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Short communication

Comparative pharmacokinetics of three marker compounds in mBHT and single-herb extract after oral administration to rats

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ABSTRACT

Modified Bo-Yang-Hwan-O-Tang (mBHT) is a decoction of 12 herbs traditionally used in the treatment of cerebral and cardiac stroke and vascular dementia. Paeoniflorin (PF), calycosin-7-O-β-D-glycoside (CY), and salvianolic acid B (SB) are marker compounds for extracts of the herbs Paeoniae Radix, Astragali Radix, and Salviae Miltiorrhizae Radix, respectively, and are used to assess the quality of mBHT. This study examined the pharmacokinetics of these three marker compounds following oral administration of each herb extract alone and in combination as mBHT in rats. The concentrations of the three compounds in rat plasma were determined by high-performance liquid chromatography, using a C18 column (2.1 × 150 mm, 5 μm) and mobile phases of methanol–water–formic acid (10:90:0.05, v/v) and methanol–water (90:10, v/v). The results indicated that the pharmacokinetic parameters of Paeoniae Radix extract group and mBHT group were very similar, while those of Salviae Miltiorrhizae Radix extract group and mBHT group were significantly different ($P < 0.05$, *t*-test). The T_{max}, AUC and $T_{1/2}$ of SB for Salviae Miltiorrhizae Radix extract group were 54.7 min, 598.7 μg min/ml and 37.4 min, respectively. However, these values increased to 77.6 min, 915.9 μg min/ml and 53.7 min for mBHT group, supposing that excretion of SB could be more retarded when administered in mBHT than in Salviae Miltiorrhizae Radix extract.

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1. Introduction

Based on an initial diagnosis, a compound prescription to treat the condition is formulated by surmising the identity and amount of the proper medicines, which may act synergistically or antagonistically in a composite formula [1]. Pharmacokinetic studies are useful for understanding the actions and interactions of drugs with regard to efficacy and toxicity, and for evaluating the rationality and compatibility of herbs and/or prescriptions [2,3]. Owing to the complexity of chemicals in compound prescriptions, one or several representative compounds may be chosen as markers to be used to investigate the pharmacokinetics of the whole prescription [4,5], allowing the interactions of herbs or prescriptions to be clarified based on the pharmacokinetic behavior of the selected compounds.

Bo-Yang-Hwan-O-Tang (BHT), originating from traditional Chinese medicines, is a decoction of seven herbs traditionally used in the treatment of cerebral and cardiac stroke and vascular dementia. Recently, modified Bo-Yang-Hwan-O-Tang (mBHT) was formulated by adding five herbs to the original BHT recipe. The biological effects of mBHT in stroke, senility, vascular dementia, and heart damage as well as thrombosis and immune modulation have been previously investigated [6,7]. Paeoniae Radix, Astragali Radix, and Salviae Miltiorrhizae Radix are the key ingredients of the mBHT formulation. As primary components of these herbs, paeoniflorin (PF), calycosin-7-O-β-D-glycoside (CY), and salvianolic acid B (SB) have been used as marker compounds for assessing the quality of a mBHT decoction. PF is a marker compound for Paeonia Radix, which is a traditional herbal treatment for dementia according to the Chinese pharmacopoeia [8–10]. Astragali Radix is widely used in traditional Chinese medicine as a treatment for inflammation, fever, hepatitis, and allergic diseases, and CY is a marker compound for Astragali Radix [11–13]. Salviae Miltiorrhizae Radix is used to treat coronary heart disease, cerebrovascular disease, hepatitis, cirrhosis, and chronic renal failure, with recent studies suggesting that its main pharmacologically active ingredients are water-soluble salvianolic acids [14,15]; thus, SB serves as a marker compound for this herbal treatment. The behaviors of individual marker compounds have been studied separately [16–18], but there is little precedence for comparing the pharmacokinetic behaviors of marker compounds

