Journal of Chromatography B, 878 (2010) 1982–1986

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination and pharmacokinetics of gastrodin in human plasma by HPLC coupled with photodiode array detector

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ARTICLE INFO

Article history:
Received 15 October 2009
Accepted 22 May 2010
Available online 31 May 2010

Keywords:
Gastrodin
HPLC
Pharmacokinetics
Human plasma

ABSTRACT

In present study, an HPLC method coupled with photodiode array detector (HPLC-PDA) was established for determination and pharmacokinetics of gastrodin (GAS) in human plasma after an oral administration of GAS capsule. In the method, ethanol and dichloromethane were respectively used for deproteinization and purification during the sample preparation procedure. Separation of GAS was achieved on an AichromBond-AQ C18 column (5 μm, 150 mm × 4.6 mm) with the mobile phase of methanol–0.1% phosphoric acid solution (2:98, v/v) at a flow rate of 0.8 ml/min. The wavelength was set at 220 nm and the injection volume was 20 μl. Under the conditions, the calibration curve was linear within the concentration range of 50–4000 ng/ml with the correlation coefficient (r) of 0.99554 (weight = $1/X^2$) and the lower limit of quantification (LLOQ) was 50 ng/ml. The inter- and intra-day precisions were less than 11% and the accuracies (%) were within the range of 95.55–103.78%. The extraction recoveries were over 65% with RSDs less than 5.50%. The GAS was proved to be stable under tested conditions. Thus, the method was valid enough to be applied for pharmacokinetic study of GAS in human plasma. The pharmacokinetic parameters of GAS in human plasma after an oral administration of 200 mg GAS capsule were described as: C_{max}, 1484.55 ± 285.05 ng/ml; T_{max}, 0.81 ± 0.16 h; $t_{1/2\alpha}$, 3.78 ± 2.33 h; $t_{1/2\beta}$, 6.06 ± 3.20 h; $t_{1/2Ka}$, 0.18 ± 0.53 h; K_{12}, 0.18 ± 0.41/h; K_{21}, 0.20 ± 0.16/h; K_{10}, 4.11 ± 15.81/h; V1/F, 180.35 ± 89.44 L; CL/F, 62.50 ± 140.03 l/h; $AUC_{0\to t}$, 5619.41 ± 1972.88 (ng/ml) h; and $AUC_{0\to\infty}$, 7210.26 ± 3472.74 (ng/ml) h, respectively. These will be useful for the clinical application of GAS.

1. Introduction

Tianma, the dried rhizome of *Gastrodia elata* Bl., is an important and notable Chinese herb, which has been used for the treatment of neurological diseases and psychiatric disorders in China, Japan, Taiwan and eastern Asia for centuries [1–3]. Gastrodin (GAS), one of the major and active constituents with advantageous properties, could be applied for the treatment of neurasthenia, dizziness, amnesia, dementia, headache, tetanus, and as adjunctive therapy for epilepsy [3]. GAS was involved in many pharmacological activities, such as memory consolidation and retrieval [4], as well as the effects of anti-convulsion, neuroprotection [5,6], relaxation on smooth muscle [7], anti-cell death [8], anti-apoptosis [5,9], antidepressant-like [10], anti-angiogenesis, anti-inflammation and analgesia, and in vivo and in vitro inhibitory on NO production [11].

The increasing studies on pharmacological activities and clinical uses of GAS require relevant quantitative method to assay its level in biological samples and its pharmacokinetics. The methods of HPLC coupled with UV [12–16], electrospray ionization mass spectrometry (ESI-MS) [17], and ESI-MS–MS [18,19] were established and applied for determination and pharmacokinetic studies of GAS in dog plasma [12] and rat biological samples [13–19], such as rat plasma, blood, brain, brain dialysate, cerebrospinal fluid (CSF), bile, liver, urine and faeces. The previous results suggest that GAS was rapidly eliminated from the plasma, quickly distributed to brain tissues and bile, and quickly transformed into *p*-hydroxybenzylalcohol (HBA) after administration; the majority of GAS was excreted to urine but not bile [12–19]. These pharmacokinetic studies were all in animal models, there has been few report on pharmacokinetics of GAS in human body. It was only reported that the main pharmacokinetic parameters were: C_{max} = 94.66 ± 23.52 μg/ml, T_{max} = 1.0 ± 0.0 h, $t_{1/2}$ = 4.16 ± 0.82 h, $AUC_{0\to t}$ = 383.97 ± 78.07 μg/ml h, respectively, after a single dose injection of 600 mg to volunteers, according with two-compartment model [20]. Therefore, the pharmacoki-

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doi:10.1016/j.jchromb.2010.05.034

netic studies of GAS in human should be performed to provide more information for its clinical use.

