Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 779–785
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

HPLC–APCI–MS for the determination of teprenone in human plasma: Method and clinical application

Li Ding [a,*], Tian Zhu [a], Qinxin Song [a], Yindi Zhang [b], Jianping Shen [b]

[a] *Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China*

[b] *Organization for State Drug Clinical Trial, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China*

Received 11 February 2007; received in revised form 18 March 2007; accepted 21 March 2007
Available online 30 March 2007

Abstract

A sensitive high performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (HPLC–APCI–MS) method for the determination of teprenone (GGA) in human plasma using menatetrenone as the internal standard (I.S.) was established. After protein precipitation with ethanol, the plasma sample was extracted by cyclohexane and separated by high performance liquid chromatography on a reversed phase C_8 HPLC column with a mobile phase of water–methanol (4:96, v/v). GGA was determined with atmospheric pressure chemical ionisation–mass spectrometry (APCI–MS). HPLC–APCI–MS was performed in the selected ion monitoring (SIM) mode using target ions at $[M+H]^+$ m/z 331.3 for GGA and $[M+H]^+$ m/z 445.4 for the I.S. Calibration curve was linear over the range of 0.3–1000 ng/ml. The lower limit of quantification was 0.3 ng/ml. The intra- and inter-batch variability values were less than 7.8% and 8.7%, respectively. The method was successfully applied in the pharmacokinetic study in which plasma concentrations of GGA in 20 healthy Chinese volunteers were determined up to 24 h after administration of capsule containing 50 mg GGA. The maximum GGA plasma concentration (C_{max}) was 246.9 ± 85.4 ng/ml, the elimination half-life ($t_{1/2}$) was 3.38 ± 1.20 h, and the time to the C_{max} was 5.35 ± 1.39 h.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Teprenone; HPLC–APCI–MS; Pharmacokineics

1. Introduction

Teprenone (Fig. 1A), 6,10,14,18-tetramethyl-5,9,13,17-nonadecatetaene-2-one, an acyllic polyisopresnoid, which is known as tetraprenylacetone or geranylgeranylacetone (GGA), is an anti-ulcer drug developed in Japan, and clinically used for the treatment of gastric ulcer and gastritis [1]. This drug is also known to increase gastric mucus level in the ulcerated and intact regions of the stomach of patients. It has been shown that teprenone exerts a protective effect against acute gastric mucosal lesions in various in vivo experimental models through preservation of gastric mucus synthesis and secretion. It has also been reported that teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats [2]. The pharmacokinetic profile of GGA in humans has not been reported so far. As entrusted by Eisai China Inc., the investigation of the pharmacokinetics of the teprenone capsule was carried out. To evaluate the pharmacokinetics of GGA in humans, an extremely sensitive method is required. A GC–MS method with a lower limit of quantification (LLOQ) of 5 ng/ml [3] and an HPLC-fluorescence derivatization method with an LLOQ 20 ng/ml [4] were reported to determine the concentration of GGA in human plasma. These two methods offer the reliable analysis of GGA in human plasma. But the sample preparation procedures of the two methods were tedious and time-consuming. Moreover, the LLOQ of 5 ng/ml or 20 ng/ml was not sensitive enough for the pharmacokinetic study of GGA, because the plasma concentration levels of GGA on the terminal elimination phase were below 5 ng/ml. In this paper, we reported a simple and sensitive HPLC–APCI–MS method that can determine GGA plasma concentration as low as 0.3 ng/ml and has been used to evaluate the pharmacokinetics of GGA in humans.

* Corresponding author. Tel.: +86 25 8327 1289; fax: +86 25 8327 1289.
E-mail address: dinglidl@hotmail.com (L. Ding).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.03.021

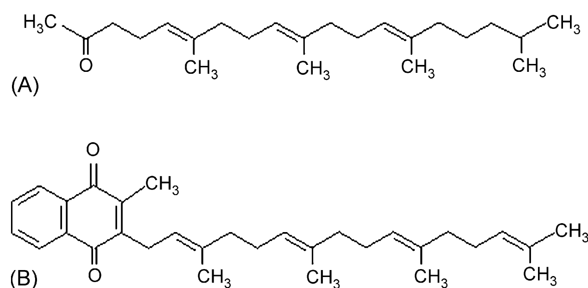

Fig. 1. Chemical structures of GGA (A) and menatetrenone (B).