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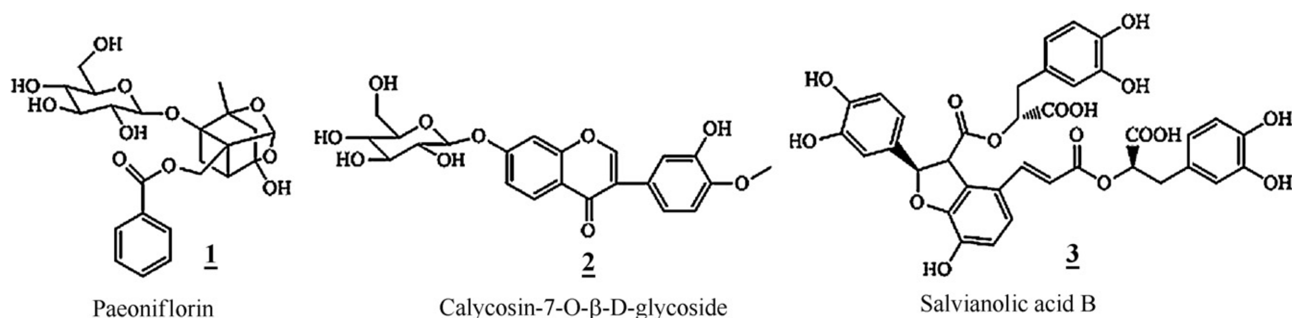

Fig. 1. Chemical structures of the marker compounds paeoniflorin, calycosin-7-O-β-D-glycoside, and salvianolic acid B.

acting individually in single-herb preparations versus together in a compound herbal preparation.

The present study compared the pharmacokinetics of the three main herbs of mBHT–Paeoniae Radix, Astragali Radix, and Salviae Miltiorrhizae Radix–administered orally to rats either individually as single-herb extracts or together in mBHT, using PF, CY, and SB as marker compounds.

2. Experimental

2.1. Reagents and materials

HPLC-grade methanol was purchased from Burdick & Jackson (Morristown, NJ, USA), and analytical grade formic acid was from Sigma–Aldrich (St. Louis, MO, USA). All water was purified via ultra-filtration (Sinhan, Korea). Astragali Radix, Salviae Miltiorrhizae Radix, Angelicae Gigantis Radix, Paeoniae Radix, Achyranthis Radix, Lumbricus, Cnidii Rhizoma, Persicae Semen, Carthami Flos, Cinnamomi Ramulus, Polygalae Radix, and Acori Graminei Rhizoma were purchased in a Korean herbal market, identified by Professor Y.K. Park (College of Oriental Medicine, Dongguk University), and stored at the College of Pharmacy, Chungnam National University. Paeoniflorin (PF), calycosin-7-O-β-D-glycoside (CY), and salvianolic acid B (SB) (Fig. 1) were isolated from Paeoniae Radix, Astragali Radix, and Salviae Miltiorrhizae Radix, respectively, at the Laboratory of Pharmacognosy, College of Pharmacy, Chungnam National University, and their structures were confirmed by spectroscopic analysis and comparison with published data. The purity of the three compounds was at least 98%, as confirmed by HPLC.

2.2. Animals

Sprague-Dawley male rats (310–320 g) were obtained from the Department of Laboratory Animal Medicine (Seoul, Korea). Animals were housed under standard conditions of temperature, humidity, and light; were provided with plentiful food and water; and were acclimated in the laboratory for at least one week prior to experimentation. Before being used in the study, the rats were fasted overnight with free access to water.

2.3. Instrumentation

High-performance liquid chromatography (HPLC) was performed with a Shimadzu LC-10AD series HPLC system (Shimadzu, Kyoto, Japan) consisting of an LC-10AD quaternary pump, an SPD-M10AVP diode array detector, a C18 column, and a CTO-10AS column oven. A high-speed centrifuge (Biospin, Hanil, Korea), an electronic balance (UM3, Mettler, Switzerland), and a vortex-mixing mill (Vortex-2; Chang Shin Co., Korea) were also used.

2.4. Sample preparation

A mixture of 12 herbal drugs (37.5 g of Astragali Radix, 15.0 g of Salviae Miltiorrhizae Radix, 7.5 g of Angelicae Gigantis Radix, 5.6 g of Paeoniae Radix, 5.6 g of Achyranthis Radix, and 3.8 g of other herbs) was coarsely ground, extracted in 2 L of boiling water for 3 h, filtered through a two-layer mesh, and concentrated under vacuum for 15 h. The same amounts of Astragali Radix, Salviae Miltiorrhizae Radix, and Paeoniae Radix were extracted individually using this same method. All extracts were freeze-dried for storage.

