Simultaneous determination and pharmacokinetics of eight ginsenosides by LC-MS/MS after intravenously infusion of 'SHENMAI' injection in dogs

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Abstract: SHENMAI injection, a prescription comprised of Panax ginseng and Ophiopogon japonicas, is being extensively applied in the field of cardio-protection and immune-modulation in China. Ginsenosides are the main active components in SHENMAI injection. In order to capture and analyze the pharmacokinetic profile of major ginsenosides of SHENMAI injection in Beagle dogs, liquid chromatography equipped with electro-spray ionization and tandem mass spectrometry method was applied in simultaneous determination for protopanaxatriol type ginsenoside (Re, Rf, Rg1), protopanaxadiol type ginsenoside (Rb2, Rb1, Rd, Rc) and oleanolic acid type ginsenoside (Ro). A C18 column (150 × 2.1mm, 5μm) and a linear gradient program were used to achieve chromatographic separation, with 0.02% acetic acid solution and acetonitrile. I.S. and ginsenosides were detected by LC-MS/MS in selective reaction mode. Good linearity spanning 5- 1500ng/mL was achieved with the R^2 values higher than 0.99 for all analytes. Limit of quantification of all analytes were 3ng/mL. Intra- and inter-day precisions ranges from 0.47 to15.68 % and accuracies were within the range of 85.27-117.57%. Validated analyzing method was then used in the pharmacokinetic experiment for SMI in dogs. The results showed that the pharmacokinetic profile of protopanaxadiol, protopanaxatriol and oleanolic acid type ginsenoside were significant difference in dogs. Protopanaxadiol type ginsenosides exhibited an extremely higher level of exposure and a much slower elimination process. Whereas protopanaxatriol type ginsenosides were quickly eliminated. We concluded that 20 (S) - protopanaxadiol type ginseno sides could be a potential pharmacokinetic marker of SHENMAI injection.

Keywords*:* 'SHENMAI' injection, HPLC-ESI-MS/MS, ginsenosides, dogs, pharmacokinetics.

INTRODUCTION

Shenmai San (also known as SMS), which is an ancient prescription comprised of *Ophiopogon japonicas* and *Panax ginseng*, is a popular traditional Chinese medicine with plenty of clinical usage. It has been wildly used as a cardio-protection and immune-modulating drug for centuries. (Chen, Yao *et al*., 2012, Li, Wang *et al*., 2011, Wu, Ding *et al*., 2010, Zhang, Hu *et al*., 2010) SHENMAI injection (SMI), which is an injection derived from SMS for better efficacy, is being widely used in China for its prominent pharmacological activities. (Zhang, Hu *et al*., 2010) Ginsenosides, which is contained in *Panax ginseng,* are the main active components in SMI. (Zhang, Hu *et al*., 2010) Currently, more than 30 types of ginsenoside have already been characterized from SMI. (Haijiang, Yongjiang *et al*., 2003) Among these ginsenosides, PPD (20 (*S*) protopanaxadiol) type (ginsenoside Rb1, Rb2, Rc, Rd), PPT (20 (*S*) protopanaxatriol) type (ginsenoside Rg1, Rf, Re) and oleanolic acid type ginsenoside (ginsenoside Ro), which are categorized according to their different core structures, are the major ginsenosides. (Haijiang, Yongjiang *et al*., 2003) Structures of major ginsenosides (ginsenoside Rb1, Rb2, Rc, Rd, Rg1, Rf, Re, Ro) in SMI are shown in fig. 1.

Researchers have been focusing on several kinds of PPD and PPT type ginsenosides in the pharmacokinetic study of SMI in rodents. (Xia, Wang *et al*., 2008, Yu, Ma *et al*., 2007) However, several active ginsenosides weren't fully studied in these studies. For example, although the content of ginsenoside Ro is almost as high as the content of gisenoside Rg1 and Rb1, (Yu, Xu *et al*., 2013) never had they been monitored in the pharmacokinetic study of SMI. Therefore, oleanolic acid type ginsenoside is both important and abundant active component. (Matsuda, Samukawa *et al*., 1990, Murata, Takeshita *et al*., 2012).