In the present study, HPLC-PDA method was developed and applied to pharmacokinetic study of GAS in human plasma after an oral administration of GAS capsule, and useful and meaningful results were obtained.

2. Experimental

2.1. Materials and reagents

Standard GAS (GAS, >99.8%) was obtained from China's National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). Methanol, dichloromethane, phosphoric acid, and ethanol were purchased from Fisher ChemAlert Guide (NJ, USA), Tedia (Fairfield, OH, USA), Samtec Tianjin Chemical Reagent Co., Ltd. (Tianjin, China), and Shandong Institute of Chemical Industry (Jinan, Shandong Province, China), respectively. Methanol and dichloromethane were HPLC grade, and other reagents were AR grade. Water was purified by Millipore water purification system. Blank plasma was obtained from the volunteers before the administration of GAS.

2.2. Instruments and chromatographic conditions

The chromatographic system consisted of two waters 515 pumps, a waters 2996 Photodiode Array (PDA) detector, 7725i sampling valve with 20 μl loop and Waters Empower 2.0 chromatography software. Separation was performed on an AichromBond-AQ C18 column (5 μm, 150 mm × 4.6 mm, Able Industries, Miami, FL, USA). The mobile phase consisted of methanol–0.1% phosphoric acid solution (2:98, v/v) at a flow rate of 0.8 ml/min. The wavelength was set at 220 nm. The injection volume was 20 μl.

2.3. Preparation of stock and working solutions

Standard stock solution was prepared by dissolving standard GAS in methanol at the concentration of 1 mg/ml and stored at 4 °C. Working standard solutions were prepared freshly by diluting stock solution in purified water at concentrations of 500, 1000, 2000, 2500, 5000, 10,000, 15,000, 20,000, 30,000 and 40,000 ng/ml for GAS.

2.4. Preparation of standard and quality control (QC) samples

Eight calibration standards were prepared by spiking 900 μl of blank plasma with 100 μl of each working solution to obtain GAS plasma concentrations of 50, 100, 200, 500, 1000, 2000, 3000 and 4000 ng/ml. Quality control (QC) samples were prepared to obtain GAS plasma concentrations of 100, 500 and 3000 ng/ml. The spiked plasma samples (standards and QC samples) were pretreated and detected in each analytical batch along with the unknown samples.

2.5. Sample preparation for the analysis of GAS

To a 1 ml plasma sample, 4 ml of ethanol was added, and the content was vigorously mixed for 2 min. After centrifugation at 10,000 rpm for 5 min, the supernatant was all transferred and dried under a nitrogen stream in a water bath at 60 °C. The residue was reconstituted in 200 μl of purified water and followed by the addition of 200 μl of dichloromethane. The content was mixed for 1 min and centrifuged for 5 min at 10,000 rpm. The supernatant was transferred and 20 μl was injected into the HPLC system for analysis.

2.6. Assay validation

Chromatogram comparison of blank plasma, standard solution, blank plasma spiked with standard and human plasma samples was conducted to evaluate the specificity and selectivity of the method. Calibration curves were constructed from by the peak area versus plasma concentrations using a $1/X^2$ weighted linear least-squares regression model. A correlation of more than 0.99 was desirable. Concentrations of QC and unknown samples were calculated by interpolation from the calibration curves. Intra- and inter-day precisions were determined by assessing measured results of QC samples at low, medium and high concentrations. Precision was expressed as the relative standard deviation (RSD, %) and expected to be less than 15% at all concentrations. Accuracy (%) was determined as the percentage difference between the mean measured concentrations and the spiked concentrations. The LLOQ of the assay, defined as the lowest concentration on the standard curve, could be quantified with accuracy within 20% of nominal and precision not exceeding 20%. Extraction recoveries were determined by comparing peak areas obtained from blank plasmas spiked with GAS standard with those of un-extracted standard solutions at the same nominal concentrations of GAS. Freeze-thaw stability was checked after two cycles. Short-term stability and long-term stability were determined by assessing QC samples stored at −20 °C for 1 and 7 days, respectively. Stability was checked by comparing measured results with those of freshly prepared samples of the same concentration.

