GC-FID Determination and Pharmacokinetic studies of Oleanolic Acid in Human Serum

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ABSTRACT

Analytical interest of oleanolic acid determination in human serum has increased owing to the increasing interest in pharmaceutical research by pharmaceutical properties. A simple, specific, precise and accurate GC method with flame ionization detection is developed and validated for the determination of oleanolic acid (OA) in human serum (HS). To an aliquot of HS, internal standard was added and a combination of liquid/liquid extraction with a mixture of diethyl ether-isopropyl alcohol, filtration, and consecutive GC resulted in separation and quantification of OA. The organic phase was analyzed by a GC system equipped with a 30 x 0.25 mm i.d. Rtx-65TG capillary column and FID detection. Total chromatographic time was ten minutes and no interfering peaks from endogenous components in blank serum were observed. The OA/internal standard peak area ratio was linearly fitted to the OA concentration ($r = 0.992$) over the range 10-1500 ng/mL. The mean serum extraction recovery of OA was $96.7 \pm 1.0\%$ and lower limit of quantification based on 5mL of serum was 10.7 ng/mL. The intraday coefficient of variation ranged from 1.3 to 3.6% and interday varied from 1.4 to 4.5%. The developed method was used to study the pharmacokinetics of OA after oral administration in humans. The assay was simple, sensitive, precise and accurate for the use in the study of the mechanisms of absorption and distribution of OA in humans.

Keywords: *betulinic acid; GC-FID determination; human serum; oleanolic acid; pharmacokinetics.*

1. Introduction

Oleanolic acid (3 β -hydroxy-olean-12-en-28-oic acid), a secondary metabolite ubiquitously distributed throughout the plant kingdom, as free acid or forming part of triterpenoid saponins is isolated from more than 120 plant species. In particular, there are 146 families, 698 genus and 1620 species of plants containing OA (Fai *et al.*, 2009). It is noteworthy that OA is the major triterpenic component and appears in very important quantities as free acid in olive leaf (ca. 3 % dry matter) (Guinda *et al.*, 2010). This triterpene displays various pharmacological effects (Dzubak *et al.*, 2006; Pollier *et al.*, 2012) being this way, a leading compound for the development of new multi-targeting bioactive agents. The pharmacological relevance of OA has increased during the last two decades. Recently, triterpenes supplements, including OA, are reported to have antineoplastic effects and to reinforce the activity of anticancer drugs against several tumor cell lines (Yamai, *et al.*, 2009; Ma *et al.*, 2014). In addition, OA exerts beneficial effects against diabetes and metabolic syndrome through complex and multifactorial mechanisms according to Castellano *et al.* (2013). Triterpene treatment possesses therapeutic effects on diet-induced hyperlipidemia by inhibiting the intestinal absorption and storage of cholesterol. Recently, Wang *et al.*, (2013) reported that OA improves hepatic insulin resistance via antioxidant, hypolipidemic and anti-inflammatory effects. Furthermore, OA has been successfully used as an orally administrated drug to treat human liver diseases in China (Liu *et al.*, 2005). The administration of the natural triterpenoid OA reduces and limits the severity and development of experimental autoimmune encephalomyelitis. Therefore, OA therapy might be of clinical interest for human multiple sclerosis and other Th1 cell mediated inflammatory diseases (Martin *et al.*, 2010). Consequently, OA is of increasing interest in pharmaceutical research circles because it may have distinct pharmaceutical properties of its own or provide a basis for the development of new pharmaceutical drugs by partial structure variation. Until now, limited researches of OA determination in HS (Song *et al.*, 2006; Chen *et al.*, 2010; Rada *et al.*, 2011) and in experimental animal serum (Jeong *et al.*, 2007; Ji *et al.*, 2009) are reported. The technique employed by these authors to determinate OA in serum is mainly HPLC-MS. However, to our knowledge, there is still no report on the determination of OA on HS samples by GC-FID.

At the view of these precedents, this study had two objectives: to develop and validate a rapid, sensitive and accurate method for the GC-FID determination of OA in HS and to apply this method to a pharmacokinetic study of OA after oral administration. Furthermore, in the present study the binding percentage with human serum proteins (HSP) was also investigated.

2. Experimental

2.1. Materials and Reagents

Oleanolic acid (98% pure, Fig. 1b) was obtained in our lab (Guinda *et al.*, 2001). Betulinic acid (97% pure, Fig. 1a) used as internal standard (IS) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was of chromatographic grade. All the other reagents were of analytical grade. Distilled water, prepared from demineralised water, was used throughout the study.