2.5. Chromatography

The PF, CY, and SB concentrations in extracts and plasma samples were determined using the HPLC system with a C18 column (2.1 × 150 mm, 5 μm; Phenomenex, CA, USA) held at a constant temperature of 25 °C and two mobile phases: A, consisting of methanol–water–formic acid (10:90:0.05, v/v), and B, consisting of methanol–water (90:10, v/v). Each sample was eluted at a flow rate of 0.4 mL/min using the following gradient program: a linear increase from 0% to 40% B over the first 30 min, followed by a linear increase to 75% B from 30 to 60 min, and holding at 75% B for 5 min. The eluent was monitored at 250 nm, and UV spectra were recorded from 190 to 400 nm. The separated peaks were identified by comparing retention times with those of standard compounds and by analyzing LC–MS spectra. LC–MS analysis to identify the three marker compounds was performed using a LCMS-2010EV system (Shimadzu, Japan) linked to an electrospray ionization source operating in both negative and positive modes.

2.6. Administration of extracts and sampling of blood

A total of 20 rats were randomly divided into four groups, and each group was orally administered a different extract by gavage with a syringe: mBHT extract (1.5 g/100 g body weight) containing 0.25% PF, 0.32% CY, and 0.35% SB; Paeoniae Radix extract (0.11 g/100 g body weight); Astragali Radix extract (0.23 g/100 g body weight); and Salviae Miltiorrhizae Radix extract (0.12 g/100 g body weight). Blood samples (about 1 mL) were collected from the orbital vein at scheduled times after administration (0, 10, 20, 30, 45, 60, 90, 120, 180, 240, and 360 min post-administration). The plasma was separated by centrifugation at 17,000 rpm for 10 min at 4 °C, and 0.1 mL of plasma was withdrawn and diluted with 0.4 mL of methanol. After 30 min, the mixture was clarified by centrifugation at 17,000 rpm for 10 min at 4 °C. The upper layer was dried under a stream of N_2, and the residue was dissolved in 0.1 mL of methanol and stored at −20 °C for further analysis. This animal

experiment procedure was approved by the Animal Ethics Board of Chungnam National University.

2.7. Method validation

2.7.1. Preparation of standards

A stock standard solution of PF, SB, and CY was prepared in methanol and calibration samples were prepared by spiking blank plasma samples with this stock solution to obtain final concentrations of 0.4, 0.8, 1.25, 2.5, 5.0 and 10 μg/mL for PF and SB, and 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 μg/mL for CY. All solutions were stored at 4 °C before analysis. Quality control (QC) samples containing various concentrations of PF, SB, and CY (PF and SB: 0.4, 1.25, and 10 μg/mL; CY: 0.25, 2.0, and 8.0 μg/mL) were prepared in a similar manner.

2.7.2. Precision and accuracy

Intra-day precision was determined by obtaining three concentrations of PF, CY, and SB in QC samples, five times on the same day. Inter-day precision was determined by successive concentration determinations over a 5-day period. Precision is indicated as the coefficient of variation (RSD). Accuracy was calculated from the mean value of the observed concentrations and the theoretical concentrations. The limit of detection (LOD) and limit of quantification (LOQ) for each standard solution were calculated with signal-to-noise ratios of 3 and 10, respectively.

2.7.3. Extraction recovery and stability

The recoveries of PF, CY, and SB were determined by comparing the peak areas obtained for QC samples subjected to the extraction procedure with those obtained for blank plasma extracts spiked post-extraction to the same nominal concentrations (PF and SB: 0.4, 1.25, and 10 μg/mL; CY: 0.25, 2.0, and 8.0 μg/mL). Freeze/thaw stability was evaluated by analyzing QC samples at three concentrations after multiple cycles of freezing (at −80 °C) and thawing (at room temperature) over a 5-day period.

2.8. Pharmacokinetic analysis

Experimental data and pharmacokinetic parameters are expressed as means ± S.D. Plasma concentration–time curves were plotted and pharmacokinetic parameters were calculated using WinNonlin Standard Edition software (Ver. 2.1). Comparison of parameters between single-herb extract group and mBHT group were carried out by a *t*-test.