Based on the fact that only simultaneous determination of seldom ginsenosides in plasma was built and used to the its pharmacokinetics study of SMI in rats and the pharmacokinetics of SMI or SMS was seldom documented in various species, (Xia, Wang *et al*., 2008, Yu, Xin *et al*., 2014, Yu, Ma *et al*., 2007) it's been more curious to us that whether there'll be any differences between the property of SMI in rodents and non-rodents.

In order to examine the pharmacokinetic profile of major ginsenosides in SMI in dogs, simultaneously

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determination of PPD, PPT and oleanolic acid type ginsenoside by LC-MS/MS was performed for the first time in present study. Additionally, pharmacokinetic experiment of SMI in dogs was also performed in present study.

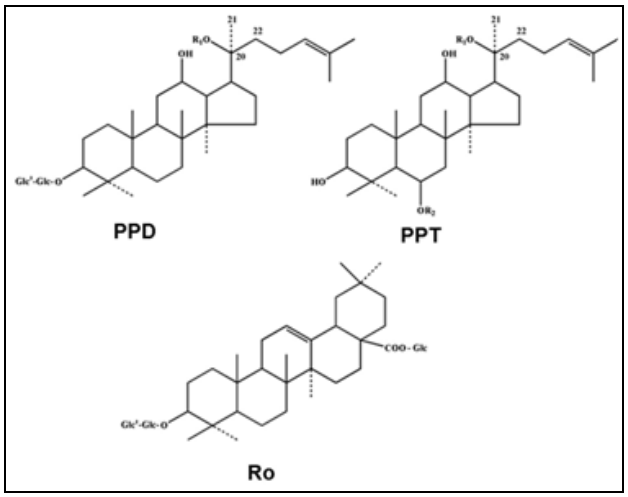

Fig. 1: Chemical structures of eight ginsenoside. (ginsenoside Rb1, R_1 = Glc^6-Glc; ginsenoside Rb2, R_1= Glc^2-Arap; Ginsenoside Rc, R_1 = Glc^6-Araf; ginsenoside Rd, R_1 = Glc; Ginsenoside Re, R_1=Glc, R_2 = O-Glc^2-Rha; ginsenoside Rf, R_1=H, R_2=O-Glc^2-Glc; ginsenoside Rg1, R_1=Glc, R_2=O-Glc; Glc=β-D-glucose; Arap=α-L-arabinose(pyranose); Araf =α-L-arabinose (furanose); Rha =α-L-rhannose)

MATERIALS AND METHODS

Chemicals and reagents

Digoxin and Ginsenoside Rb1, Re, Rg1, Ro were purchased from National Insitutes for Food and Drug Control (Beijing, China). Other Ginsenosides such as Rb2, Rc, Rd, Rf were purchased from Shanghai YuanYe Co., Ltd. (Shanghai, China). SMI (Lot No. 1207091, 10 mL/bottle, 0.2g crude drug containing 0.1g *Panax ginseng* and 0.1g *Ophiopogon japonicas*/mL) was kindly provided by Chiatai Qingchun Bao Pharmaceutical Co., Ltd (Hangzhou, China). Concentration of ginsenoside Rg1, Re, Rf, Rc, Rd, Rb1, Rb2, Ro in SMI were 0.33, 0.23, 0.08, 0.13, 0.08, 0.33, 0.13, 0.26mg/mL, respectively. Acetonitrile and methanol (both chromatographic pure) were obtained from Tedia Company (Fairfield, USA). *N*-butanol and acetic acid (both analytical pure) were purchased from Huadong Medicine Co., Ltd (Hangzhou, China). Deionized water was obtained with MilliQ Ultrapure Water Purification System (Millipore, Billerica, MA).

Equipment and chromatographic conditions

TSQ Quantum Access LC-MS/MS system (Thermo Electron Co.,) which consists of auto-sampler, quaternary pump and triple tandem quadrupole mass detector was used for the determination. Separating of analytes was achieved by a Finnigan Surveyor^{TM} HPLC system and a C18 column (Thermo BDS Hypersil, 150mm × 2.1mm, 5μm, 30^{o}C). Acetonitrile (A) and aqueous solution (pH = 5; 0.02% acetic acid, *v/v*) (B) were adobted. Flow rate was set as 200μL/min. Gradient elution program was used as follows: 0-13min, A% 30-35; 13-13.5min, A% 35-90; 13.5-15.5min, A% 90- 90; 15.5-16 min, A% 90-30; 16-20 min, A% 30- 30.

