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Determination of Maslinic Acid, a Pentacyclic Triterpene from Olives, in Rat Plasma by High-Performance Liquid Chromatography

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ABSTRACT: Maslinic acid, a pentacyclic triterpene from olives, has been reported to exert beneficial effects on health, including anticarcinogenic activity. Despite its importance, little is known about its bioavailability in both humans and animals. A fundamental step for this evaluation consisted of measuring this compound in blood. Therefore, a simple high-performance liquid chromatography (HPLC) method with diode array detection has been developed. Maslinic acid contained in plasma was extracted twice using ethyl acetate. After centrifugation, the organic fraction was evaporated to dryness and the residue was reconstituted with methanol/water (75:25, v/v) and analyzed by HPLC. The method was validated by obtaining a linear correlation (r^2 = 0.999) and an average recovery of 99%. Precision expressed as the coefficient of variation ranged from 1.23 to 9.06%. The oral administration of maslinic acid (50 mg/kg) to rats and its subsequent detection in plasma showed that the method is suitable for absorption, distribution, and metabolism studies.

KEYWORDS: *Maslinic acid, HPLC, pentacyclic triterpenic acids, olives, rat*

INTRODUCTION

The different regions of the Mediterranean basin have their own dietary traditions, but in all of them, olives and olive oil are regularly consumed.[1] Olive oil, rich in monounsaturated fatty acids and bioactive compounds, has been associated with a reduced risk of cardiovascular disease and certain cancers.[2,3] However, scant attention has been given to the fruit of *Olea europaea* L., which yields the nutritious edible oil and harbors compounds with important biological properties.[4,5] In addition to their fatty acid profile, table olives contain tocopherols, phospholipids, carotenoids, phenols, and triterpenic acids.[6] Among the latter, maslinic acid (Figure 1) is the most abundant pentacyclic triterpene,[7] which is mainly localized in the cuticular lipid layer of the drupe, where it protects the integrity of the fruit by acting as an insect antifeedant[8] and antimicrobial agent.[6] The concentration of this compound in commercial table olives ranges from 287.1 ± 66.6 to 1318.4 ± 401.0 mg/kg depending upon the variety and the method of processing.[9] The amount of maslinic acid in the oil is much lower than in the fruit, and its concentration depends upon the oil extraction process. In extra virgin olive oil with acidity inferior to 1%, maslinic acid can be found at 64.2 ± 8.1 mg/kg, whereas it increases to 193.9 ± 14.0 mg/kg in virgin olive oil.[10]

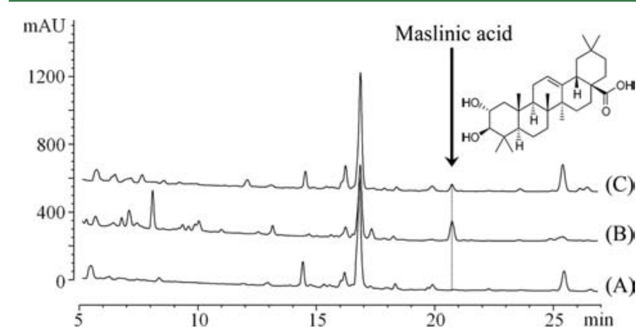

Figure 1. HPLC chromatograms of (A) rat blank plasma, (B) blank plasma spiked with 25 μM maslinic acid, and (C) plasma obtained 60 min after the oral administration of 50 mg/kg of maslinic acid to Sprague−Dawley rats.

Recently, much attention has been paid to maslinic acid because of its beneficial effects on health.[4,11−14] An extract from the skin of olive fruits containing 73.25% maslinic acid and 25.75% oleanolic acid inhibits proliferation and induces apoptosis in HT-29 human colon cancer cells.[4] Further studies demonstrated that maslinic acid was the compound with the highest antitumoral effect that inhibits cell growth with an EC_{50} of 101.2 ± 7.8 μM without cytotoxicity and with pro-apoptotic activity at 25 μM.[12] The antitumor effect has also been reported in different human tumor cell lines, such as astrocytoma,[15] pancreatic cancer cells,[13] and human breast cancer cells.[16] Moreover, maslinic acid, an inhibitor of glycogen phosphorylase,[17] has been proposed as a promising antidiabetic agent for its prevention of hyperglycemia in mice treated with adrenalin[18] and in KK-A^y mice, an animal model of genetic type 2 diabetes.[11] Recently, a neuroprotective activity has been described, because this pentacyclic triterpene reduces cerebral ischemic injury in hyperglycemic rats.[14] In addition, maslinic acid elicits multiple biological activities, such as antioxidant,[19] antihipertensive,[20] and antiparasitic.[21]

