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Analysis of the Chemical Constituents of BaZhen Decoction in Rat Urine by HPLC-ESI/MS

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High performance liquid chromatography/mass spectrometry (HPLC-ESI/MS) method was used to study BaZhen decoction and its metabolites in rat urine. 30 of major fragment ion peaks were marked in the total ion chromatogram of urine containing BaZhen decoction. According to the molecular weight and characteristic fragmentation information, 20 peaks were identified, containing 7 prototype constituents: Glycyol, glyuranolide, paeoniflorin, ginsenoside Rb_2, liquiritin, PGG, ginsenoside Rd; and 13 metabolites: *p*-Hydroxycinnamic acid, 5-FDCA, pinicolic acid, 3α-hydroxyglycyrrhehetic acid, paeonimetabolin I, M1, Compound K, M2, tumulosic acid, M3, *n*-γ-hydroxy-butyl phthalide, M4, Ginsenoside Rh_1. The result shows that the final metabolites in rat urine of BaZhen decoction, which is quite valuable for the further studies on effective constituents and the effecting mechanism as well as for clinical application of Bazhen decoction.

Key Words: BaZhen decoction, HPLC-ESI/MS, Chemical constituents, Metabolites, Urine.

INTRODUCTION

Ba Zhen decoction is first recorded in 'Zheng Ti Lei Yao' written in Ming Dynasty. It is composed of 'Si Jun Zi Decoction' which restore vital energy and the blood tonic 'Si Wu Decoction'. The modern pharmacology research discoveres that Ba Zhen decoction can remarkably enhance organism hemopoiesis, promote white blood cell to multiply and balance the organism internal secretion. This is annotate the tonifying qi-blood of Ba Zhen Decoction. Because the consitituent of traditional Chinese medicinent is extremely complex, blends after the compound prescription is unpredictable. After oral administration, the metabolites of traditional Chinese medicine passing through I and II biochemical reaction are possible material base of drug efficacy[1-3]. So it is significance for research of drug efficacy material base and expansion of clinical application to clear the metabolites of Ba Zhen decoction and the corresponding metabolism mechanism.

It is high sensitivity and selective superiority to combined the high performance liquid chromatography and electrospray ion trap mass spectrometry technology. We take advantage of it to directly access the excimer-ion fragments of the analytes in mild conditions and gradually increase the relative collision energy of selected parent ion. So we obtained the chemical relationship between the parent ion and the fragment ions[4]. It provide abundant chemical information to the study of the structure of metabolites *in vivo*. Therefore, it is systematic understanding of the metabolism *in vivo* and providing scientific basis for researching the material basis of Ba Zhen decoction by HPLC-ESI/MS.

EXPERIMENTAL

Homemade ultra-pure water, formic acid, acetonitrile and methanol were HPLC grade. Reference substance: paeoniflorin (110736-200420), liquiritin (111610-200402) were from China Pharmaceutical and Biological Products; Chinese herb medicine are purchased from Baofeng Medicine commercial Limited company.

Agilent 1100 LC system materials; Agilent1100 liquid chromatography/VL ion trap mass spectrometer (with atmospheric pressure electrospray ionization); Milli-Q Ultra-pure water machine; TDL80-2B Feige centrifuge; H66MC ultrasonic vibration apparatus. animal: body weight 200 ± 20 g, male, Wistar rats, Changchun National Biological Industry Base Laboratory Animal Center, Lot: SCXK-(Kyrgyzstan) 2003-2004.

Standard preparation: Weigh accurately *ca.* 0.5 mg of paeoniflorin and liquiritin, respectively into a 2 mL volumetric flask and dilute to volume with methanol, shake, filtrate with 0.22 μm membrane filter.

Preparation of sample solution *in vitro*: Weigh accurately about 7.5 g of Ba Zhen decoction, extracted with methanol for 2 times (in the condition of 70 ºC), first 8 times amount, refluxed 2 h, then 6 times amount, refluxed for 1.5 h combine the filtrate, then the filtrate is concentrated on a rotary evaporator

and the concentrate was transferred into a 10 mL volumetric flask and diluted with methanol to the mark, the solution were filtered with 0.22 μm membrane filter.

Preparation of the solution for intragastric administration: BaZhen decoction (Ginseng, Rehmannia, Atractylodes, white peony, angelica root, Chuan Xiong, Poria 10g, respectively, Baked Licorice 5 g) all parties 75 g, treated following the same progress as before, filtered, combined filtrate and vacuum recovery to dry and further diluted with distilled water to get final concentration of 3 g/mL of Ba Zhen decoction for intragastric administration.