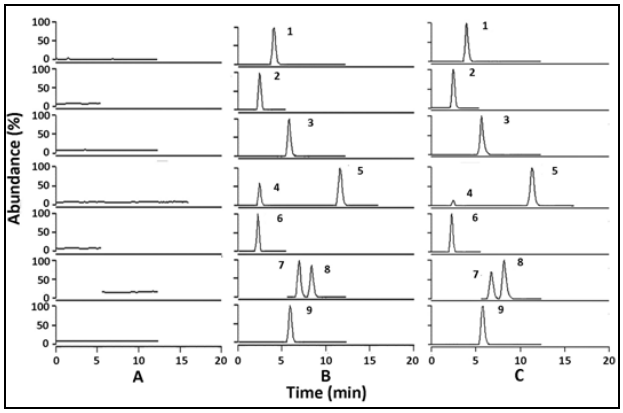

Fig. 2: Representative SRM chromatograms of the ginsenosides: (A) blank plasma; (B) plasma spiked with 500ng/mL digoxin (1), ginsenoside Rg1 (2), ginsenoside Rf (3), ginsenoside Re (4), ginsenoside Rd (5), ginsenoside Ro (6), ginsenoside Rc (7), ginsenoside Rb2 (8), ginsenoside Rb1 (9); (C) plasma sample after intravenous dripping of 'SHENMAI' injection.

Mass spectrometer used in present study was TSQ Quantum Access system. Mass spectrometer was operated in ESI positive-ion mode. SRM (Selective reaction monitoring) mode was used in quantification to detect the iron transition of m/z 1131.0→ 371.80 for Rb1, 1101.7 →343.35 for Rb2, 1101.7→343.17 for Rc, 969.4 → 789.46 for Rd, 969.4→ 789.46 for Re, 823.6 → 371.94 for Rf, 823.5 → 643.75 for Rg1, 979.1 → 641.65 for Ro and 803.0 → 392.70 for digoxin (IS). The optimized MS parameters were set as following: Spray Voltage =3000 V, Sheath Gas Pressure =35.0, Ion Sweep Gas Pressure = 0.0, Aux Gas Pressure =5, Capillary Temperature =394^{o}C, Tube Lenz Offset: -107 V, Collision Pressure =1.5 mTor, Collision Energy =44V (digoxin, ginsenoside Rf, Re, Rd), 37V (ginsenoside Rg1), 36 V (ginsenoside Ro), 57V (ginsenoside Rb1), 56V (ginsenoside Rc), 53V (ginsenoside Rb2).

Standard solution and QC (quality control) samples

Ginsenoside and I.S. (digoxin) were dissolved in methanol with the concentration of 1.01, 1.01, 1.07, 1.01, 1.06, 1.09, 1.00, 1.01 and 1.04mg/mL, respectively. Working solution of ginsenosides was obtained by further diluting. The concentration of Rb1, Rb2, Rc, Rd, Re, Rg1, Rf and Ro for standard curve ranges from 5ng/mL to 1500ng/mL. In order to validate the method, three different levels of QC samples were prepared with the

concentration of 10ng/mL, 1000ng/mL, 1500ng/mL of ginsenoside Rb1, Rb2, Rc, Rd, Re, Rg1, Rf, Ro respectively.

Plasma sample preparation

In our experiment, regular LLE (liquid liquid extraction) method was used for the preparation of plasma samples. After 100μL plasma sample was transferred into a 1.5mL tube. 10μL I.S. solution was added in followed by adding of 1.2mL *n*-butanol. All ginsenosides and IS were extracted by 10min vortexing. 10000g centrifuging was performed for 10min for each sample. Organic layer was transferred into another 1.5mL tube. Evaporation at 50°C for each sample was preceded. Finally, residue was reconstituted with 100μL mobile phase and centrifuging at 10000g was performed for 10min. Then, 10μL aliquot was injected into LC-MS/MS for analysis.