Despite the beneficial effects on health described for maslinic acid, there is a lack of knowledge about its absorption, distribution, and metabolism in either humans or animals. The

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first step in *in vivo* physiological and pharmacokinetic studies is to set up a method to determine this pentacyclic triterpene in plasma. Therefore, the aim of the present study was to develop a procedure to determine maslinic acid in rat plasma by liquid−liquid extraction followed by high-performance liquid chromatography (HPLC) analysis with diode array detection. The method was fully validated and applied to determine the concentration of maslinic acid in rat plasma after the oral administration of 50 mg/kg.

■ MATERIALS AND METHODS

Instrument. HPLC analyses were performed on an Agilent model 1100 gradient liquid chromatograph equipped with an automatic injector and a diode array ultraviolet−visible (UV−vis) detector (Agilent Technologies, Palo Alto, CA). A ChemStation for LC 3D software system (Rev. A.10.02) from Agilent Technologies controlled the equipment and performed the data processing. Separation was performed on a reversed-phase Luna C18 column (250 × 4.6 mm; 4 μm) preceded by a security guard cartridge of the same material, both provided by Phenomenex (Torrance, CA).

Chemicals and Reagents. Maslinic acid was obtained from olive pomace according to the patented method from García-Granados.[22] Briefly, the process comprises selective extractions and fractionation of the resulting mixtures with the use of hexane and ethyl acetate as solvents. The product obtained is a chemically pure white powder comprising 99.9% maslinic acid. Acetonitrile (ultra-gradient HPLC grade) and methanol were from J.T. Baker (Deventer, The Netherlands), and glacial acetic acid was from Scharlau Chemie S.A. (Barcelona, Spain). All of the solvents used were HPLC-grade. Other chemicals employed were analytical-grade and obtained from Sigma-Aldrich (St. Louis, MO). Water used in all experiments was passed through a Milli-Q water purification system (18 mΩ) (Millipore, Milan, Italy).

Animals. Male adult Sprague−Dawley rats (275−300 g) were housed in cages ($n = 2$/cage) under controlled conditions, which were 12 h light/12 h dark cycles, temperature of 22 ± 3 °C, and relative humidity of 40−70%. They received a standard diet (2014 Teklad Global 14%, Harlan, Barcelona, Spain) and water *ad libitum*. Handling and killing of rats were in full accordance with the European Community guidelines for the care and management of laboratory animals. The studies were approved by the Ethic Committee of Animal Experimentation of the Universitat de Barcelona. Rats were fasted overnight and anesthetized by intramuscular injection of 90 mg/kg of ketamine (Imalgene 1000, Merial Laboratorios S.A., Barcelona, Spain) and 10 mg/kg of xylacine (Rompun 2%, Química Farmacéutica Bayer S.A., Barcelona, Spain). All rat manipulations were carried out in the morning to minimize the effects of circadian rhythms.

Preparation of Plasma Samples. Plasma samples were treated by liquid−liquid extraction with ethyl acetate. An aliquot of 500 μL was added with 4 mL of ethyl acetate in glass tubes, which were vigorously vortex-mixed for 3 min and centrifuged at 1500×*g* for 30 min at 4 °C. The supernatant was transferred to another tube, and the pellet underwent a second extraction. The organic layers were combined and evaporated to dryness with a Concentrator 5301 (Eppendorf Ibérica S.L., San Sebastián de los Reyes, Spain) at 45 °C. The residue was reconstituted in 400 μL of methanol/water (75:25, v/v), vortex-mixed for 30 s, and centrifuged at 12000×*g* for 5 min at 4 °C. The supernatant was transferred to an amber vial for HPLC analysis.