2.2. Sample preparation

To 5 mL of serum sample, 12.5 μ L methanolic solution of betulinic acid (0.5 mg/mL, IS) was added. The solution was mixed for 30 seconds and dry nitrogen was used to eliminate methanol traces. The mixture was filtered in a cellulose acetate membrane filter with a pore size 0.20 μ m (AC02047BL). After the filtration was completed the sample was extracted with 5.0 mL of 5% isopropyl alcohol in diethyl ether through vigorous vortex mixing for 3 min, followed by centrifugation at 3000 rpm for 10 min. This extraction procedure was repeated three times. Supernatants were accurately combined and concentrated to dryness by

evaporation (Büchi Rotavapor R-205, Switzerland). The residue was derivatized by adding 75 μ L BSTFA [N, O-bis-(trimethylsilyl) trifluoroacetamide] +1 % TMCS (trimethylchlorosilane), 25 μ L of pyridine and by sonication at 40 °C for 10 min (ultrasonic bath ULTRASONS H-D, Pselecta, Spain). A 1 μ L aliquot of the derivatized sample was injected into the GC.

2.3. GC/FID Analysis

2.3.1. GC-MS identification of oleanolic acid. The analysis of silylated OA was performed using a coupled gas chromatography-mass spectrometry detector (GC-MS) QP2010 Ultra (Shimadzu Europa GmbH) fitted with an AOC-20i autosampler, an ion source of electron impact and a quadrupole detector. The splitless mode was used and the injector temperature was set at 290 °C. Helium as a carrier gas at a pressure of 53.1 kPa and a flow of 1 mL/min was used. The oven temperature program was as follow: initial temperature 50 °C for 1 min, 50-200 °C at 40 °C/min, 200-280 °C at 10 °C/min and finally held for 2 min. Total run time: 14,75 min. The MS conditions were: interface temperature: 280 °C; ion source temperature: 220 °C; electron impact: 70 eV; acquisition mode: scan (m/z 50-600). The identification of OA were accomplished by comparing the retention times and abundance ratios of two fragments ions (203 and 189 m/z).

2.3.2. Determination of oleanolic acid. The quantification was carried out by a modification of the method previously established in our lab (Rada *et al.*, 2011). One microliter of the silylated sample was injected in an Agilent 6890N GC (Agilent Technologies, Santa Clara, CA), equipped with a Rtx-65TG Crossbond capillary column (30 m x 0.25 mm I.D.; 0.1 mm film thickness;) coated with 35 % dimethyl and 65 % diphenyl polysiloxane as stationary phase (Restek, Co.; Bellefonte, PA) and a FID detector. The injection was realized in split mode and hydrogen was used as carrier gas (pressure at column head 140 kPa). The oven temperature was isothermally established at 260 °C for 10 min. The injector and detector temperatures were established at 300 °C. These triterpenic compounds were previously identified in our laboratory by means of GC-MS using the same Rtx-65TG Crossbond capillary column and a Finnigan MAT95's (Finnigan, Bremen, Germany) high resolution mass spectrometer interfaced with a HP 5890 Series II GC (Guinda *et al.*, 2004).

2.4. Assay validation

2.4.1. Limits of Detection (LOD) and Quantification (LOQ). Detection and quantitation limits were calculated by measuring the analytical background response, running six blank serum samples using maximum sensitivity allowed by the system. The signal-to-noise ratio was used to determine the LOD, and it was estimated as the concentration of oleanolic acid in serum samples that generated a peak with an area at least 3 times higher than the baseline noise. The LOQ was afterward validated by the analysis of six serum samples known to be near the LOQ.

2.4.2. Stability. Drug free serum was spiked with known amount of the drug to achieve the concentration of 10, 100, 200, 300, 500, 1000, 1500 ng/mL ($n=4$) and stored at 4°C. Those samples were used to investigate the stability of OA over a period of two months. No internal standard was added prior to the analysis.

2.4.3. Specificity. The specificity of the method was determined by comparing the chromatograms obtained from the samples containing OA and internal standard with those obtained from blank HS. Four samples from five lots of blank HS were processed with and without the OA and IS to evaluate presence of interfering peaks.

2.4.4. Linearity and range. The linear detector response for the assay was tested. The determination ($n = 4$) from seven concentration levels (10, 100, 200, 300, 500, 1000, 1500 ng/mL) of the analyte were made. Detector response was correlated against analyte concentration by least squares regression. A weight of $1/y$ was used to determine slopes,

intercept and correlation coefficients. The minimum acceptable coefficient to establish linearity was set at 0.95 a priori.