Validating of the method

LLOQ (lower limit of quantification) was defined as the lowest concentration on the calibration curve with S/N≥ 10. Interference from the plasma was observed to determine the specificity of the method. Standard curves for all 8 analytes were obtained in six different days from six independent batches. Standard curve ranging from 3 to 1500ng/mL was constructed with weighted ($1/X^2$ and $1/X$) linear regressions of the peak areas of the analyte (y) against the plasma concentration of the analytes (x, ng/mL). The ratio between ginsenosides and digoxin were obtained for calibration. Standard curves obtained were described as the form $y = a + bx$. Value of R2 was used for the determination of linearity. For evaluating inter-day precision and accuracy, RSD and recovery of quality control (QC) samples in three different levels were analyzed on three different days respectively. Meanwhile, intra-day accuracy and precision were also evaluated to determine the stability of present method. Extraction recovery of ginsenosides were further calculated by comparing the peak areas of the analytes in plasma carried through the complete preparation procedure to those spiked into the prepared blank plasma of the same concentration as those of QCs. Furthermore, evaluation of matrix effect was also preceded.

Stability of all eight ginsenosides was also investigated with all three different QC levels. Storing stability was evaluated after samples had been reserved in -80°C for 30 days. Meanwhile, freeze-thaw stability was also evaluated after three freeze-thaw cycles (-80°C to 20°C).

Pharmacokinetic application

Six Beagle dogs, weighting from 6 to 7kg, were purchased from Zhejiang Jiaxing Institution of Experimental animal (Jiaxing, China), were adopted in present study. Dogs were raised under conditions with temperature ranges from 16 to 26°C and humidity ranges from 40 to 70%. Dogs were raised with commercial diets and tap water *ad libtum* during the study.

All animal experiments were performed in strict compliance with the Institutional Animal Care and Use Committee guidelines and "Guide for the Care and Use of Laboratory Animals" by the National Research Council's (Washington, USA). Dogs were intravenously infused with 0.64 g crude drug/kg of SMI in 60 minutes. Blood were collected as following times points: Pre-dose (0 h); 0.17, 0.5, and 1 hours after starting the infusion; and 0.17, 0.5, 1, 1.5, 2, 4, 6, 9, 24, 48 and 72 hours post-administration.

Pharmacokinetic analyzing

Working models was developed by graphically evaluation of the concentration of ginsenosides in plasma. Three- and two-compartment were applied for different ginsenosides by evaluating AIC parameter (Akaike's Information Criterion). Non-compartmental parameters were calculated by Drug and Statistics software (Wannan Medical College, Anhui, China, DAS, Version 2.1.1). Data in this article were all expressed in the form of mean ± S.E.M..

RESULTS

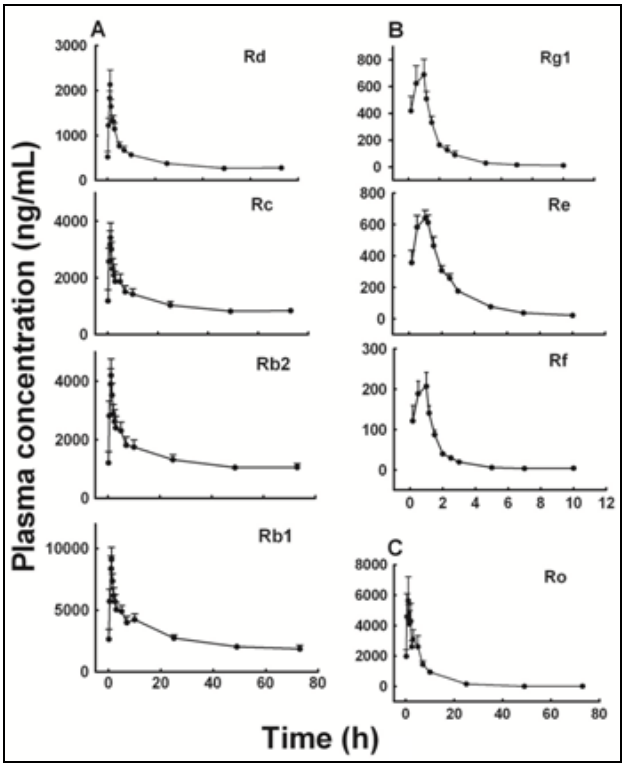

Fig. 3: The plasma concentration time curves of ginsenosides after the intravenous dripping of 'SHENMAI' injection. Each data point was shown as the form of mean ± S.E.M. (Rg1 =ginsenoside Rg1; Re =ginsenoside Re; Rf = ginsenoside Rf; Rb1 =ginsenoside; Rb2 =ginsenoside; Rc =ginsenoside Rc; Rd =ginsenoside Rd; Ro =ginsenoside Ro; A, PPD type ginsenoside; B, PPT type ginsenoside; C, oleanolic acid type ginsenoside)