2.7. Pharmacokinetic study

Eighteen Chinese healthy male subjects, aged 20.9 ± 1.7 years, weighted 62.5 ± 5.4 kg and heighted 171.1 ± 6.0 cm, were randomly selected into pharmacokinetic study. GAS capsule was orally administered at a dose of 200 mg. Blood samples (4 ml) were collected prior to administration, and at 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0 and 16.0 h after a single dose, respectively. Blood samples were immediately heparinized and centrifuged at 3000 rpm for 10 min, and the supernatants were placed into 2-ml polypropylene tubes and stored at −20 °C until analysis.

The concentrations of GAS in plasma were determined by the established method, and the main pharmacokinetic parameters were calculated and analyzed using Drug and Statistics Software (DAS), version 2.0 (Mathematical Pharmacology Professional Committee of China).

3. Results and discussion

3.1. Analytical characteristics

3.1.1. Optimization of chromatographic conditions

Different mobile phases were previously used to obtain well separation of GAS from endogenous-related substances [12–19]. In present method, several mobile phases were also investigated to optimize analytical performance. Methanol–0.1% phosphoric acid solution (2:98, v/v) at a flow rate of 0.8 ml/min on an analytical column (AichromBond-AQ C18 column; 5 μm, 150 mm × 4.6 mm) was proved to be the best chromatographic conditions. The maximum absorption wavelength for GAS was at 220 nm. Under these conditions, the GAS obtained better peak shape and higher response, and could be eluted within 25 min with retention time of 18.74 ± 0.27 min.

3.1.2. Extraction procedure development

To reduce the endogenous-related substances in plasma the method of precipitation of protein is usually performed. Good chromatographic results could be obtained for GAS with the extracts

