduced by UPLC-HDMS technology combined with pattern recognition, which can be used as potential targets for disease treatment.

YDPGC is the national protected variety of traditional Chinese medicine for the treatment of alcoholic liver, fatty liver, hepatitis B, and other hepatobiliary diseases. In recent years, the effectiveness of YDPGC has been widely studied. However, at present, the research on its chemical compounds is limited to the determination of chlorogenic acid, geniposide, gentiopicroside, ferulic acid, baicalin, ammonium glycyrrhizinate, and paeoniflorin and still lacks a comprehensive analysis of its active components. Therefore, a method for total component analysis of YDPGC supported by high-throughput UPLC-HDMS has been established in this study to scan and characterize the chemical components in YDPGC quickly, sensitively, and comprehensively. Through the analysis of the components absorbed into the blood of YHS mice after oral administration of YDPGC, the prototype ingredients, and their metabolites have been found. On this basis, through the analysis of the components absorbed into the

blood of YHS mice after oral administration of YDPGC, the prototype components, and their metabolites were found. At the same time, on this basis, through the analysis of the components absorbed into the blood of YHS mice after oral administration of YDPGC, the prototype components, and their metabolites were found. Through further correlation analysis, the ingredients absorbed in blood highly related to the biomarker expressing the essence of YHS have been selected as the potential bioactive components of YDPGC, so a quick method for screening the bioactive substances of YDPGC has been established. This method is helpful to further explore the mechanism and innovative drug research of YDPGC to provide a shred of scientific evidence for improving its quality.

6. Conclusion

Combined with high-throughput HPLC-HDMS technology, the chemical compounds and intergradients absorbed in blood of YDPGC have been rapidly and comprehensively analyzed based on the mature mice model of YHS. Using the mature animal model of damp-heat jaundice, combined with high-throughput UPLC-MS technology, the in vivo and in vitro components of YDPGC were analyzed and characterized rapidly. Through correlation analysis, the effective constituents of YDPGC in the treatment of YHS have been confirmed, which provided a basis for the development of innovative drugs and quality improvement.

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