Method validation

Specificity Separation of different analyst could be reviewed through specificity test. Representative chromatograms of eight ginsenosides and I.S. are shown in fig. 2. According to fig. 2, excellent separation of ginsenoside Rb2, Rb1, Rd, Rc, Rf, Re, Ro, Rg1 and Digoxin have been achieved. No significant endogenous interference from plasma could be seen. Corresponding retention time of Rb2, Rb1, Rd, Rc, Rf, Re, Ro, Rg1 and Digoxin in plasma were as follows: 10, 7.23, 13.32, 8.4, 6.34, 2.5, 2.94, 2.6 and 4.43 min.

Linearity and LLQQ Linearity and LLQQ are essential indicators for the precision for present quantification. Standard curve and LLOQ of all eight ginsenosides were shown in table 1. Correlation coefficients of each eight standard curves were all higher than 0.99. LLOQ of all ginsenosides were all 3ng/mL, which were sufficient enough to determine the ginsenosides in dog plasma.

Stability and extraction recovery Recoveries for extraction of ginsenosides were all shown in table 2. Extraction recoveries of all ginsenosides were within the range of 91.22-115.72%. Classic liquid-liquid extraction method in which *n*-butanol was involved was proven suitable for present analysis. Meanwhile, storing and freeze-thaw stability for all eight ginsenosides were also investigated. Every ginsenosides were proved stable in the experiment.

Accuray and precision Intra- & inter-day accuracy and precision at different QC concentration levels for each ginsenosides were shown in table 3. Intra-day and inter-day accuracy for all 8 ginsenosides were within the range of 85.27-119.58% and 87.10-117.57%, respectively. The intra-day & inter-day precision for all eight ginsenosides were within the range of 2.40-15.68% and 0.47-10.76%, respectively. Present method is validated both accuracy and precise for the quantification of all eight ginsenosides.

Pharmacokinetic study

The pharmacokinetics features of three different types of ginsenosides in SMI were simultaneously studied for the first time in dogs with the validated method. According to our previous research, the abundance of three different type ginsenosides in SMI was reported as follows: PPD type ginsenoside: 41.03%; PPT type ginsenoside: 42.95%; oleanolic acid type ginsenoside: 16.03%. (Yu, Xu *et al*., 2013) Although the core structure of PPD, PPT and oleanolic acid type was similar and both PPD & PPT type ginsenoside shared CYP3A4 as predominant isomer responsible for their metabolism, (Chiu, Guns *et al*., 2014, Hao, Lai *et al*., 2010, Xia, Wang *et al*., 2008) the pharmacokinetic profile of three different types of ginsenoside were proved to exhibit significant difference in dogs in the present study.

Mean plasma concentration - time curves for ginsenoside Rb1, Rb2, Rc, Rd, Re, Rg1, Rf and Ro were shown in fig. 3. Parameters for pharmacokinetic were calculated and listed in table 4.

DISCUSSION

Optimization of method

In order to develop a highly sensitive method for simultaneously determination of ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Ro and I.S., highly of ionization of all analytes at the same time is required. Although negative-ion mode have been used for the determination of several ginsenosides, (Xia, Wang *et al*., 2008, Yu, Ma *et al*., 2007) signals of analytes was much more significant when the adduction ions $[M + Na]^+$ instead of other adduct ions were monitored. It can be observed that sodium ion addition of ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Ro) and I.S. with *m/z* 1131, 1101.7, 1101.7, 969.4, 969.4, 823.6, 823.5, 979.1 and 803 in full-scan mode. Sensitivity of sodium ion adduction is sufficient for present study.