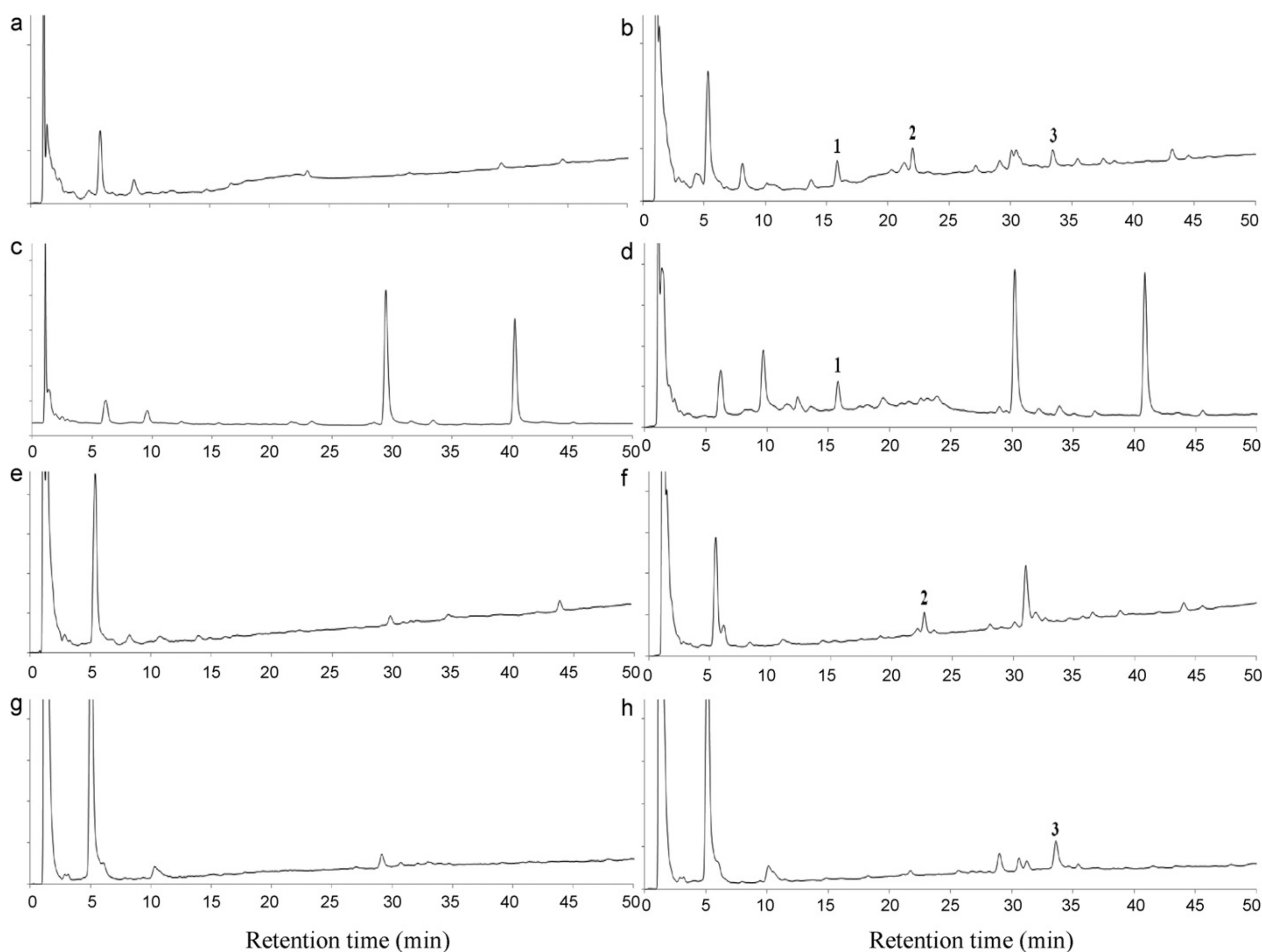

Fig. 2. Representative HPLC chromatograms of plasma samples. Panels on the left (a, c, e, and g) show chromatograms of blank plasma corresponding respectively to the following panels on the right: plasma sample after oral administration of (b) mBHT, (d) Paeoniae Radix extract, (f) Astragali Radix extract, and (h) Salviae Miltiorrhizae Radix extract. Peaks: 1. Paeoniflorin (PF), 2. Calycosin-7-O-β-D-glycoside (CY), and 3. Salvianolic acid B (SB).