2.4.5. Precision and accuracy. The precision of the assay was determined at low, medium and high concentrations of OA by replicate analyses of the quality control (QC) samples. Intra-day precision was determined by replicated analysis of each QC sample on 1 day ($n = 5$). Inter-day precision was determined by replicated analysis on five consecutive days ($n = 1$ series per day). Accuracy is defined as the relative deviation in the calculated value of a standard from that of its true value expressed as a percentage (RE %). Precision was calculated as inter and intra-day coefficient of variation [% CV = (SD/mean) x100].

2.4.6. Recovery. The extraction recovery of oleanolic acid was determined at low, medium and high concentrations, respectively. Recovery was calculated by comparison of the peak areas of oleanolic acid extracted from serum samples with those of injected standards.

2.5. System suitability test

Prior to running each batch of clinical serum samples, the instrument performance (e.g. sensitivity, reproducibility of chromatographic retention and separation, plate number and tailing factor) was determined by analysis of the reference standard of OA, IS, blank serum and serum spiked with OA and IS (US Department of Health and Human Services et al., 2001).

2.6. Application of the GC Method and Pharmacokinetic Study

2.6.1. Clinical study design and pharmacokinetic analysis. To study the pharmacokinetic profile of OA, aliquots (5mL) of HS samples provided by Dr. Ruiz-Gutierrez (Instituto de la Grasa-CSIC) was analyzed. Nine healthy men aged 26.2 ± 4.3 with body mass index $23.7 \pm 2.0 \text{ kg/m}^2$ participated in the study. The subjects were recruited after screening for fasting plasma triglycerides, cholesterol, and glucose concentration, all of which were within the normal limits proposed by the Atherosclerosis, Hypertension, and Obesity in Youths Committee (Pearson *et al.* 2002). Medical history verified that volunteers did not suffer from any digestive or metabolic disorder. The participants gave written informed consent to a protocol approved by the Institutional Committee on Human Research of the Virgen del Rocío University Hospital, Sevilla-Spain. The samples were obtained as described by Cabello-Moruno *et al.* (2007). Each one of healthy volunteers received a test meal including 70 g of pomace olive oil containing 30 mg of dissolved OA, after overnight fasting. Blood was sampled pre-dose and at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 hour post-dose for determination of serum concentration of OA and pharmacokinetic parameters were calculated. The serum concentrations of OA at different time points were expressed as mean $\pm$ SD ($n = 9$), and the mean concentration-time curve was plotted. One-compartment pharmacokinetic parameters were calculated for OA. The maximum serum concentration (C_{max}) and the time to reach the peak concentration (t_{max}) were obtained directly from the observed values. The apparent elimination rate constant (K_e) was calculated using fitting mean data at four terminal points of the serum concentration profile with a log-linear regression equation using the least-squares method. The $t_{1/2}$ was calculated as $0.693/K_e$. The area under the serum concentration-time curve from zero to the time of the final measurable sample (AUC_{0-t}) was calculated using the linear-trapezoidal rule up to the last sampling point with detectable levels (C). The area under the serum concentration-time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the trapezoidal rule with extrapolation to infinity with K_e . The mean residence time (MRT) was calculated as the ratio of the area under the first moment curve ($AUMC_{0-t}$) to AUC_{0-t} . The apparent total body clearance (CL) after oral administration was calculated using the equation $CL = \text{Dose} / AUC_{0-t}$. The volume of distribution (V_d) after oral administration of OA was calculated using the equation $V_d = \text{Dose} / C_0$.

2.6.2. Oleanolic acid binding study. The extent of binding of OA to serum proteins was investigated by spiking blank HS with OA at 1, 5 and 20 $\mu\text{g/mL}$ (each level in triplicate) and then allowing equilibration to take place for 15 min at 37 °C prior to ultrafiltration. An Amicon (Millipore, Bedford, MA, USA) YM-30 ultrafiltration centrifuge filter with a molecular cut-off of 30 000 was used to separate the protein-bound from the free OA. A 2 mL aliquot of each equilibrated serum solution was placed in a filter unit and was centrifuged at 3000 g (37°C) for 30 min. Next, a 500 μL aliquot of each ultrafiltrate was subjected to the protocol described in the section 2.5 for the determination of the concentration of free OA.

3. Results and discussion

The present study covers OA determination in biological samples, binding percentage and pharmacokinetics studies.