Generally, when mass spectrometry is equipped, separations between different analytes were not necessary unless isomers were found. However, in current study, the *m/z* of the adduction and production ion for ginsenoside Rb2 & Rc and Rd & Re were identical. In order to simultaneously determinate the plasma concentration of ginsenoside Rb2 & Rc and Rd & Re, separation between ginsenoside Rb2 & Rc and Rd & Re must be achieved. For ginsenoside Rd and Re which belonged to different type of ginsenosides, separation of these two were easily achieved. Unfortunately, for both ginsenoside Rb2, Rc belonged to PPD type ginsenosides and both of them shared a highly similar structure, the retention time of ginsenoside Rb2 and Rc were extremely close. Therefore, an extremely long gradient program had to be employed to achieve separation for ginsenoside Rb2 and Rc. 70% A phase was chosen as the initiating ratio for gradient program and the percentage of aqueous phase only dropped 5% in the first 13 minutes of the gradient program. Only in this way was the separation of ginsenoside Rb2 and Rc accomplished. Furthermore, 0.02 % acetic acid solution was chosen as aqueous phase for the high sensitivity of ginsenoside Ro to pH. Otherwise, the peak of ginsenoside Ro would split into two individual peaks without acetic acid. Conventionally, I.S. was employed in present study for its similar chemical form, ionization efficiency, retention time, and extraction ability to ginsenosides.

Pharmacokinetic study

It's proven in present study that the values of AUC, Cmax and t1/2 of PPT type ginsenosides (Rg1, Re, Rf) were much lower than those of PPD type (Rb1, Rb2, Rc, Rd) and oleanolic acid type ginsenosides. However, the values

Table 1: Standard curves for eight ginsenosides from 'SHENMAI' injection in dog plasma. (Rg1 = ginsenoside Rg1; Re = ginsenoside Re; Rf = ginsenoside Rf; Rb1 = ginsenoside; Rb2 = ginsenoside; Rc = ginsenoside Rc; Rd = ginsenoside Rd; Ro = ginsenoside Ro)

Component	Standard curve	Linear range (ng/mL)	R^2	LOQ (ng/mL)
Rg1	$y = 0.001 + 0.011\ x$	3- 1500	0.999	5
Rf	$y = -0.001 + 0.014\ x$	3- 1500	1.000	5
Re	$y = -0.003 + 0.009\ x$	3- 1500	0.997	5
Rd	$y = -0.020 + 0.017\ x$	3- 1500	0.999	5
Ro	$y = 0.0003 + 0.0003\ x$	3- 1500	0.998	5
Rc	$y = - 0.027 + 0.017\ x$	3- 1500	1.000	5
Rb2	$y = 0.064 + 0.018\ x$	3- 1500	0.998	5
Rb1	$y = -0.020 + 0.011\ x$	3- 1500	0.995	5

Table 2: The extraction recovery of ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Ro in dog plasma at three concentration levels. (Rg1 = ginsenoside Rg1; Re = ginsenoside Re; Rf = ginsenoside Rf; Rb1 = ginsenoside; Rb2 = ginsenoside; Rc = ginsenoside Rc; Rd = ginsenoside Rd; Ro = ginsenoside Ro; *n*=6)

Component	Spiked concentration (ng/mL)	Recovery (%)
Rg1	10	110.28
	1000	114.51
	1500	115.84
Rf	10	96.36
	1000	111.63
	1500	106.47
Re	10	105.52
	1000	99.28
	1500	98.12
Rd	10	105.22
	1000	109.88
	1500	110.71
Ro	10	108.62
	1000	96.77
	1500	105.50
Rc	10	115.72
	1000	104.62
	1500	101.50
Rb2	10	116.31
	1000	111.14
	1500	106.88
Rb1	10	91.22
	1000	112.77
	1500	106.99

of Cl for PPT type ginsenosides (Rg1, Re, Rf) were much larger than other ginsenosides. Based on the fact that PPD type ginsenosides exhibited an extremely higher level of exposure and a much slower elimination process, PPD type ginsenosides could be considered as potential PK marker in pharmacokinetic research of SMI. On the other hand, pharmacokinetic profile of ginsenoside Ro, which is a kind of abundant content, was reported for the first time in experimental animals.