2. Experimental

2.1. Materials

Both of GGA and menatetrenone were obtained from Eisai Co., Ltd. The test formulation was teprenone capsule (each capsule containing 50 mg GGA) provided by Eisai China Inc. Methanol was of HPLC grade (Scharlau Chemie S.A., Spain). Ethanol was of analytic-grade purity and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Cyclohexane was of analytic-grade purity and purchased from Shanghai No.4 Reagent & H.V. Chemical Co., Ltd. (Shanghai, China). Distilled water was used throughout the study.

2.2. Instrumentation

The HPLC–APCI–MS method was performed using an Agilent Technologies Series 1100 LC/MSD SL system (Agilent Technologies, Palo Alto, CA) with a Sepax GP-C8 column, 5 μm, 250 mm × 4.6 mm i.d. (Sepax Technologies, Inc., USA). The signal acquisition and peak integration were performed using the ChemStation software (10.02 A) supplied by Agilent. The LC run time was 12.5 min.

2.3. HPLC–APCI–MS conditions

The mobile phase was methanol–water (96:4, v/v) at a flow rate of 1.0 ml/min. The column temperature was maintained at 25 °C. The HPLC–APCI–MS was carried out using nitrogen to assist nebulization. A single quadruple mass spectrometer equipped with an APCI source was set with a drying gas (N_2) flow of 4 l/min, nebulizer pressure of 30 psi, drying gas temperature of 300 °C, vaporizer temperature of 350 °C, capillary voltage of 4.0 kV, corona current of 3.0 μA and the positive ion mode. The fragmentor voltage was 70 V. HPLC–APCI–MS was performed in the selected-ion monitoring (SIM) mode using the target ions at $[M+H]^+$ m/z 331.3 for GGA and $[M+H]^+$ m/z 445.4 for the I.S. Fig. 2 shows the typical full-scan APCI mass spectrum of GGA and the I.S.

2.4. Preparation of standard solutions

The stock solutions of GGA and the I.S. were prepared at the concentration of 1.0 mg/ml in methanol, respectively. The stock solution of GGA was diluted with methanol to prepare the working solutions at the concentrations of 10 μg/ml, 1.0 μg/ml, 100 ng/ml, and 10 ng/ml. Each of the standard solutions contained 2 mg/ml alpha-tocopherol as the antioxidant. The I.S. solution at the concentration level of 5 μg/ml was also prepared by further diluting the stock solution with methanol. All of the solutions were stored at −20 °C.