3.1. Method development

Liquid-liquid extraction was used for the sample preparation in this method to avoid matrix interferences. Various organic solvents, such as ethyl ether, ethyl acetate, dichloromethane, and their mixtures in different combinations and ratios were evaluated. Finally, a mixture of ethyl ether–2-propanol (95:5, v/v) was found to be optimal for producing a clean blank serum chromatogram and yielding the highest recovery for OA and the betulinic acid (IS). The extraction procedure was repeated three times and a fourth extraction was carried out to confirm the extraction completeness. No OA was detected in the fourth extraction. To improve the recovery of OA, the serum sample was micro-membrane filtered before the extraction procedure. The filtration by 0.20 μm cellulose acetate membrane of HS retained proteins, which helped to release into the extracting solvent the bound organic components with Van der Waals interaction (Peng *et al.*, 2014), including OA. The addition of this previous step in the method, also avoided the possible matrix interferences especially in the identification by GC-MS. Matrix effects were evaluated by comparing the peak areas of analyte in extracted samples of blank serum spiked after extraction with the corresponding areas obtained by direct injection of standard solutions. No endogenous species interfered with the analyte and IS.

For the gas chromatographic analysis of OA, a preliminary derivatization process is necessary due to its low volatility and high molecular weight. Derivatization of the terpene was time-reduced at only 10 min by involving sonication of the sample at 40 °C. The experimental chromatographic variables were optimized. The operating conditions are described in Section 2.3. Splitless and split injections were tested to check the influence of the split ratio on the GC–FID analysis. The sensitivity depends on the volume injected and on the split ratio. In this work, to prolong the shelf-life of the chromatographic column, the volume injected was 1 μL and the split ratio was 1:25. For more sensitive determinations, 2 μL or even larger volumes can be injected and the split ratio can also be decreased. Betulinic acid, a pentacyclic triterpene as well as OA, was used as internal standard due to its chemical behaviour similar to that of the target analyte (relative response factor (RRF) = 1). Identification of the chromatographic peaks was made by comparison of mass spectra with those provided by standard solutions and also by GC retention times. The GC-MS identification of OA trimethylsilyl derivative were accomplished by comparing the retention times and abundance ratios of two fragments ions (203 and 189 m/z).

Representative GC chromatograms of blank serum and serum spiked with the terpenic acids (oleanolic and IS) are shown in Fig. 2a and 2b, respectively. The retention times of OA and the IS were 7.0 and 7.5 min, respectively. It can be observed that blank serum chromatogram shows no peak at these retention times, being this way the assay free of interference from compounds in the biological matrix. A GC chromatogram of HS sample after OA oral administration is presented in Fig. 2c. This sample has been collected in the pharmacokinetic

study, 2 hours after administration of 30 mg of OA in 70 g of pomace olive oil, as described in section 2.6. As shown in Figure 2, the selected chromatographic conditions resulted in a good chromatographic resolution, with good peak separation. In addition, the good chromatographic separation between the studied compounds (OA and BA) and other important HS compounds such as cholesterol derivatives evidence the interest of this method. However, to our knowledge, there is still no report on the gas chromatography determination of OA in serum samples. The developed and validated GC method resulted rapid, sensitive, and accurate for the determination of OA in HS. This method is very interesting for future clinical studies and research using common techniques; furthermore it could be applied to several aqueous matrices.

There are a limited number of reports on the analysis of OA in human samples (Song *et al.*, 2006; Rada *et al.*, 2011) and only a few reports in animal serum (Jeong *et al.*, 2007; Ji *et al.*, 2009) the reported articles were based mainly on HPLC-MS determination. Jeong *et al.*, (2007) evaluated the pharmacokinetics of OA after intravenous and oral administration in rats using the HPLC-MS method reported by Song *et al.*, (2006). As well, the same method was used by Ji *et al.*, (2009) to study OA pharmacokinetics in experimental animals (mice, rats, rabbits and dogs) and prediction of human pharmacokinetics. In addition, Chen *et al.*, (2010) reported pharmacokinetic profiles of OA formulations in healthy Chinese male volunteers using the Song's method.

In our previous work (Rada *et al.*, 2011) a method for separation, identification and quantification of pentacyclic triterpenoids in HS employing HPLC technique was reported. The quantification limits were in the same range as the ones obtained in the present study, 10.7-14.3 ng/mL, and the linearity values ranged up to 1 µg/mL.