HPLC Procedure. An aliquot of 100 μL of the sample was injected into a C18 reversed-phase column kept at 40 °C. The mobile phase included solvent A, consisting of 0.05% acetic acid in deionized water, and solvent B, containing 100% acetonitrile. The flow rate was 1 mL/min. Separation was performed using the gradient elution program: 0 min, 60% A and 40% B; 5 min, 50% A and 50% B; 10 min, 40% A and 60% B; 15 min, 30% A and 70% B; 20 min, 30% A and 70% B; 25 min, 25% A and 75% B; 30 min, 20% A and 80% B; 35 min, 0% A and 100% B; and 40 min, 0% A and 100% B. There was a 5 min delay prior to the injection of the next sample to ensure re-equilibration of the column.

The chromatograms were obtained according to the retention time, with detection at 200 nm, at which the absorbance of maslinic acid presents its maximum. Peak identification of this compound was carried out by comparison of the retention time and its UV spectra (from 200 to 400 nm). Quantification of maslinic acid was performed using the external standard method. Calibration curves were constructed after spiking relevant concentrations of this compound in blank plasma. The standard curves were characterized by regression coefficients of r^2 equal to 0.99 or above. The results of the analyses are expressed in micromoles of maslinic acid per liter.

Method Validation. To prove the suitability of the developed analytical method, validation was performed according to *The United States Pharmacopeia*.[23]

Samples. A pool of plasma obtained from fasted Sprague−Dawley rats that were not administered with maslinic acid was used. Aliquots of the pooled plasma were stored at −20 °C until the analyses were performed. A total of 475 μL of plasma was spiked with 25 μL of working standard solutions to obtain the calibration standards at final concentrations of 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 15, and 25 μM and was stirred in the vortex for 1 min before being extracted, as indicated in sample preparation. Calibration standards were freshly prepared before use. A stock solution of maslinic acid was obtained by dissolving the pure standard in 80% (v/v) methanol, stored at 4 °C, and used within 4 weeks.

Linearity. Calibration curves of maslinic acid were prepared in the range of application of the analytical method. Integrated peak areas were plotted against the analyte concentration, and linear regression analysis was performed by the least-squares method.

Recovery. Recoveries were measured by spiking blank plasma samples with 1, 2.5, 5, 10, 15, and 25 μM maslinic acid. Absolute recoveries were calculated by comparing the peak area ratio from spiked samples to those of the corresponding concentrations injected directly without extraction into the HPLC system.

Precision and Accuracy. The precision of the analytical method was determined by assaying a sufficient number of plasma samples ($n = 4-6$) at six different concentrations of maslinic acid ranging from 1 to 25 μM and was expressed as the relative standard deviation (% RSD, coefficient of variation). Peak areas were considered to calculate the concentration and establish the precision. The intra- and interday precisions were determined by analyzing the spiked samples prepared within a day and on three different days, respectively.

Accuracy was estimated on the basis of the mean percentage of error of the measured concentration (con_M) to the theoretical concentration (con_T) according to the following equation:

$$\text{bias (\%)} = \left(\frac{\text{con}_T - \text{con}_M}{\text{con}_T}\right) \times 100$$

Sensitivity. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by measuring the analytical background response, running six blank plasma samples using the 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 maslinic acid in plasma samples that generated a peak with an area at least 3 times higher than the baseline noise. LOQ was considered to be 10 times superior to the baseline noise. The LOQ was subsequently validated by the analysis of six plasma samples known to be near the LOQ.

Selectivity. The specificity of the method was determined by comparing the chromatograms of six independent blank plasma samples to the corresponding spiked samples.

Method Application. Maslinic acid was administered orally to overnight fasted rats at a dose of 50 mg/kg. Given that this compound is insoluble in water, it was solubilized using (2-hydroxypropyl)-β-cyclodextrin at 40% and sodium carboxymethylcellulose at 0.5%, following a patented procedure described for betulinic acid.[24] Blood was collected from the saphenous vein[25] at 0, 10, 30, and 60 min and placed in Microvette CB300 tubes (Sarstedt, Granollers, Spain)

containing EDTA-K2 as an anticoagulant. Plasma was obtained by centrifugation at 1500*g* for 15 min at 4 °C, and the analyte was extracted using the protocol described above.