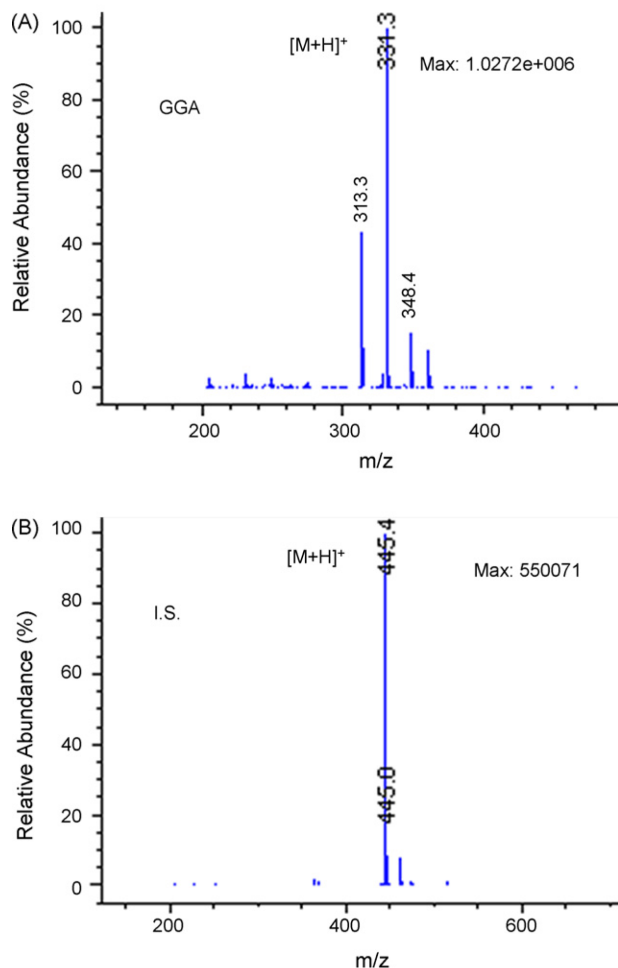

Fig. 2. Mass spectra of the positive ions of GGA (A) and the I.S. (B) at 70 V fragmentor voltage.

2.5. Sample preparation

Aliquot of 0.5-ml plasma sample and aliquot of 50 μl I.S. (5 μg/ml) were both placed into a 10 ml glass centrifuge tube and vortex-mixed for 10 s, then to which 2 ml ethanol was added and vortex-mixed for 30 s to precipitate the protein. After precipitation the protein, the plasma sample was extracted with 3 ml cyclohexane by vortex mixing for 3 min, and then centrifuged for 10 min. The cyclohexane phase was separated and evaporated to dryness under a stream of nitrogen in a water bath of 25 °C. The residue was reconstituted with aliquot of 150 μl of the mobile phase, and a 20 μl aliquot of the reconstituted solution was injected onto the HPLC–APCI–MS for analysis.

2.6. Preparation of calibration curves and quality control samples

The calibration standards of GGA were prepared by spiking appropriate amounts of the standard solutions in 0.5 ml blank plasma obtained from the healthy volunteers. The standard curves were prepared in the range of 0.3–1000 ng/ml for GGA at concentrations of 0.3 ng/ml, 1.0 ng/ml, 3.0 ng/ml, 10 ng/ml, 30 ng/ml, 100 ng/ml, 300 ng/ml, 600 ng/ml and 1000 ng/ml. The calibration curve was prepared and assayed along with the quality control (QC) samples. The QC samples were prepared in 0.5 ml blank plasma at concentrations of 0.6 ng/ml, 16 ng/ml, 160 ng/ml and 900 ng/ml, and stored at −20 °C. The QC samples were assayed along with clinical samples to monitor the performance of the assay and to assess the integrity and validity of the result of the unknown clinical samples analyzed.

2.7. Assay validation

2.7.1. Selectivity

The selectivity of the method was checked by comparing the chromatograms of the blank plasma samples from six different batches with the corresponding spiked plasma samples. Each blank plasma sample was tested using the proposed extraction procedure and HPLC–APCI–MS conditions to ensure no interference of GGA and the I.S. from the plasma.

2.7.2. Linearity of calibration curves and lower limit of quantification

The calibration standards of nine GGA concentration levels at 0.3 ng/ml, 1 ng/ml, 3 ng/ml, 10 ng/ml, 30 ng/ml, 100 ng/ml, 300 ng/ml, 600 ng/ml and 1000 ng/ml were extracted and assayed. To evaluate the linearity, plasma calibration curves were prepared and assayed on 5 days. The calibration curve was constructed by plotting the peak-area ratios of GGA to the I.S. versus the concentrations of GGA, using weighed least squares linear regression (weighing factor was $1/C^2$). The LLOQ was defined as the lowest concentration on the calibration curve at which precision was within 20% and accuracy was within ±20% [5], and it was established using five samples independent of standards.

2.7.3. Precision and accuracy

Validation samples were prepared and analyzed on three batches to evaluate the accuracy, intra- and inter-batch precisions of the method. The accuracy, intra- and inter-batch precisions of the method were determined by analyzing five replicates at 0.6 ng/ml, 16 ng/ml, 160 ng/ml and 900 ng/ml of GGA along with one calibration curve on each of three batches. Assay precision was calculated using the relative standard deviation (R.S.D.%). The accuracy is the degree of closeness of the determined value to the nominal or known true value under prescribed conditions. The accuracy is defined as the relative deviation in the calculated value (E) of a standard from that of its true value (T) expressed as a percentage (R.E.%). It was calculated using the formula: R.E.% = $(E - T)/T \times 100$.