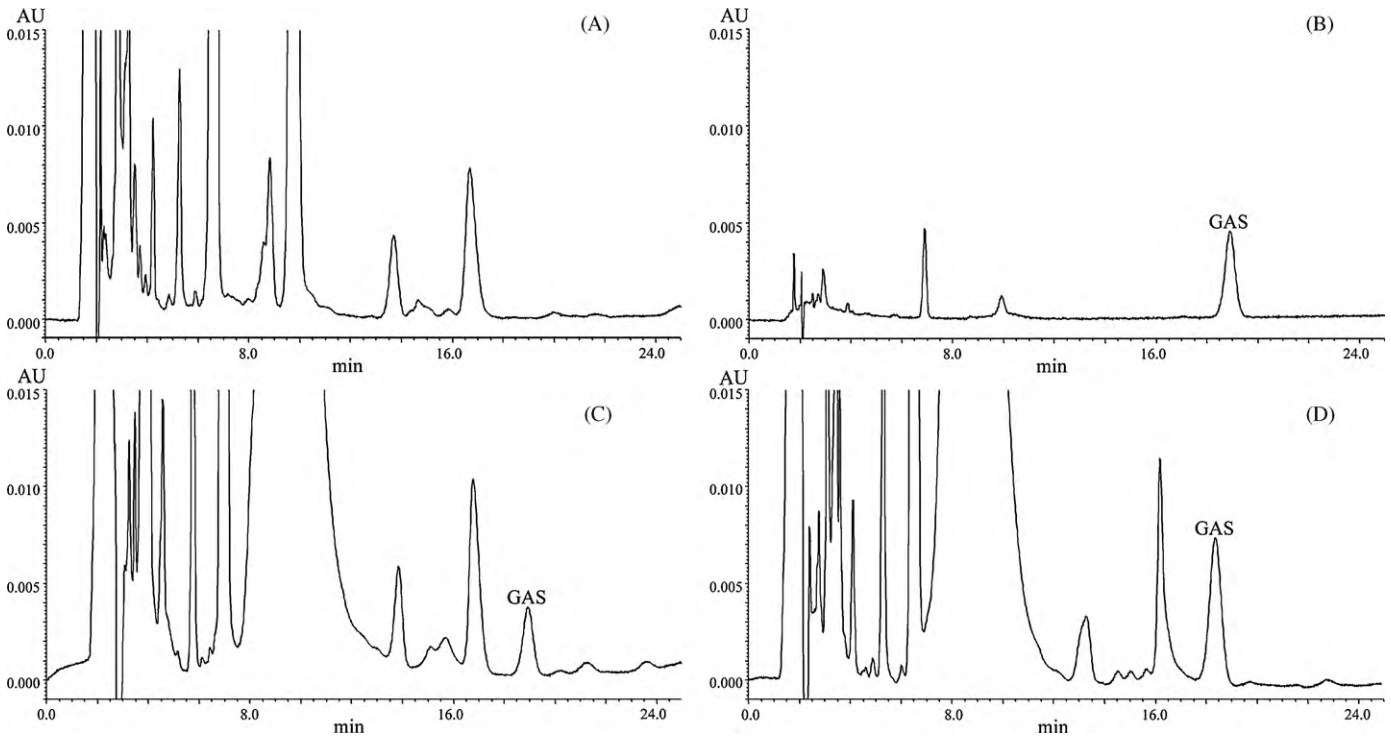

Fig. 1. Typical chromatograms obtained from samples of blank plasma (A), standard solution at 2500 ng/ml (B), blank plasma spiked with GAS standard at 500 ng/ml (C), and plasma at 0.5 h after an oral administration of GAS capsule (D).

of sample deproteinized merely by methanol, ethanol, acetonitrile or perchloric acid [12–19]. In present method, ethanol was used as the precipitant and the ratio of ethanol to plasma was explored. The results showed that the best protein precipitation was achieved at the ratio of 4:1, but some endogenous compounds affected the separation of GAS. The dichloromethane was used to remove endogenous compounds after reconstitution of the residue, and good performance was obtained.

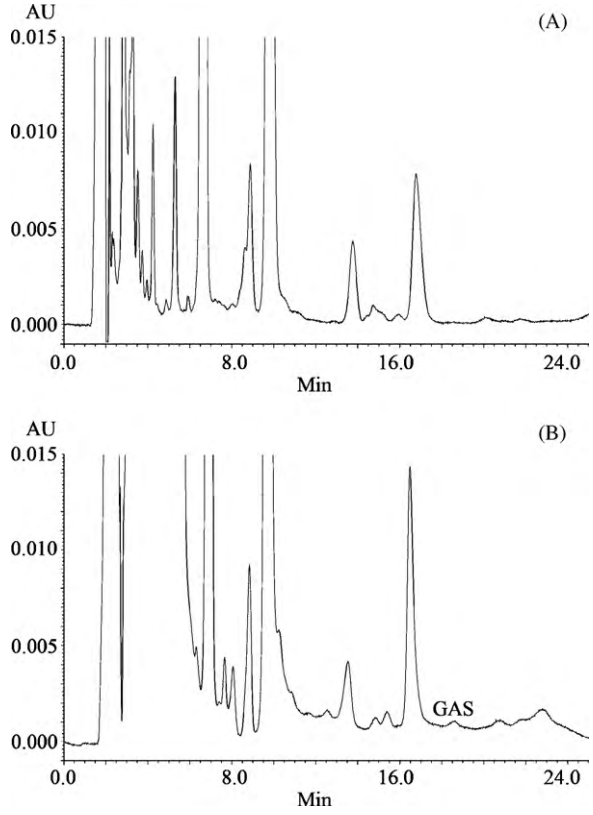

Fig. 2. The chromatograms obtained from blank plasma (A) and blank plasma spiked with GAS standard at 50 ng/ml (LLOQ, B).

It should be notice that no internal standard was used in present study, although phloroglucinolum [16] and zolpidem [18] were used as internal standards previously. An internal standard, though desirable, is not essential in the method as described. Since external standards could be prepared along with the unknown samples and subsequently treated in parallel with the latter, an internal standard may not be necessary. In present study, the correlation was satisfactory and there appeared to be no need for an internal standard.

3.2. Method validation

3.2.1. Specificity and selectivity

Typical chromatograms are shown in Fig. 1, which were obtained from samples of blank plasma, standard solution at 2500 ng/ml, blank plasma spiked with GAS standard at 500 ng/ml, and plasma at 0.5 h after an oral administration of GAS capsule. In these chromatograms, there was little interference observed. The identification of the GAS peak was achieved by comparing retention time with GAS standard. The absence of endogenous interference clearly demonstrated the good specificity and selectivity of the method.