RESULTS

Optimization of the Chromatographic Conditions. The chromatographic conditions were optimized to achieve the maximum sensitivity and selectivity allowed by the system. The analysis of maslinic acid was attempted using reversed-phase liquid chromatography (LC), with gradient elution of aqueous and organic mobile phases. To assay acidic compounds by HPLC, the mobile phase is often acidified to improve the chromatographic peak shape and the retention time of the analytes. Therefore, water with 0.05, 0.1, and 0.5% formic acid or 0.05, 1.5, and 3% acetic acid was evaluated as the aqueous solvent. Acidification with 0.05% acetic acid provided the best chromatograms with a retention time for maslinic acid of 20.9 min as well as good peak shapes and areas. In contrast, no peak of maslinic acid was observed when higher amounts of acetic acid were used. Formic acid was not selected because it gave lower peak areas and the retention time of maslinic acid was delayed to 29 min. With regard to the organic solvents, methanol and acetonitrile were evaluated. Acetonitrile was chosen instead of methanol because of its lower UV absorbance in the region of 200−210 nm. In addition, methanol was also discarded given that a carryover effect was always observed after the injections with maslinic acid that did not take place when acetonitrile was employed.

Different gradients were evaluated until obtaining the adequate conditions that did not produce any significant interference from blank plasma at the retention time of maslinic acid. Consequently, a compromise between an appropriate retention time for maslinic acid (20.9 min) and adequate separation from compounds present in the biological matrix was achieved. The flow rate was also assessed to advance the elution of maslinic acid. A flow of 1.5 mL/min gave a retention time of 17.2 min; however, the peak areas were reduced by 33% with respect to 1 mL/min, which was finally selected. A difference in the wavelength of maximum absorbance for maslinic acid was observed depending upon the organic solvent of the mobile phase. When methanol was used, the maximum was observed at 204 nm, whereas the use of acetonitrile gave a value of 197 nm. Once acetonitrile was selected, the optimal wavelength to measure maslinic acid was assessed. Analysis at 195, 200, 205, and 210 nm was performed, and the wavelength was set at 200 nm. Peak areas obtained at 195 and 200 nm only differed by 2%, while peak areas at 205 and 210 nm were reduced by 10 and 30%, respectively. The column temperature was evaluated by keeping it at 25, 30, or 40 °C. The selected temperature was 40 °C because it yielded the highest signal intensity.

Optimization of the Extraction Procedure. Sample preparation is a crucial step for a successful analysis. Consequently, different extraction approaches were investigated to obtain the best extraction efficiency. Solid-phase extraction that was first assessed using reversed-phase C18 cartridges yielded recoveries of only 5% ($n = 3$). Ultrafiltration of plasma samples was also evaluated. Microcon or Ultrafree-MC centrifugal filter devices were applied to plasma samples spiked with maslinic acid, and the recoveries obtained were of 1.30 ± 0.21% ($n = 3$) and 13.5 ± 1.5% ($n = 3$), respectively. Finally, liquid−liquid extraction was assessed. Various solvents, including methanol, ethanol, acetonitrile, and ethyl acetate, were screened. Acetonitrile and ethanol gave recoveries lower than 75% ($n = 3$). Protein precipitation with methanol provided recoveries of 89.3 ± 1.4% ($n = 6$). However, ethyl acetate was selected, because it yielded recoveries superior to 95% ($n = 6$) and gave less interfering peaks. To optimize the extraction procedure, the volume of ethyl acetate, times of extraction, and duration of the same were also evaluated. Plasma samples were initially extracted 3 times, and the individual recoveries were 95.1 ± 6.2% ($n = 3$), 3.98 ± 0.91% ($n = 3$), and 0.30 ± 0.02% ($n = 3$), respectively. The results indicated that maslinic acid was mainly recovered in the first two extractions; thus, the last one was discarded. The established extraction method in which plasma samples were extracted 2 times with 4 mL of ethyl acetate by vigorous vortex-mixing for 3 min was appropriate for the analysis.

Method Validation. The analytical performance parameters assessed for the overall assay were linearity, recovery, precision, accuracy, sensitivity, and selectivity.

Linearity. Good linearity of the assay in plasma was found over the investigated calibration range of 0.5−25 μM. The calibration curves were characterized by coefficients of correlation (r^2) of 0.99 or above.

Recovery. The extraction recoveries of maslinic acid were conducted in plasma samples spiked with six different concentrations (Table 1). The mean recovery was 98.8 ± 0.74% ($n = 37$).