2.7.4. Extraction recovery

The extraction recovery of GGA was evaluated by analyzing five replicates at 0.6 ng/ml, 16 ng/ml, 160 ng/ml and 900 ng/ml of GGA. Recovery was calculated by comparison of the peak areas of GGA extracted from plasma samples with those of injected standards.

2.7.5. Stability

The stability of GGA in plasma was studied under a variety of storage and handling conditions using the low (0.6 ng/ml) and high (900 ng/ml) QC samples. The short-term temperature stability was tested at ambient temperature for 8 h. The stability of samples in autosampler was conducted by reanalyzing the extracted QC samples kept under the autosampler conditions (15 °C) for 24 h. Freeze–thaw stability (−20 °C in plasma) was checked through three cycles. The QC samples were stored at −20 °C for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated three times, and then analyzed on the third cycle. The long-term stability was performed at −20 °C in plasma for 4 weeks.

2.7.6. Application

The method described above was applied to the pharmacokinetic study in which plasma concentrations of GGA in 20 healthy Chinese male volunteers were determined up to 24 h after administration of the teprenone capsule containing 50 mg GGA. The drug was administrated 30 min after the breakfast. The blood was sampled pre-dose and at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 10 h, 12 h, 15 h and 24 h post-dose. To 2-ml aliquot plasma, 50 μl alpha-tocopherol solution (0.5 mg/ml) was added as the antioxidant, and kept at −20 °C until the determination. The Ethic committee of the first affiliated hospital of Nanjing Medical University approved the clinical pharmacokinetic study. All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki. Model-independent pharmacokinetic parameters were calculated for GGA. The maximum plasma concentration (C_{max}) and the time to it (t_{max}) were noted directly. The elimination rate constant (k_{el}) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k_{el}$. The area under the plasma concentration–time curve $AUC_{0–24}$ to the last measurable plasma concentration was calculated by the linear trapezoidal rule.

3. Results and discussion

3.1. Sample preparation

The protein precipitation (PPT) and liquid–liquid extraction (LLE) methods were tested in the sample preparation procedure, respectively. The results showed that the extraction efficiency of these two kinds of sample preparation methods is very low. However, to extract the analytes using the method of an LLE following a PPT, the higher extraction recovery of the analytes may

be achieved. Thus, the method of an LLE following a PPT was applied to prepare the plasma samples. Several reagents were tested as the protein precipitation and liquid–liquid extraction. The test results showed that using ethanol as the precipitant and cyclohexane as the extractant gave higher recoveries and less interference of the analytes. Therefore, ethanol and cyclohexane were finally chosen as the reagents in sample preparation procedure.

3.2. Conditions of HPLC

As a proper internal standard, it should be structurally or chemically similar to the analyte, have similar retention to the analyte, be well resolved from the analyte and other peaks, and mimic the analyte in any sample preparation steps [6]. Menatetrenone was chosen as the internal standard for the assay because of its similarity of structure, retention and ionization to GGA.

Methanol and acetonitrile were tested as the organic portion of the mobile phase. The experiment results indicated that the MS response of GGA was higher when chosen methanol. The aqueous portion was also investigated. The results showed that using pure water as the aqueous portion of the mobile phase could sufficiently achieve the symmetric chromatographic peak sharp and high MS sensitivity for GGA and the I.S. So, the mobile phase of water–methanol was chosen. When the ratio of the water was less than 4%, the peak of the I.S. cannot reach the basic separation with the interference's peak. The more the ratio of the water in the mobile phase was added, the longer the retention time of the analytes was encountered. According to this, a mobile phase of water–methanol (4:96, v/v) was selected in the method. It is important to choose the suitable column temperature for the HPLC. The different column temperatures of 20 °C, 25 °C and 30 °C were tested. The result showed that GGA could be separated from the interference of the endogenous substance when the column temperature was selected at 25 °C. Finally, the acceptable retention and separation of GGA was obtained by using an elution system of water–methanol (4:96, v/v) as the mobile phase at the column temperature of 25 °C. The representative selected-ion chromatograms were shown in Fig. 3. Typical retention times were about 6.9 min for GGA and 10.8 min for the I.S.