3.2.2. Linearity and sensitivity

The calibration curve was linear within the concentration range of 50–4000 ng/ml in human plasma. The regression equation was $Y = 158.18833X - 524.21814$ ($n = 5$) and the correlation coefficient (r) was 0.99554 (weight = $1/X^2$). The LLOQ of the assay was 50 ng/ml for GAS. The reproducibility of LLOQ was determined by examining five LLOQ samples independent from the calibration curve, and the accuracy and precision was 105.46 and 8.92%, respectively. The typical chromatograms of blank plasma and LLOQ sample are shown

Table 1
Precision, accuracy and extraction recovery of GAS in human plasma.

Spiked (ng/ml)	Intra-day (n = 5)			Inter-day (n = 25)			Extraction recovery (%)[a,c] (n = 5)
	Measured (ng/ml)[a]	RSD (%)	Accuracy (%)[b]	Measured (ng/ml)[a]	RSD (%)	Accuracy (%)[b]	
100	96.326 ± 8.552	8.88	96.33	95.551 ± 9.941	10.40	95.55	70.94 ± 3.90
500	514.922 ± 15.851	3.08	102.98	513.022 ± 18.348	3.58	102.60	76.02 ± 3.44
3000	3113.304 ± 57.260	1.84	103.78	2997.731 ± 79.693	2.66	99.92	78.35 ± 2.16

[a] Mean ± SD.
[b] Accuracy (%) = (mean of measured concentration/spiked concentration) × 100.
[c] Extraction recovery (%) = (peak area obtained from blank plasma spiked with GAS)/(peak area obtained from GAS standard solution at the same nominal concentration) × 100.

Table 2
Stability of GAS in human plasma (n = 5).

Spiked (ng/ml)	Freeze-thaw bias (%)		Stored at −20 °C bias (%)	
	Once	Twice	1 day	7 days
100	−0.20	−3.91	3.38	−3.91
500	4.76	1.80	1.80	1.77
3000	5.54	−1.29	3.23	−1.29

in Fig. 2. The quantitative data suggested that the LLOQ of 50 ng/ml met the criteria. This method obtained the similar sensitivity with the analysis by microdialysis sampling system and UV detector [13], and it is more sensitive than that with UV detector [12,14,15], but less sensitive than that coupled with MS [16,17]. In all, the method is sensitive enough to determine GAS concentrations after an oral administration of GAS capsule.

3.2.3. Precision, accuracy and extraction recovery

The overall inter- and intra-day precision, accuracy and extraction recoveries of GAS in human plasma at QC concentrations are shown in Table 1. The precisions (RSD, %) for all were less than 11% and the accuracies (%) were within the range of 95.55–103.78%. The results indicated that the assay has remarkable reproducibility with acceptable accuracy and precision. The mean extraction efficiencies were all over 65% with RSDs less than 5.50%. The extraction efficiency is within the acceptance criteria.

3.2.4. Stability

The GAS was found to be stable in human plasma kept at −20 °C for 7 days, or two freeze-thaw cycles at −20 °C. The results are similar with previous reports [12,14–16]. All values of bias obtained were within ±15%, which are shown in Table 2.

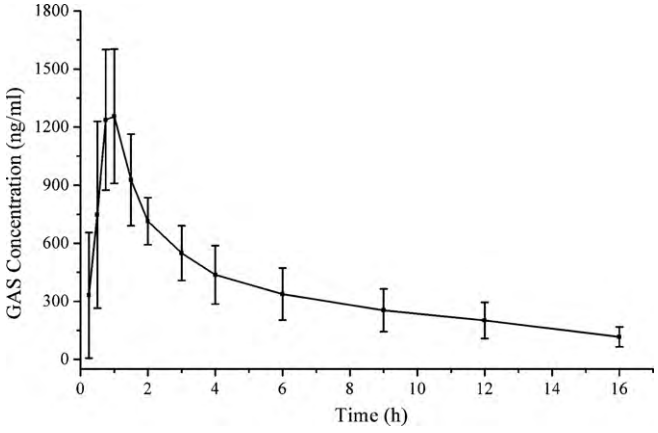

Fig. 3. Mean plasma–time profile of GAS in human plasma after an oral administration of 200 mg GAS capsule. Each point represents the mean ± SD (n = 6).