Knowing that the chromatographic determination of OA by HPLC using UV or fluorescence detection is limited since this pentacyclic triterpene lacks UV-absorbing chromophores; application of GC-FID resulted appropriate because it does not depend on the presence of a particular chromophore in the molecule. It is worth noting that the recovery of OA by GC-FID method described is higher than that obtained by Rada *et al.*, (2011).

Song *et al.* (2006) reported a method for the analysis of OA in human plasma by HPLC-MS. The assay was validated in the concentration range of 0.02–30.0 ng/mL for OA when 0.5 mL of plasma was determined. The validation of method described in this study was established in the range of 10-1500 ng/mL when 5 mL of HS was analysed by GC-FID. The relative standard deviation was less than 5 % and the limit of quantification (LOQ) was 10.7 ng/mL, whereas the relative standard deviation by HPLC-MS was less than 15 % and the limit of quantification was 0.02 ng/mL. Furthermore, the recovery results of the samples analysed by the method described hereby is higher than the results obtained by HPLC-MS. In consonance with these previous methods, the technique described in the present study enables the GC-FID analysis of OA in HS with good precision and accuracy. On the other hand, taking into consideration that GC is a technique widely established in laboratories for numerous applications, easy handling and of low cost, which also avoids the use of solvents resulted another possible choice for our purpose. In addition, GC presents high resolution power ($N \sim 1.3 \times 10^6$) and high linear range (FID) and the retention index standards can be applied, which is standardized using Kovats index, LC is still lacking.

3.2. Calibration and validation

The intra- and interday precision and accuracy results are shown in Table 1. The intra- and interday precision was less than 3.3 and 4.5 %, respectively, and the accuracy was within 3.6 % for the QC samples. The values obtained were lower than the limits required for biological sample analysis. The results indicated that the LOQ of 10.7 ng/mL was achieved. The results showed that the extraction recoveries of OA from HS were from 95.7 to 98.4 at concentrations of 20 and 1000 ng/mL, respectively. The mean recovery was 96.7 ± 1.0 %.

Results of the stability experiments indicated that OA storage at $-20\text{ }^{\circ}\text{C}$ was stable at least four weeks in HS. This storage period and conditions should be adequate for most in vivo studies of OA. In addition, the corresponding relative errors from the same three concentrations indicated that OA was stable in HS after three freeze-thaw cycles.

3.3. Application of the method in pharmacokinetic studies

The developed and validated GC-FID method was used to determine OA in HS after oral administration of pomace olive oil at a dose containing OA 30 mg. Using the established GC method with the degree of sensitivity (LOQ of OA, 10.7 ng/mL) a pharmacokinetic study of OA in humans was successfully performed. The OA concentration analysed resulted above the LOQ during 7 h post-dose. The mean serum concentration-time curve profile is shown in Figure 3 and its pharmacokinetic parameters are listed in Table 2. The pharmacokinetic results show that the absorption of OA presents the peak concentration at 3.0 h ($t_{\max}$) after oral administration of prepared meal. These data are in accordance to Chen *et al.*, (2010), in a randomized, crossover and self-control study, where 18 male volunteers received orally 20 mg of OA. Likewise, the mean residence time (MRT) reported by this author is very similar (13.6 ± 8.6 and 18.3 ± 11.8 h for the OA normal tablets and dispersible tablets, respectively). However, the results obtained in our study for the AUC and the $C_{\max}$ are higher. These results could be due to the higher dose (50 %) administered to volunteers in our assay. It is noteworthy, that the curves of OA concentration versus time, in both trials have the same profile. The pharmacokinetic parameters for the administered OA dose revealed distribution volumes (V_d) of 81.4 ± 9.7 L, and lengthy elimination half-lives of 4.6 ± 1.1 h. The 30 mg OA oral dose yielded total clearance of 35.1 ± 4.2 L/h. The different oral administration of OA single dose studied by Song *et al.* (2006) and Chen *et al.*, (2010), were 40 mg-capsules and 20 mg normal/ dispersible tablets, respectively and the OA concentrations determined in serum volunteers ranged between 12.1 ± 6.8 and 18.9 ± 8.0 ng/mL. In our pharmacokinetic study was tested a single dose of OA within the above-mentioned range (30 mg-oil solution) and the concentrations of OA determined in HS volunteers were higher (598.2 ± 176.7 ng/mL). In this experience, the higher values obtained could be due to the way that the triterpenic acid is supplied, OA was provided in solution in an edible vegetable oil. When supplied as a capsule or tablet, particle size of OA is higher than the molecular size present in the oil and presents different bioavailability. We assume that the smaller size of the OA facilitates the absorption of the terpene. Obviously, alternative formulations are possible and it will be of interest to examine the pharmacokinetics of OA in other formulations. This is the first report of OA determination in HS by GC-FID and the utility of this method for the quantitative analysis of OA in HS in support of in vivo studies was demonstrated.