3.3. Conditions for APCI–MS

Usually, electrospray ionization (ESI) is used for medium- to high-polarity analytes and atmospheric pressure chemical ion-

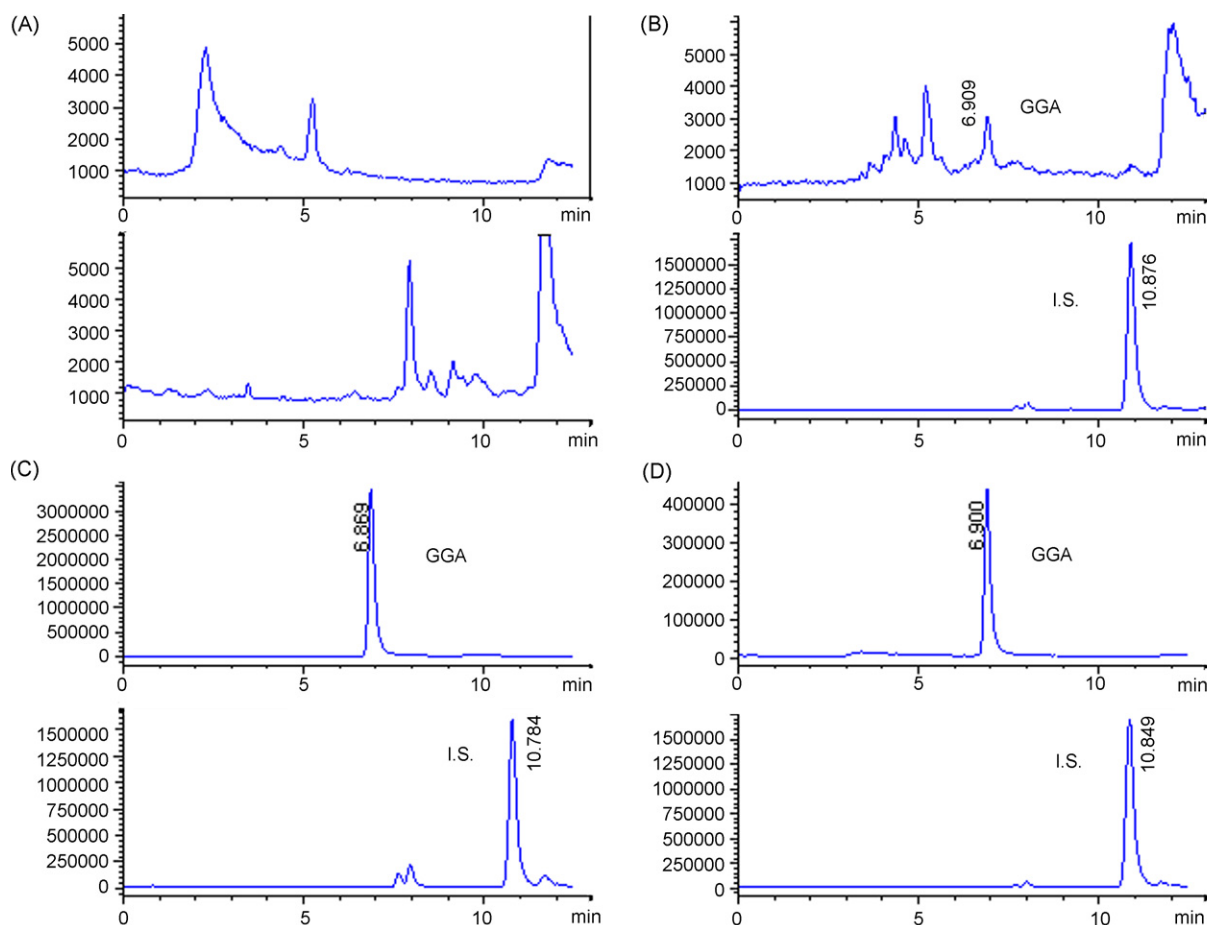

Fig. 3. Typical SIM mass chromatograms of blank plasma (A), LLOQ for GGA in plasma (0.3 ng/ml) and the I.S. (B), plasma spiked with GGA (1000 ng/ml) and the I.S. (C), plasma obtained from a volunteer at 6 h after orally administration teprenone capsule, the plasma concentration of GGA was estimated to be 114.2 ng/ml (D).