3.2.5. Application – pharmacokinetic study

The established method was applied to analysis of plasma samples after an oral administration of 200 mg GAS capsule. The concentrations of GAS and the mean plasma concentration–time profile of GAS are respectively shown in Table 3 and Fig. 3. A two-compartment model was used to estimate the pharmacokinetic parameters of GAS in human plasma, which are shown in Table 4. The results differed from previous reports [12–17], which might be due to different animals and biological samples. After the oral

Table 3
Quantization of GAS in human plasma by HPLC (ng/ml).

No.	t (h)											
	0.25	0.5	0.75	1	1.5	2	3	4	6	9	12	16
1	808.905	1278.543	1535.579	1015.919	815.161	736.337	578.103	544.680	397.228	261.257	214.036	178.002
2	789.125	1288.114	1573.420	1283.687	1180.883	1021.127	944.693	815.161	702.939	536.558	378.002	132.797
3	1285.348	1463.523	1222.018	1006.926	718.950	599.069	412.246	366.967	274.912	120.486	115.801	81.554
4	426.892	1290.626	1518.955	959.894	728.335	602.018	336.973	229.315	172.163	136.947	107.171	78.961
5	536.016	1501.597	1259.376	1145.446	1017.812	659.814	583.815	571.563	542.861	471.025	424.312	203.661
6	166.385	245.743	908.989	1082.298	887.119	637.603	519.560	441.752	258.984	204.145	177.928	93.378
7	210.245	745.159	1468.304	1067.675	899.617	845.901	740.127	674.554	449.453	247.183	216.662	74.076
8	233.659	561.391	1039.235	845.257	732.023	695.111	535.368	519.827	480.331	328.147	221.425	132.797
9	179.231	473.216	1228.035	1277.452	1006.275	845.901	676.754	445.326	305.657	239.914	181.059	69.048
10	165.711	1256.486	2015.036	1731.025	850.495	732.794	513.010	313.184	247.905	174.410	100.544	74.724
11	160.041	635.036	1162.910	1797.335	1595.372	716.296	546.686	511.661	404.735	374.093	357.547	255.839
12	161.113	994.195	1453.363	1009.674	794.051	618.192	535.368	450.233	305.657	220.849	174.632	85.720
13	163.332	248.022	950.626	862.533	670.935	569.978	420.525	358.747	261.571	185.725	179.312	101.589
14	136.522	355.028	526.774	1899.815	1339.176	808.617	548.582	280.319	267.811	254.536	179.529	145.349
15	127.995	394.078	1035.876	1811.143	793.032	632.208	425.542	325.756	243.003	176.098	152.146	107.982
16	102.442	164.180	583.331	1548.800	950.626	820.117	549.437	341.909	231.307	181.940	117.711	75.978
17	190.665	320.958	1381.628	1057.026	813.916	767.136	626.789	415.349	296.259	255.327	178.086	91.959
18	119.483	233.259	1403.854	1199.647	891.657	552.587	392.875	261.332	235.351	201.956	144.455	110.489
Mean	331.284	747.175	1237.073	1255.642	926.969	714.489	549.247	437.091	337.674	253.922	201.131	116.328
SD	314.792	469.034	353.035	336.693	230.079	117.982	137.407	146.937	130.635	107.299	90.766	49.801

Table 4
Pharmacokinetic parameters of GAS in human plasma ($n = 18$) after an oral administration of 200 mg GAS capsule (mean ± SD).

Parameter	Unit	Value
C_{max}	ng/ml	1484.55 ± 285.05
T_{max}	h	0.81 ± 0.16
$t_{1/2\alpha}$	h	3.78 ± 2.33
$t_{1/2\beta}$	h	6.06 ± 3.20
$t_{1/2Ka}$	h	0.18 ± 0.53
K_{12}	1/h	0.18 ± 0.41
K_{21}	1/h	0.20 ± 0.16
K_{10}	1/h	4.11 ± 15.81
V1/F	l	180.35 ± 89.44
CL/F	l/h	62.50 ± 140.03
$AUC_{0\to t}$	(ng/ml) h	5619.41 ± 1,972.88
$AUC_{0\to\infty}$	(ng/ml) h	7210.26 ± 3,472.74

C_{max}, the maximum plasma concentration; T_{max}, the time to reach C_{max}; $t_{1/2\alpha}$, distribution half-life (the half-life of the α phase); $t_{1/2\beta}$, elimination half-life (the half-life of the β phase); $t_{1/2Ka}$, absorption half-life; K_{12}, distribution rate constant for transferring the drug from the central to peripheral compartment; K_{21}, transfer rate constant from peripheral to central compartment; K_{10}, elimination rate constant; V1/F, apparent central volume of distribution; CL/F, the apparent oral elimination clearance; $AUC_{0\to t}$, the area under the plasma concentration–time curve from time zero to the last sampling time; $AUC_{0\to\infty}$, the area under the plasma concentration–time curve from time zero to infinity.