Pharmacokinetic profile of SMI in dogs was firstly described in present research. Comparing with pharmacokinetic profiles of SMI in the rats and rabbits, (Xia, Wang *et al*., 2008, Yu, Xin *et al*., 2014, Yu, Ma *et al*., 2007) the elimination of PPD type ginsenosides in dogs was proved significantly longer. $T_{1/2}$ of ginsenoside Rd and Rb1 in rats were about 22 hours while the values were about four-time in beagle dogs. Since cytochrome P450 was crucial to the metabolism of ginsenosides and significant difference of the activity of cytochrome P450 between different species have already been reported, (Chiu, Guns *et al*., 2014, Hao, Lai *et al*., 2010, Shimada, Mimura *et al*., 1997) the difference of pharmacokinetic profile between rodent and large non-rodent animals was hypothesized to be induced by different activities of cytochrome P450 in different species.

Table 3: The intra-day and inter-day precision and accuracy of ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Ro at three concentration levels in dog plasma. (Rg1 =ginsenoside Rg1; Re =ginsenoside Re; Rf =ginsenoside Rf; Rb1 =ginsenoside; Rb2 = ginsenoside; Rc = ginsenoside Rc; Rd = ginsenoside Rd; Ro = ginsenoside Ro; *n*=6)

Component	Spiked concentration (ng/mL)	Intra-day (*n*=5)		Inter-day (*n*=5)	
		Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Rg1	10	103.75	15.68	106.95	10.76
	1000	111.15	5.40	104.23	8.80
	1500	112.44	7.78	106.55	6.58
Rf	10	107.88	2.40	108.82	1.88
	1000	106.68	6.82	104.15	2.49
	1500	106.02	7.62	105.54	0.47
Re	10	119.58	3.66	117.57	2.31
	1000	106.17	3.84	103.13	6.01
	1500	100.73	8.70	101.65	6.58
Rd	10	119.45	5.23	112.87	5.25
	1000	109.11	7.46	105.03	5.38
	1500	109.73	8.97	109.41	4.08
Ro	10	110.85	9.85	92.66	2.79
	1000	85.27	10.19	87.78	3.31
	1500	86.49	13.14	87.10	2.90
Rc	10	106.03	4.03	109.44	7.53
	1000	106.25	4.67	104.79	1.88
	1500	110.96	7.24	109.63	2.01
Rb2	10	105.77	9.30	112.70	5.59
	1000	111.32	5.18	109.17	7.77
	1500	118.95	5.18	114.79	6.08
Rb1	10	98.70	8.13	109.20	8.88
	1000	108.33	7.12	107.74	2.78
	1500	113.96	6.26	113.04	3.74

Table 4: Pharmacokinetic parameters for ginsenosides from SMI in dogs plasma. (Rg1 = ginsenoside Rg1; Re = ginsenoside Re; Rf = ginsenoside Rf; Rb1 = ginsenoside; Rb2 = ginsenoside; Rc = ginsenoside Rc; Rd = ginsenoside Rd; Ro = ginsenoside Ro; *n*=6)

Component	AUC (μg/L·h)	$t_{1/2}$ (h)	Cl (L/h/kg)	C_{max} (μg/L)
Rg1	1225±189	1.7±0.18	568±75	735±107
Re	1678±105	1.7±0.25	380±24	683±46
Rf	326±133	1.14±0.5	2095±856	228±93
Rc	79051±7844	66.6±9.03	4.7±0.3	3436±540
Rd	30862±2264	54.2±11.8	13.9±2.0	2101±293
Rb1	199055±20540	52.0±10.6	2.2±0.4	9263±1013
Rb2	99590±10755	97.61±36.5	3.4±0.5	4255±567
Ro	33520±4010	8.8±2.4	6.0±1.2	6374±1392

CONCLUSION

A sensitive and rapid analytical method capable of determining PPD, PPT and oleanolic acid type ginsenoside from SMI in canine plasma was developed in present research. The validated method was then performed in the pharmacokinetic study of SMI in dogs. The pharmacokinetic study of SMI was performed in dogs for the first time. The pharmacokinetic profile of ginsenoside Ro was reported in the experimental animals as a kind of both abundant and important content in SMI. The results showed that the pharmacokinetic profile of PPD, PPT and oleanolic acid type ginsenoside were significant different in dogs. PPD type ginsenosides exhibited an extremely higher level of exposure and a much slower elimination process. Whereas PPT type ginsenosides were quickly eliminated. We concluded that 20 (S) - protopanaxadiol type ginsenosides could be a potential PK marker of SMI.

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