HPLC-ESI/MS analysis conditions: Column: hydrosphere C_{18} (5 μm, 4.6 mm × 250 mm); mobile phase: acetonitrile-0.15 % formic acid (Table-1); column temperature: 30 ºC; flow rate: 0.5 mL min^{-1}; injection volume: 20 μL. Mass spectrometer ion source: electrospray ionization (ESI); atomization air pressure: 0.17 MPa; drying gas flow rate: 9.0 L min^{-1}; capillary voltage: 4 KV; transmission voltage: 70 V. Scan mode: negative ion scan, scan range: *m/z* 100-1200.

TABLE-1
GRADIENT ELUTION PROGRAM OF MOBILE PHASE

Time (min)	Acetonitrile (%)	0.15 % formic acid solution (%)
0	5	95
45	35	65
55	40	60
70	55	45
115	80	20

Collection and treatment of urine: Male Wistar rats were kept individually in metabolic cages for 2 days time, then were fasted (free water) for 12 h from the 3rd day. Then the rats in treated group were given Ba Zhen decoction (1.5 mL 100 g^{-1}) by intragastric administration and those in the blank group were given distilled water of the same volume.All the rats continue fasting for 16 h (free water), urine in 8-16 h were collected, then were stored in a freezer at -20 ºC.

Thaw frozen urine samples at room temperature, centrifuged for 10 min at 3000 rpm, transfer 1 mL of top layer accurately to a conditioned SPE cartridge, After washing with 1 mL of distilled water, the cartridge was eluted with 2 mL of methanol and 1 mL acetonitrile, the elution was dried under decompression and was dissolved with 1 mL acetonitrile, then filtered through 0.22 μm membrane filters to analysis by HPLC with 10 μL.

RESULTS AND DISCUSSION

Based on recorded *m/z* values and much more fragment information, compared with some reference substance or the literature[5-9]. By analyzing total ion chromatogram (TIC) of Bazhen decoction samples *in vitro* and urine samples. Thirty molecular ion peaks were obtained. Fourteen components is the prototype, they are peak 2, 3, 7, 8, 13, 16, 18, 21, 22, 24, 27, 28, 29, 30. 16 metabolites, They are peak 1, 4, 5, 6, 9, 10, 11, 12, 14, 15, 17, 19, 20, 23, 25, 26 (Fig. 1).

Based on recorded *m/z* values, quasi-molecular ion peak and much more fragment information, compared with some reference substance or the literature[10-15] reported the composition of the control, thus it is possible to infer the structure of

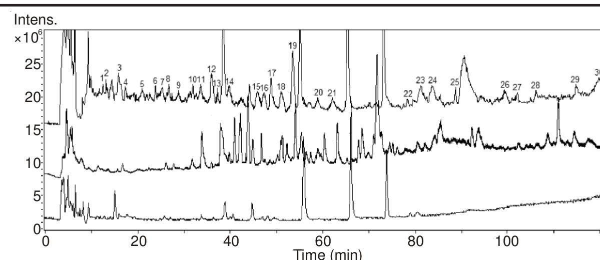

Fig. 1. Mass spectrometry-total ion current chromatograph in negative ion mode for blank urine (S1), Bazhen decoction (S2) and drug-containing urine sample (S3)

20 compounds, including 7 prototype components, *i.e.*, glycyol, glyuranolide, paeoniflorin, ginsenoside Rb2, liquiritin, PGG, ginsenoside Rd and 13 metabolites: *p*-Hydroxycinnamic acid, 5-FDCA, pinicolic acid, 3α-hydroxyglycyrrhehetic acid, paeonimetabolin I, M1, compound K, M2, Tumulosic acid, M3, *n*-γ-hydroxy-butyl phthalide, M4, ginsenoside Rh_1 (Table-2).