Table 1
Mean inter-day back-calculated standard and standard curve results of GGA ($n = 5$)

Added C (ng/ml)	Found C (ng/ml)					Mean	S.D.	R.S.D. (%)	R.E. (%)
	I	II	III	IV	V				
Mean inter-day back-calculated standard results									
0.3000	0.2955	0.2915	0.2912	0.2794	0.2892	0.2894	0.01	2.1	−2.3
1.000	0.9467	1.078	1.043	1.116	1.043	1.045	0.06	6.0	5.9
3.000	3.397	3.047	3.105	3.000	3.052	3.120	0.16	5.1	5.4
10.00	11.05	10.47	9.340	10.95	10.32	10.43	0.68	6.5	5.7
30.00	27.83	26.85	28.32	27.21	26.99	27.44	0.62	2.3	−7.3
100.0	88.85	89.33	95.67	103.4	95.22	94.49	5.91	6.3	−4.3
300.0	295.1	308.5	288.9	281.4	296.6	294.1	10.04	3.4	−0.7
600.0	590.6	564.2	607.6	575.8	621.7	592.0	23.25	3.9	0.0
1000	1025	1111	1059	973.0	986.2	1031	56.10	5.4	4.5
Standard curve results									
Slope	0.001819	0.001728	0.001716	0.001764	0.001856	0.001777	0.0001	3.4	
Y-intercept	0.0001612	−0.0001363	−0.000003177	0.0002666	0.0001151	0.00008068	0.0002	NA	
r	0.9967	0.9965	0.9988	0.9975	0.9987	0.9976	0.001	0.1	

Note: Calibration curves were weighted $1/C^2$. R.S.D., relative standard deviation; R.E., relative error; NA, not applicable; n, number of calibration curves.

ization (APCI) is used for low- to medium-polarity analytes. In contrast to the ESI process where ions are primarily formed by desorbing preformed ions from a solution, APCI is an ionization technique in which ions are produced at atmospheric pressure, by gas-phase ion–molecule reactions between analyte molecule and solvent-based reagent gas. In the APCI process, ionization of solvent molecules is initiated by a corona discharge at the tip of the corona needle. The analytes arriving in the ionization source are chemically ionized through proton transfer in the positive mode and through proton loss in the negative mode [7,8]. GGA is a low-polarity and neutral compound, and does not exist in the LC mobile phase as the preformed ions. According to the chemical character of GGA, APCI in positive ion mode was adopted for the assay of GGA. In order to determine the optimal fragmentor voltage which is set for the in-source fragmentation, the intensities of GGA protonated molecular $[M+H]^+$ m/z 331.3 were compared at fragmentor voltages of 30 V, 50 V, 70 V, 90 V, 120 V and 150 V in the SIM mode. The highest sensitivities could be obtained by using a 70 V fragmentor voltage. At this fragmentor voltage, the base peak in the mass spectrum of the I.S. was the protonated molecular $[M+H]^+$ m/z 445.4. So, the protonated molecular $[M+H]^+$ at m/z 445.4 was selected as the target ion for the I.S. in the method. The nebulizer pressure can also influence the sensitivity of the analytes. When the nebulizer pressure was decreased from 60 psi to 10 psi, the MS response of GGA increased, but the ratio of the signal to noise (S/N) decreased when the nebulizer pressure was less than 30 psi. So, 30 psi was chosen as the nebulizer pressure.

3.4. Method validation

3.4.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Fig. 3 showed the typical chromatograms of a blank, a spiked plasma sample with GGA (0.3 ng/ml) and the I.S., a spiked plasma sample with GGA (1000 ng/ml) and the I.S., and a plasma sample from a healthy volunteer. There was no significant interference from endogenous substances observed at the retention times of the analytes. The selectivity test results also showed that there was no interference of internal standard on GGA and vice versa.