Table 1. Precision, Accuracy, and Recovery of Maslinic Acid in Spiked Rat Blank Plasma Samples

maslinic acid (μM)	accuracy (% bias)	precision (% RSD) intraday	precision (% RSD) interday	recovery (%)
1 ($n = 5$)	0.28	5.20	9.06	99.7 ± 4.0
2.5 ($n = 6$)	0.59	2.87	3.54	99.4 ± 1.4
5 ($n = 6$)	1.89	3.75	4.24	98.1 ± 1.7
10 ($n = 6$)	0.59	3.36	1.23	99.4 ± 0.5
15 ($n = 8$)	0.98	2.35	4.68	99.0 ± 1.6
25 ($n = 6$)	2.57	3.81	3.67	97.4 ± 1.5

Precision and Accuracy. The precision and accuracy data for the analytical procedure are shown in Table 1. Intra- and interday precisions were lower than 10% and were within the acceptable limit to meet the guidelines for bioanalytical method validation, which is considered to be ≤15%.[26] The accuracy was also good with the deviation between the nominal concentration and calculated concentration for maslinic acid well below the limit of 15%. Precision and accuracy data indicated that the method to extract maslinic acid from plasma is highly reproducible and robust.

Sensitivity. The LOD and LOQ were calculated by measuring the magnitude of the analytical background response, running six blank plasma samples, and analyzing them using the maximum sensitivity allowed by the system. At 200 nm, the LOD for maslinic acid was 0.02 μM and the LOQ was 0.32 μM. The LOQ was subsequently validated by the analysis of six blank plasma samples spiked with the triterpene at 0.5 μM.

Selectivity. The chromatogram at 200 nm shown in Figure 1 indicated that maslinic acid in plasma samples was well-resolved and free from interfering peaks. No interferences from endogenous substances were observed at the retention time of the analyte. The use of a diode array detector allowed for the

confirmation of the identity of the chromatographic peak by not only its retention time but also its spectrum.

Verification of the Method. Because the method provided good accuracy, precision, and recovery during the validation procedure, the question of the availability of maslinic acid after the oral administration could be approached. Therefore, maslinic acid at 50 mg/kg was orally administered to rats, and blood samples collected at different time points were analyzed following the validated analytical method. The representative HPLC chromatogram (Figure 1) showed that maslinic acid eluted at 20.9 min and was well-resolved from other compounds. After the administration of 50 mg/kg to overnight fasted rats, this pentacyclic triterpene reached the blood (Figure 2) and was still detected in plasma at 60 min (3.08 ± 0.02 μM). The results indicate that the method developed is reliable, reproducible, and easily applied to biological samples.

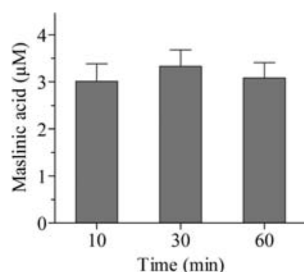

Figure 2. Plasmatic concentrations of maslinic acid after oral administration of 50 mg/kg of the triterpene. Values are represented as means + standard error of the mean (SEM) (n = 3).

■ DISCUSSION

Table olives are regular dietary components of the different countries bordering the Mediterranean Sea and are being recognized as healthy foods from a nutritional point of view because of their content in monounsaturated fatty acids as well as bioactive components, such as polyphenols and pentacyclic triterpenes.[5] The fruit of *O. europaea* L. is a particularly rich source of maslinic acid,[9] which is a hydroxy pentacyclic triterpenic acid with health-protecting properties, such as antitumor,[4,12,13] antioxidant,[19] antidiabetic,[14,17] and cardioprotective.[20] Notwithstanding the promising effects described, the mechanisms of absorption, bioavailability, and tissue distribution of maslinic acid in either humans or experimental animals are still undefined. The absence of this knowledge is mainly due to the lack of an analytical method to determine maslinic acid in biological fluids. Consequently, the present study develops and validates a procedure that allows for the measurement of this compound in plasma by applying liquid−liquid extraction followed by HPLC analysis with diode array detection.