Secondary ion mass spectrometry analysis of the main debris as follows: peak 1 was *p*-hydroxycinnamic acid (t_R =13. 5 min) m.w. 164, $[M-H]^-$ *m/z* 163, $[M-OH]^-$ *m/z* 147; peak 2 was glycyol (t_R = 14.4 min) m.w. 366, $[M-H]^-$ *m/z* 365, $[M-CH_3]^-$ *m/z* 351, $[M-CH_3-C_5H_8]^-$ *m/z* 283, peak 3 was glyuranolide (t_R = 15.6 min) m.w. 514, $[M-H]^-$ *m/z* 513, $[M-CH_3]^-$ *m/z* 499, $[M-CH_3-COO]^-$ *m/z* 455, peak 4 was 5-FDCA (t_R =17.4 min) m.w. 156, $[M-H]^-$ *m/z* 155, $[M-COOH]^-$ *m/z* 111, peak 5 was pinicolic acid (t_R = 21.5 min) m.w.454, $[M-H]^-$ *m/z* 453, $[M-H-C_6H_{10}]^-$ *m/z* 371, $[M-C_6H_{10}-COOH]^-$ *m/z* 327, peak 9 was 3α-hydroxyglycyrrhehetic acid (t_R = 28.6 min) m.w. 470, $[M-H]^-$ *m/z* 469, $[M-COOH]^-$ *m/z* 425, peak 10 was paeonimetabolin I (t_R = 32.3 min) m.w. 198, $[M-H]^-$ *m/z* 197, $[M-H-OH]^-$ *m/z* 180, $[M-H-OH-C_4H_9]^-$ *m/z* 123, peak 11 was metabolites of atractylenolide-II (t_R = 33.6 min) m.w. 584, $[M-H]^-$ *m/z* 583, $[M-H-(Glc\ acid)]^-$ *m/z* 407, $[M-H-2(Glc\ acid)]^-$ *m/z* 231, peak 12 was compound K (t_R = 36.7 min) m.w.622, $[M-H]^-$ *m/z* 621, $[M-H-Glc]^-$ *m/z* 459, $[M-H-Glc-C_6H_{12}]^-$ *m/z* 375, peak 13 was paeoniflorin (t_R = 37.4 min) m.w.480, $[2M-H]^-$ *m/z* 959, $[M-H]^-$ *m/z* 479, $[M-H-CH_2O]^-$ *m/z* 449, $[M-H-CH_2O-(benzoic\ acid)]^-$ *m/z* 327, consistent with the standard control; peak 14 was metabolites of licorice-saponin F3 (t_R = 40.9 min) m.w. 630, $[M-H]^-$ *m/z* 629, $[M-H-(Glc\ acid)]^-$ *m/z* 453, peak 16 was ginsenoside Rb2 (t_R = 47.8 min) m.w.1078, $[M-H]^-$ *m/z* 1077, $[M-H-Ara(p)]^-$ *m/z* 945, $[M-H-Ara(p)-Glc]^-$ *m/z* 783, $[M-H-Ara(p)-2Glc]^-$ *m/z*621, peak 17 was tumulosic acid (t_R = 49.1 min) m.w. 486, $[M-H]^-$ *m/z* 485, $[M-H-H_2O]^-$ *m/z* 467, $[M-H-H_2O-C_7H_{12}]^-$ *m/z* 371; peak 19 was metabolites of ferulic acid (t_R = 53.8 min) m.w.546, $[M-H]^-$ *m/z* 545, $[M-H-(Glc\ acid)]^-$ *m/z* 369, $[M-H-2(Glc\ acid)]^-$ *m/z* 193, peak 20 was n-γ-hydroxy-butyl phthalide (t_R = 58.8 min) m.w.206, $[M-H]^-$ *m/z* 205, $[M-OH]^-$ *m/z*189, $[M-OH-C_4H_8]^-$ *m/z* 133, peak 21 was liquiritin (t_R = 62.5 min) m.w. 418, $[M-H]^-$ *m/z* 417, $[M-H-Glc]^-$ *m/z* 255, consistent with the standard control; peak 22 was PGG (t_R = 78.6 min) m.w. 940, $[M-H]^-$ *m/z* 939, $[M-H-2H_2O]^-$ *m/z* 903, $[M-H-(Gallic\ acid)]^-$ *m/z* 769, peak 23 (t_R = 81.6 min) m.w. 836, $[M-H]^-$ *m/z* 835, $[M-H-(Glc\ acid)]^-$ *m/z* 659, $[M-H-2(Glc\ acid)]^-$ *m/z* 483, revealing its structure was metabolites, peak 26 was Ginsenoside Rh1 (t_R = 98.8 min) m.w. 638, $[M-H]^-$ *m/z* 637, $[M-H-Glc]^-$ *m/z* 475,