3.4.2. Calibration curve and sensitivity

Five calibration analyses were performed on 5 days and the back-calculated values for each level were recorded (see Table 1). The calibration curves did not exhibit any non-linearity within the chosen range. The back-calculated results showed good day-to-day accuracy and precision. The limit of detection (LOD) was 0.1 ng/ml, which was established based on an S/N ratio of 3. The LLOQ for GGA in plasma was 0.3 ng/ml. The data of the LLOQ was shown in Table 2.

Table 2
Accuracy and precision for the analysis of the LLOQ ($n = 5$)

Concentration level (ng/ml)	Calculated concentration (ng/ml)	Mean (ng/ml)	R.S.D. (%)	R.E. (%)
0.3000	0.2544			−14.1
0.3000	0.3176			7.3
0.3000	0.3096	0.2970	11.0	4.6
0.3000	0.2717			−8.2
0.3000	0.3317			12.0

Note: R.S.D., relative standard; R.E., relative error; n, number of replicates.

Table 3
Accuracy and precision for the analysis of GGA in human plasma (in prestudy validation, three batches, five replicates per run)

Added C (ng/ml)	Found C (ng/ml)	Intra-batch R.S.D. (%)	Inter-batch R.S.D. (%)	R.E. (%)
0.6000	0.6106	7.8	4.7	3.1
16.00	14.40	7.5	6.4	−8.8
160.0	172.0	4.4	3.6	8.9
900.0	951.8	4.1	8.7	7.1

Note: R.S.D.: relative standard deviation; R.E.: relative error.

Table 4
Stability data of GGA in human plasma under the various storage conditions ($n = 3$)

Storage conditions	Concentration level (ng/ml)	Calculated concentration (ng/ml)	R.E. (%)
Ambient temperature for 8 h	0.6000	0.6377	7.7
	900.0	889.7	0.2
Autosampler for 24 h	0.6000	0.6556	10.7
	900.0	1019	14.7
Three freeze–thaw cycles	0.6000	0.5813	−1.8
	900.0	933.3	5.1
4 weeks at −20 °C	0.6000	0.5799	−2.1
	900.0	823.1	−7.3

Note: R.E., relative error; *n*, number of replicates.

3.4.3. Assay precision and accuracy

Table 3 summarizes the intra- and inter-batch precision and accuracy for GGA evaluated by assaying the QC samples. The precision was calculated by using one-way ANOVA. In this assay, the intra-batch precision was 7.8% or less, and the inter-batch precision was 8.7% or less for each QC level of GGA. The results above demonstrated that the values are within the acceptable range and the method is accurate and precise.

3.4.4. Extraction recovery

Methanol and cyclohexane were chosen as the solvents for the higher extraction efficiency to the two target compounds. It cannot only eliminate the interference of endogenous substances, but also meet the requirement of sensitivity for the method. The recovery of GGA, determined at four concentrations of 0.6 ng/ml, 16 ng/ml, 160 ng/ml and 900 ng/ml were 87.8 ± 10.2%, 96.5 ± 4.9%, 91.4 ± 4.9% and 83.8 ± 9.9% ($n = 5$), respectively.

3.4.5. Stability

The stability of GGA was studied under a variety of storage and handling conditions. The results (see Table 4) showed that no significant degradation occurred at ambient temperature for 8 h. The samples in autosampler were stable for at least 24 h. And there were also no significant degradation occurred during the three freeze–thaw cycles for GGA plasma samples. GGA in plasma at −20 °C was stable for 4 weeks.