3. Results and discussion

3.1. Optimization of chromatographic conditions

HPLC with isocratic elution failed to fully separate the three marker compounds in the extracts and plasma samples. However, gradient elution with mobile phases consisting of methanol–water–formic acid (10:90:0.05, v/v) and methanol–water (90:10, v/v) yielded baseline resolution for all three compounds. Among the different mobile phase modifications tested, formic acid gave the best separation, as it prevented peak tailing. The extent to which endogenous plasma constituents interfered with PF, CY, and SB was assessed by inspecting chromatograms derived from processed blank plasma samples. Typical chromatograms of blank plasma and plasma samples after administration of herbal drug extracts are shown in Fig. 2. The retention times of PF, CY, and SB were 16.1, 22.6, and 33.8 min, with no endogenous interference or matrix effect.

3.2. Linearity, precision, and accuracy

Calibration curves were generated by plotting chromatographic peak area as a function of marker compound concentration. Peak areas of PF, CY, and SB in rat plasma all displayed good linear relationships, described by the following regression lines: $y = 220.4x + 0.4$ for PF; $y = 1754.1x - 0.6$ for CY; and $y = 646.2x - 0.2$ for SB (where y is peak area, and x is marker compound concentration in μg/mL). The LOD values for PF, CY, and SB as determined from the pooled plasma samples were 0.15, 0.12, and 0.16 μg/mL, respectively, and the LOQ values, 0.31, 0.25, and 0.32 μg/mL. The intra-day and inter-day precision determined from replicate analyses of QC samples were less than 4.2% and 6.8%, and the accuracy of the method ranged from 96.0% to 107.2%, as shown in Table 1. The recoveries of PF, CY, and SB in extractions of the medium-concentration QC samples were 94.2%, 84.3% and 90.4%, respectively. In the freeze/thaw stability test, the concentrations of the marker compounds were between 95.7% and 105.4% of the initial values, indicating that the analytes are stable in rat plasma for at least 5 days with freeze/thaw cycles.

3.3. Pharmacokinetic applications

The validated method was successfully applied to the pharmacokinetic study of PF, CY, and SB in rat plasma after oral administration of Astragali Radix, Salviae Miltiorrhizae Radix, Paeoniae Radix, and mBHT extracts. The mean concentration–time curves are shown in Fig. 3, and the pharmacokinetic parameters are presented in Table 2. After oral administration of single-herb extracts, the marker compounds were absorbed at a higher absorption rate with T_{max} range of 52.1–77.6 min. There was no significant difference in C_{max} between the single-herb extract group and mBHT group for PF, CY, and SB indicating that both groups were administered with same amount of marker compounds. The pharmacokinetic parameters between Paeoniae Radix extract group and mBHT group were very similar, while those between Salviae Miltiorrhizae Radix extract group and mBHT group were significantly different. The T_{max}, AUC and $T_{1/2}$ for Salviae Miltiorrhizae Radix extract group were 54.7 min, 598.7 μg min/ml and 37.4 min, respectively. However, these values increased to 77.6 min, 915.9 μg min/ml and 53.7 min for mBHT group ($P < 0.05$, t-test).

Compound herbal prescriptions are often administered in Asia, and each herbal component of a compound prescription is necessary to obtain the desired effect. Given the complexity of the chemical constituents of compound herbal prescriptions, pharmacokinetic studies usually focus on the main active constituents or marker compounds. The present pharmacokinetic study of SB provides evidence of about 42–53% increase for the pharmacokinetic parameters, indicating that excretion of SB could be more retarded when administered in mBHT than in Salviae Miltiorrhizae Radix extract. These differences in pharmacokinetic properties between the two treatments highlight the importance of investigating the pharmacological characteristics of the mBHT decoction used in clinical situations. The mechanisms accounting for the different pharmacokinetic behaviors of CY, and SB administered in mBHT versus in single-herb extracts are not clear; however, different compound–compound interactions of marker compounds in mBHT versus in single-herb extracts may be one possible explanation. For example, because it is a compound prescription, mBHT may contain some ingredients that improve the absorption of other components. Indeed, differences in the pharmacokinetic parameters of PF were observed in rats administered pure PF compared with extract of Cortex Moutan or extract of Shuang-Dan [16]. Moreover, the binding between rat plasma proteins and SB was increased and its elimination half-life was prolonged when the two extracts were