TABLE-2
INFORMATION OF HPLC-ESI/MS AND IDENTIFICATION OF COMPOUND

No.	Retention time (min)	MS (m/z)		m.w.	Compound	Note
		MS[M-H]-	MS/MS			
1	13.5	163	147	164	*p*-Hydroxycinnamic acid	M
2	14.4	365	351, 283	366	Glycyol	P
3	15.6	513	499, 455	514	Glyuranolide	P
4	17.4	155	111	156	5-FDCA	M
5	21.5	453	371, 327	454	Pinicolic acid	M
6	22.0	627	566, 475, 323	–	Unknow	M
7	25.6	499	439	–	Unknow	P
8	26.5	549	417, 255	–	Unknow	P
9	28.6	469	425	470	3α-Hydroxyglycyrrhehetic acid	M
10	32.3	197	180, 123	198	Paeonimetabolin I	M
11	33.6	583	407, 231	584	M1	M
12	36.7	621	459, 375	622	Compound K	M
13	37.4	959	479, 449, 327	480	Paeoniflorin	P
14	40.9	629	453	630	M2	M
15	47.3	555	534, 336	–	Unknow	M
16	47.8	1077	945, 783, 621	1078	Ginsenoside Rb2	P
17	49.1	485	467, 371	486	Tumulosic acid	M
18	51.2	–	–	–	Unknow	P
19	53.8	545	369 193	546	M3	M
20	58.8	205	189, 133	206	n-γ-hydroxy-butyl phthalide	M
21	62.5	417	255	418	Liquiritin	P
22	78.6	939	903, 769	940	PGG	P
23	81.6	835	659, 483	836	M4	M
24	83.7	539	522, 479	–	Unknow	P
25	89.1	–	–	–	Unknow	M
26	98.8	637	475	638	Ginsenoside Rh1	M
27	102.3	945	783, 621	946	Ginsenoside Rd	P
28	108.3	–	–	–	Unknow	P
29	115.8	–	–	–	Unknow	P
30	119.5	690	512	–	Unknow	P

peak 27 was ginsenoside Rd (t_R =102.3 min) m.w. 946, $[M-H]^-$ *m/z* 945, $[M-H-Glc]^-$ *m/z* 783, $[M-H-2Glc]^-$ *m/z* 621, peaks 6, 7, 8, 15, 18, 24, 25, 28, 29, 30 were unknown.

Chinese medicine is extremely complex, the compound is unpredictable after oral administration of Chinese herbal, including prototype components and metabolized trace elements and thus more difficult to study Chinese medicine on the increase; but the same type of compounds in the body to follow a similar metabolism of the metabolism of control can be targeted after the metabolic study. This study developed an integrated method for screening the bioactive constituents in urine after intragastric administration of Ba Zhen Tang and identified a total of 20 chemical components, including 7 prototype components and 13 metabolic components.

This study provide important scientific information for the studies on effective substances of Ba Zhen Tang and have significance for the the improvement of clinical medicine. At the same time, HPLC-ESI/MS technology combined with the chemical composition of traditional Chinese medicine rapid qualitative chemical composition of traditional Chinese medicine for the depth and breadth of research provides a new idea.

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REFERENCES

1. W.L. Li, Q.C. Dai, Y.B. Ji, Z. Sun, J. Du and J. Bai, *Chin Pharm*., **21**, 1022 (2009).
2. Z. Chun, X. Luo, D.H. Chen, M.Y. Yu, Y.F. Cheng and Z.R. Yang, *J. Biomed. Eng.*, **21**, 727 (2004).
3. H.P. Pan, *Chin Trad. Patent Med.*, **25**, 90 (2003).
4. X.R. Ran, H.H. Yang, Q.L. Liang, J. Chen, Y.M. Wang, G.A. Luo, P. Li, K.M. Li and Y.B. Chen, *Chem. J. Chin. Univ.*, **28**, 250 (2007).
5. H.M. Wen, K.J. Guo, W.L. Xing and H.M. Bian, *J. Nanjing Univ. TCM*, **23**, 96 (2007).
6. L. Yang, S.J. Xu, X. Zeng, Z.F. Wu, Y.H. Deng, Y.M. Liu, S.G. Deng and R.M. Ou, *Chem. J. Chin. Univ.*, **27**, 1042 (2006).
7. L. Yang, S.J. Xu, X. Zeng, Z.F. Wu, Y.H. Deng, Y.M. Liu, S.G. Deng and R.M. Ou, *Acta Pharm. Sin.*, **41**, 742 (2006).
8. X.F. Zhao, H.W. Kong, J.S. Wang and G.W. Xu, *World Sci. Technol.-Modern Trad. Chin. Med.*, **11**, 153 (2009).
9. F.Q. Guo, L.F. Huang and S.Y. Zhao, *Chin. J. Chromatogr*., **25**, 43 (2007).
10. B.L. Qu, H.Y. Wu, F.L. Ren, W.F. Yu and J.Z. Wang, *Food Sin. Technol.*, **34**, 92 (2009).
11. N. Yang, S.Y. Qiao and F.H. Meng, *J. Chin. Mass Spectrom*, **31**, 153 (2010).
12. Y. Liu, R.B. Shi, B. Liu and M. Dong, *J. Beijing Univ. TCM*, **31**, 334 (2008).
13. N.J. Yu, J.F. Guo, Y.M. Zhao and Y.X. Zhang, *Chin. J. Anal. Chem.*, **3**, 866 (2004).
14. L. Zhang, D.S. Xu and Y. Feng, *Chin. Mater. Med*., **32**, 1789 (2007).
15. J.J. Zhou, G.R. Xie and F.J. Yan, Traditional Chinese Medicines Molecular Structures, Natural Sources and Applications, Beijing, pp. 365, 533 (2004).