administration of GAS capsule, GAS was rapidly absorbed into blood ($t_{1/2Ka} = 0.18$ h) and reached a peak concentration (C_{max}) at 0.81 h. The concentration of GAS in plasma declined, due to its excretion to urine, and distribution to bile and brain, as well as its biotransformation into HBA [4,14–17]. The results are useful and meaningful information for the clinical administration of GAS.

4. Conclusion

The method established in the present study is simple, useful and sensitive for assay of GAS in human plasma after an oral administration of GAS capsule. The method shows good recovery, accuracy and precision, indicating that it is valid enough to meet the requirement for the pharmacokinetic study of GAS. The pharmacokinetic results provide further information for the clinical application of GAS.

Acknowledgement

We thank Professor Wang Benjie, from Institute of Clinical Pharmacology, Qilu Hospital, Shandong University, China, for his help in estimating and analyzing main pharmacokinetic parameters.

References

[1] X. Gong, N.J. Sucher, Trends Pharmacol. Sci. 20 (1991) 191.
[2] Z.J. Zhang, Life Sci. 75 (2004) 1659.
[3] L.M. Ojemann, W.L. Nelson, D.S. Shin, A.O. Rowe, R.A. Buchanan, Epilepsy Behav. 8 (2006) 376.
[4] M.T. Hsieh, C.R. Wu, C.F. Chen, J. Ethnopharmacol. 56 (1997) 45.
[5] C.L. Hsieh, J.J. Lin, S.Y. Chiang, S.Y. Su, N.Y. Tang, G.G. Lin, I.H. Lin, C.H. Liu, C.Y. Hsiang, J.C. Cheng, T.Y. Ho, J. Ethnopharmacol. 109 (2007) 241.
[6] H.J. Kim, K.D. Moon, S.Y. Oh, S.P. Kim, S.R. Lee, Neurosci. Lett. 314 (2001) 65.
[7] J. Hayashi, T. Sekine, S. Deguchi, Q. Lin, S. Horie, S. Tsuchiya, S. Yano, K. Watanabe, F. Ikegami, Phytochemistry 59 (2002) 513.
[8] H.J. Kim, K.D. Moon, D.S. Lee, S.H. Lee, J. Ethnopharmacol. 84 (2003) 95.
[9] N.K. Huang, Y.L. Lin, J.J. Cheng, W.L. Lai, Life Sci. 75 (2004) 1649.
[10] B.H. Zhou, X.J. Li, M. Liu, Z. Wu, X.M. Hu, Fitoterapia 77 (2006) 592.
[11] E.K. Ahn, H.J. Jeon, E.J. Lim, H.J. Jung, E.H. Park, J. Ethnopharmacol. 110 (2007) 476.
[12] L.L. Li, Z.R. Zhang, T. Gong, L.L. He, L. Deng, J. Pharm. Biomed. Anal. 41 (2006) 1083.
[13] L.C. Lin, Y.F. Chen, T.R. Tsai, T.H. Tsai, Anal. Chim. Acta 590 (2007) 173.
[14] Q. Wang, G. Chen, S. Zeng, Int. J. Pharm. 341 (2007) 20.
[15] Q. Wang, G. Chen, S. Zeng, J. Pharm. Biomed. Anal. 46 (2008) 399.
[16] K.X. Liu, G.Z. Han, Y.L. Chang, C.Y. Su, N.Y. Tang, Y.R. Chen, Biomed. Chromatogr. 2 (1987) 17.
[17] W. Zhang, Y.X. Sheng, J.L. Zhang, Phytomedicine 15 (2008) 844.
[18] Y.F. Lv, X. Hu, W.M. Cheng, Y.L. Ni, K.S. Bi, Chromatographia 68 (2008) 105.
[19] L.C. Lin, Y.F. Chen, W.C. Lee, Y.T. Wu, T.H. Tsai, J. Pharm. Biomed. Anal. 48 (2008) 909.
[20] J. Luo, Z.L. Wu, H.X. Wang, J. Chin. PLA 22 (2006) 391.