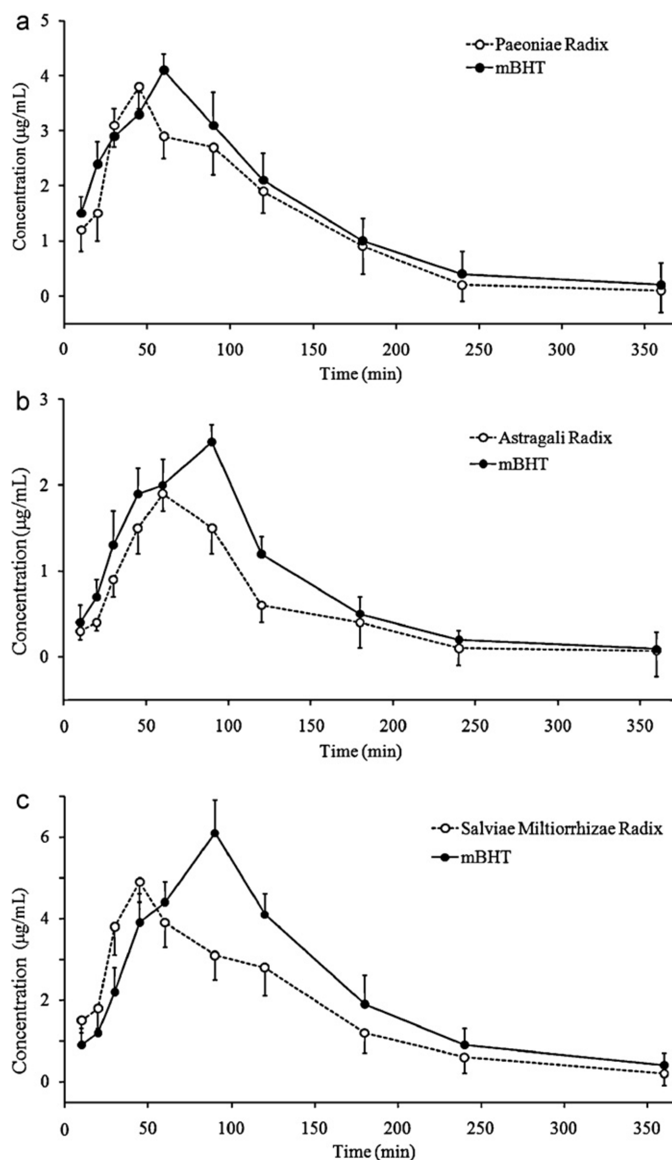

Fig. 3. Plasma concentration–time curves of (a) paeoniflorin, (b) calycosin-7-O-β-D-glycoside, and (c) salvianolic acid B in rat plasma after oral administration of mBHT (open circles) and single-herb extracts (filled circles).

Table 1
The intra-day and inter-day precision and accuracy values for three marker compounds in blank plasma.

Component[a]	Spiked (μg/ml)	Intra-day ($n=5$)			Inter-day (n = 5)		
		Measured (μg/ml)	Accuracy (%)	Precision (RSD%)	Measured (μg/ml)	Accuracy (%)	Precision (RSD%)
PF	0.40	0.39	97.5	3.21	4.12	102.5	4.33
	1.25	1.31	104.8	2.17	1.34	107.2	3.15
	10.00	10.20	102.0	2.83	9.86	98.6	5.71
CY	0.25	0.24	96.0	3.54	0.26	104.0	5.12
	2.00	1.97	98.5	2.72	2.03	101.5	4.98
	8.00	8.12	101.5	1.94	7.81	97.5	4.64
SB	0.40	0.41	102.5	2.82	0.39	97.5	3.92
	1.25	1.24	99.2	4.27	1.27	101.6	6.87
	10.00	10.10	101.0	2.23	9.82	98.2	6.11

[a] PF, paeoniflorin; CY, calycosin-7-O-β-D-glycoside; SB, salvianolic acid B.