3.4.6. Matrix effect

The matrix effect (ME) was defined as the direct or indirect alteration or interference in response due to the presence of unintended analytes or other interfering substances in the samples [5]. It was examined by comparing the peak areas of the analytes and the I.S. between two different sets of samples. In set 1, analytes were resolved in the blank plasma sample's reconstituted solution, and the obtained peak areas of analytes were defined as *A*. In set 2, analytes was resolved in mobile phase, and the obtained peak areas of analytes were defined as *B*. ME was calculated by using the formula: $\text{ME}(\%) = A/B \times 100$. The matrix effect of the assay was evaluated at four GGA concentration levels of 0.6 ng/ml, 16 ng/ml, 160 ng/ml and 900 ng/ml and the I.S. concentration level of 500 ng/ml. Five samples at each concentration level of the analytes were analyzed. The blank plasma samples used in this study were five different batches of human blank plasma. If the ME values exceed the range of 85–115%, an exogenous matrix effect is implied. As shown in

Table 5
Matrix effect data for GGA and menatetrenone in the five different lots of human plasma ($n = 5$)

Samples	Concentration level (ng/ml)	*A* (mean ± S.D.)	*B* (mean ± S.D.)	Matrix effect (%)
GGA	0.60	28688 ± 3622	28389 ± 3930	101.1
	16	624021 ± 17374	588361 ± 22356	106.1
	160	8549573 ± 728971	8460950 ± 896371	101.0
	900	42650475 ± 2415614	44504038 ± 3910689	95.8
Menatetrenone	500	25345625 ± 2650471	25828071 ± 3580303	98.1

Note: S.D., standard deviation; *n*, number of replicates.

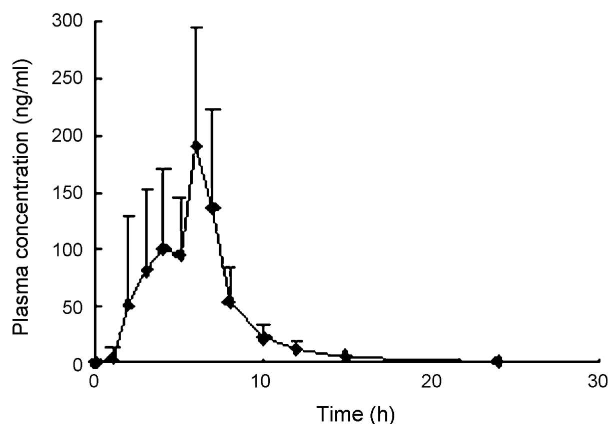

Fig. 4. Mean GGA plasma concentration–time profile in 20 healthy volunteers after oral administration of teprenone capsule. *Note*. Error bars: standard deviation.

Table 5, the results obtained were well within the acceptable limit, it indicated that there was no matrix effect of the analytes observed in this study.

3.5. Application

The method was successfully applied in the pharmacokinetic study in which plasma concentrations of GGA in 20 healthy Chinese volunteers were determined up to 24 h after administration of capsule containing 50 mg GGA. The maximum GGA plasma concentration (C_{max}) was 246.9 ± 85.4 ng/ml, $t_{1/2}$ was 3.38 ± 1.20 h, and the time to C_{max} was 5.35 ± 1.39 h. The area under the plasma concentration–time curve ($AUC_{0–24}$) was 851.7 ± 194.2 μg h/l. The mean plasma concentration–time curve of GGA is shown in Fig. 4.

4. Conclusion

The assay achieved good sensitivity and specificity for the determination of GGA in human plasma. No significant interferences caused by the endogenous compounds are observed. This simple and sensitive method is suitable for the pharmacokinetic study of GGA in human subjects.

References

[1] K. Nishida, Y. Ohta, I. Ishiguro, Pharmacol. Res. 39 (1999) 325–332.
[2] T. Kobayashi, Y. Ohta, J. Yoshino, S. Nakazawa, Pharmacol. Res. 43 (2001) 23–30.
[3] M. Tanaka, J. Hasegawa, J. Tsutsumi, J. Chromatogr. Biomed. Applic. 231 (1982) 301–310.
[4] T. Seki, N. Hashida, T. Kanazawa, J. Chromatogr. Biomed. Applic. 424 (1988) 410–415.
[5] Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001.
[6] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, John Wiley & Sons Inc., New York, 1997, pp. 657–660.
[7] S. Ahuja, M.W. Dong, Handbook of Pharmaceutical Analysis by HPLC, Elsevier Academic Press, 2005, pp. 512–515.
[8] R.E. Ardrey, Liquid Chromatography–Mass Spectrometry: An Introduction, John Wily & Sons Ltd., England, 2003, pp. 134–140.