3.4. Oleanolic acid binding study

HS albumin is an important plasma protein responsible for the binding and transport of many endogenous and exogenous substances such as hormones and fatty acids, as well as foreign molecules such as drugs. It is known that the distribution, free concentration and the metabolism of various drugs are strongly affected by drug-protein interactions in the blood stream. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. In addition, thermodynamic analyses show that the basic forces acting between bovine serum albumin and triterpenoids are hydrogen bonds, van der Waals forces, and hydrophobic interactions (Peng *et al.*, 2014; Bhattacharya *et al.*, 2000; Subramanyam *et al.*, 2009) anticipated that the complexation of plant triterpenoids with protein may be exploited as a biologically relevant model for evaluating the physiologically applicable no-covalent complexes in vivo examination of triterpenoid properties such as accumulation, bioavailability, and distribution. In a first step to learn more about the way of interaction of OA and the HSP we studied the absorbance spectrum of the system OA-HSP (Rada *et al.*, 2011). Furthermore, an additional study was

made to investigate the extent of protein binding of OA in HS was using the ultrafiltration method. In accordance to Peng *et al.*, (2014), our results showed that OA was highly protein-bound in HS. The bound fraction was 98.8 ± 3.2 , 98.9 ± 2.5 and 98.9 ± 0.3 % at 1, 5 and 20 $\mu\text{g/mL}$ concentrations.

Acknowledgements

This work was supported in part by grants from Comisión Interministerial de Ciencia y Tecnología (CICYT, AGL2008-02258) and Instituto de Salud Carlos III (FIS-ISCIII, PI010/01415) partially financed by the European Regional Development Fund of the EU. Authors also express their gratitude to Dr. Ruiz-Gutierrez for the supply of human serum samples for the pharmacokinetic study.

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Legends

Figure 1. Chemical structures of triterpenic compounds determined in HS: (a) betulinic acid (b) oleanolic acid.

Figure 2. GC Chromatograms for the determination of OA in HS. (a) Blank HS sample; (b) blank HS sample spiked with OA at 1 μ g/mL and internal standard; (c) HS sample after oral administration of OA (30mg). (1) Cholesterol; (2) OA; (3) IS.

Figure 3. Concentration-time curve of OA in HS after oral administration of 30mg. Each point and bar represent mean $\pm$ SD (n=9).

Table 1. , Intra-day and inter-day accuracy, precision and recovery of oleanolic acid (OA) from human serum (HS) (n=5)

Added to serum	Absolute recovery		Intra-day ^a		Inter-day ^b	
(ng/mL)	Mean (%)	(R.S.D.%)	RE%	Precision (R.S.D.%)	RE%	Precision (R.S.D.%)
20.0	95.5	3.7	-3.6	3.3	3.2	4.5
100.0	96.0	2.2	2.8	2.2	-1.3	2.6
500.0	96.8	2.1	2.3	1.9	3.4	1.9
1000.0	98.6	1.5	1.9	1.3	0.3	1.4

^a The sample was analyzed five times within one day.^b The sample was analyzed over five consecutive days.

Table 2. Pharmacokinetic parameters (mean $\pm$ SD) of OA in HS after oral administration at a dose of 30 mg (n = 9)

parameters	$C_{\max}$	$t_{\max}$	K_e	$t_{1/2}$	AUC_{0-t}	$AUC_{0-\infty}$	MRT	CL	V_d
	(ng/mL)	(h)	(1/h)	(h)	(ngh/mL)	(ngh/mL)	(h)	(L/h)	(L)
mean $\pm$ SD	598.2 $\pm$ 176.7	3.0 $\pm$ 0.8	0.43 $\pm$ 0.04	4.61 $\pm$ 1.1	3025.2 $\pm$ 847.1	3181.9 $\pm$ 894.3	8.89 $\pm$ 2.67	35.1 $\pm$ 4.2	81.4 $\pm$ 9.7

$C_{\max}$, maximum serum concentration; $t_{\max}$, peak concentration time; K_e , elimination rate constant; MRT, mean residence time; AUC, area under the curve; $t_{1/2}$, elimination half-lives; CL, clearance; V_d , volume of distribution.

Figure 1

Figure 2

Figure 3