Table 2
Pharmacokinetic parameters of three marker compounds in rat plasma administered as single herbal drug or mBHT ($n=5$).

Parameter	Paeoniflorin		Calycosin-7-O-β-D-glycoside		Salvianolic acid B	
	PR[a]	mBHT	AR[b]	mBHT	SM[c]	mBHT
C_{max} (μg/ml)	3.2 ± 0.4	3.6 ± 0.4	1.4 ± 0.3	1.9 ± 0.3	4.0 ± 0.3	4.3 ± 0.4
T_{max} (min)	52.1 ± 4.6	53.4 ± 5.2	59.0 ± 7.4	63.6 ± 5.2	54.7 ± 6.3	77.6 ± 8.7**
AUC (μg min/ml)	449.3 ± 48.3	517.3 ± 68.5	218.7 ± 32.7	322.9 ± 54.8*	598.7 ± 84.5	915.9 ± 85.3**
$T_{1/2}$ (min)	36.2 ± 7.5	37.4 ± 8.2	41.0 ± 7.9	44.1 ± 8.2	37.4 ± 6.2	53.7 ± 7.4*

**$P<0.01$; *$P<0.05$ when compared with corresponding single-herb extract such as PR, AR or SM.
[a] Paeoniae Radix.
[b] Astragali Radix.
[c] Salviae Miltiorrhizae Radix.

administered together [17]. However, the present study is the first report of differences in the pharmacokinetic parameters of marker compounds administered in mBHT and in single-herb extracts in rats. Possible compound–compound interactions in mBHT should be studied to further elucidate the pharmacokinetic differences between mBHT and single-herb extracts for clinical applications.

4. Conclusions

An HPLC method using a C18 column and mobile phases of methanol–water–formic acid (10:90:0.05, v/v) and methanol–water (90:10, v/v) was developed and validated to determine the concentration of three marker compounds of mBHT (PF, CY, and SB) in rat plasma after administration of a mBHT and single-herb extracts. There was no significant difference in C_{max} between the single-herb extract group and mBHT group for the marker compounds. The pharmacokinetic parameters (Tmax, AUC and $T_{1/2}$) of Paeoniae Radix extract group and mBHT group were very similar, while those of Salviae Miltiorrhizae Radix extract group and mBHT group were significantly different ($P<0.05$, *t*-test), supposing that excretion of SB could be more retarded when administered in mBHT than in Salviae Miltiorrhizae Radix extract. The present results could provide a methodical application for mBHT and the extracts of Astragali Radix, Salviae Miltiorrhizae Radix and Paeoniae Radix, alone and in combination.

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References

[1] X.G. Yang, B. Peng, G.H. Zhang, L.L. Wei, S.F. Nie, W.S. Pan, Studies of the pharmacokinetics of paeoniflorin in two Jing–Zhi–Guan–Xin formulations after oral administration to beagle dogs, J. Pharm. Biomed. Anal. 41 (2006) 320–324.
[2] L.C. Chen, M.H. Chou, M.F. Lin, L.L. Yang, Pharmacokinetics of paeoniflorin after oral administration of Shao-yao Gan-chao Tang in mice, Jpn. J. Pharmacol. 88 (2002) 250–255.
[3] C.H. Wang, R. Wang, X.M. Cheng, Y.Q. He, Z.T. Wang, C. Wu, J. Cao, Comparative pharmacokinetic study of paeoniflorin after oral administration of decoction of Radix Paeoniae Rubra and Radix Paeoniae Alba in rats, J. Ethnopharmacol. 117 (2008) 467–472.
[4] Q.F. Liao, W. Yang, Y. Jia, X.H. Chen, Q.T. Gao, K.S. Bi, LC–MS determination and pharmacokinetic studies of ursolic acid in rat plasma after administration of the traditional Chinese medicine preparation Lu-Ying extract, Yakugaku Zasshi 125 (2005) 509–515.
[5] K. Rath, K. Taxis, G. Walz, C.H. Gleiter, S.M. Li, L. Heide, Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of *Artemisia Annua* L. (AnnualWormwood), Am. J. Trop. Med. Hyg. 70 (2004) 128–132.
[6] E.H. Kim, J. Kim, Y.O. Son, G.H. Chung, Y.S. Jang, Modulation of antigen-specific immune responses by the oral administration of a traditional medicine, Bo-Yang-Hwan-O-Tang, Immunopharmacol. Immunotoxicol. 24 (2002) 423–440.
[7] Y.H. Kim, J.C. Ha, J.S. Do, Y.H. Choi, Y.K. Choo, W.H. Woo, H.K. Yi, P.H. Hwang, S.Y. Nam, In vitro immunomodulatory activity of Bo-Yang-Hwan-O-Tang, Immunopharmacol. Immunotoxicol. 26 (2004) 631–644.
[8] H. Watanabe, Candidates for cognitive enhancer extracted from medicinal plants: paeoniflorin and tetramethylpyrazine, Behav. Brain Res. 83 (1997) 135–141.
[9] J. Liu, J.S. Wang, L.Y. Kong, Comparative pharmacokinetics of paeoniflorin in plasma of vascular dementia and normal rats orally administrated with Danggui-Shaoyao-San or pure paeoniflorin, Fitoterapia 82 (2011) 466–473.
[10] L.C. Chen, M.H. Lee, M.H. Chou, M.F. Lin, L.L. Yang, Pharmacokinetic study of paeoniflorin in mice after oral administration of Paeoniae radix extract, J. Chromatogr. B 735 (1999) 33–40.
[11] Y.S. Wang, W.L. Deng, C.S. Que, Pharmacology and Application of Traditional Chinese Medicine, Human Health Press, Beijing, 1998, pp. 972–982, 1004–1029.
[12] G. Ye, R.R. Ma, Z.X. Li, H. Wang, H.Y. Zhu, Z.L. Sun, C.G. Huang, Determination of calycosin-7-O-β-D-glucopyranoside in rat plasma and urine by HPLC, Biomed. Chromatogr. 21 (2007) 762–767.
[13] Y.L. Yang, G.X. Xia, G. Ye, Determination of two metabolites of calycosin-7-O-β-glucopyranoside in rat urine by HPLC, Biomed. Chromatogr. 23 (2009) 48–53.
[14] J.L. Zhang, H.L. Yu, Y.X. Sheng, L. Li, M. Ye, D.A. Guo, HPLC determination and pharmacokinetic studies of salvianolic acid B in rat plasma after oral administrationof Radix Salviae Miltiorrhizae extract, Biomed. Chromatogr. 19 (2005) 15–18.
[15] Y.F. Chen, I. Jaw, M.S. Shiao, T.H. Tsai, Determination and pharmacokinetic analysis of salvianolic acid B in rat blood and bile by microdialysis and liquid chromatography, J. Chromatogr. A 1088 (2005) 140–145.
[16] H. Wu, Z.Y. Zhu, G.Q. Zhang, L. Zhao, H. Zhang, D.L. Zhu, Y.F. Chai, Comparative pharmacokinetic study of paeoniflorin after oral administration of pure

paeoniflorin, extract of Cortex Moutan and Shuang-Dan prescription to rats, J. Ethnopharmacol. 125 (2009) 444–449.

[17] X.N. Yang, Y.J. Wang, Y.S. Liu, X. Tang, Pharmacokinetics of salvianolic acids after intravenous injection, with and without *Panax quinquefolium* protopanaxadiol saponins, in rats, J. Ethnopharmacol. 117 (2008) 408–414.

[18] X.D. Wen, L.W. Qi, P. Li, K.D. Bao, X.W. Yan, L. Yi, C.Y. Li, Simultaneous determination of calycosin-7-O-β-D-glycoside, ononin, astragaloside IV, astragaloside I and ferulic acid in rat plasma after oral administration of Danggui Buxue Tang extract for their pharmacokinetic studies by liquid chromatography–mass spectrometry, J. Chromatogr. B 865 (2008) 99–105.