Gas chromatography (GC) coupled with different detectors has been traditionally used to determine pentacyclic triterpenes, such as maslinic and oleanolic acids, in olives[7,27] and olive oil.[10] However, the low volatility and high molecular weight of these compounds prevent its direct injection to the chromatograph, and a derivatization step is mandatory prior to GC analysis. Derivatization of triterpenes has traditionally been carried out by silylation,[7,10] which presents several disadvantages, such as an increase of time, reagents, and materials consumption. For the analysis of nonvolatile molecules, such as maslinic acid, LC constitutes an appropriate alternative that shortens analysis time because derivatization is not required.[28] We analyzed maslinic acid in plasma by HPLC, which is an appropriate technique for both nutritional and pharmacological studies. These instruments are common in many laboratories, thus providing readily available and affordable analysis that renders satisfactory separation, identification, and quantification of the triterpene. For these reasons, LC is a technique that is used in the analysis of pentacyclic triterpenic acids. In fact, the concentrations of oleanolic and maslinic acids in table olives were determined by HPLC analysis with diode array detection.[9] Analysis was carried out with an isocratic mobile phase [92:8 (v/v) methanol/phosphoric acid in water at pH 3.0] that was delivered to a reversed-phase column kept at 35 °C at 0.8 mL/min, and the eluate was monitored at 210 nm. Under these experimental conditions, the concentrations of maslinic and oleanolic acids were quantified in different types of commercial black and green olives.[9] Recently, the efficacy of different drying and extraction methods were evaluated through the quantification of maslinic and oleanolic acids in olives using similar reversed-phase HPLC conditions.[29] Moreover, HPLC with diode array detection has also been employed to determine oleanolic, ursolic, and betulinic acids in human serum.[30] After liquid extraction with diethyl ether, the triterpenes were separated on a C18 reversed-phase column at 30 °C and eluted isocratically [85:15 (v/v) acetonitrile/0.5% phosphoric acid in water] at 0.8 mL/min. The method was validated but lacks verification, because it was not applied to serum samples from patients who were administered the triterpenes.

The analytical procedure to determine maslinic acid in plasma was attempted through liquid−liquid extraction, which is a basic sample preparation method that has been commonly used to determine other pentacyclic triterpenes.[30−35] Different organic solvents were evaluated, and ethyl acetate yielded the best recovery. Ethyl acetate has also been used in the extraction from plasma samples of other pentacyclic triterpenes, such as oleanolic acid,[32] ursolic acid,[35] and glycyrrhetic acid.[31,34]

The validation of the method gave an average recovery of 98.8 ± 0.74%. This value was calculated after spiking blank plasma samples at six different concentrations to ensure that the method is adequate in both low and high concentrations.[26] The analytical method is accurate given that the average inter- and intraday precisions, expressed as RSD, were 4.40 ± 0.42 and 3.98 ± 0.29%, respectively. Furthermore, the method is selective, because no other peak elutes at the retention time of maslinic acid. In addition, the LOD and LOQ were 0.02 and 0.32 μM, respectively. Consequently, the results of the validation indicated that the method showed good linearity, recovery, precision, sensitivity, and selectivity.

Once the method was established and validated, it was verified by applying it to the detection of maslinic acid after a single oral administration of a dose of 50 mg/kg to rats. The analysis of plasma revealed that the method developed is suitable for the determination of maslinic acid because it was detected in all of the samples. The results showed that maslinic acid is absorbed in the intestine and reaches the blood, where it is found 10 min after the oral administration and can still be detected in plasma at 60 min. A recently published study determines the plasmatic concentration of maslinic acid in male C57BL/6 mice after its consumption mixed in the chow at 0.5% (approximately 25 mg/kg) for 4 or 8 weeks.[36] Maslinic acid, analyzed by HPLC tandem mass spectrometry, was not detected in plasma after 4 weeks of administration, whereas the concentration obtained in the mice administered for 8 weeks

was 1.00 ± 0.15 μM (0.47 ± 0.07 μg/mL). The paper presents little information on method validation, indicating only a detection limit of 0.21 μM (0.1 μg/mL of plasma), which is 10 times higher than the detection limit than we have reported, a precision lower than 5%, and no information about the recovery of maslinic acid from plasma.

In contrast to the scarce data existing on the bioavailability of maslinic acid, the plasmatic concentrations of other pentacyclic triterpenes have been thoroughly studied. The bioavailability of oleanolic acid, an isomer of maslinic acid that lacks a hydroxyl group at the carbon 2 position, was determined in both rats[33] and humans.[32] After the oral administration of 50 mg/kg of oleanolic acid to rats, a maximum concentration of 0.29 ± 0.26 μM was observed at 21 ± 17 min, which is a value lower than the value that we have reported for maslinic acid at the same dose.[33] These authors indicated that oleanolic acid was minimally absorbed, with an absolute oral bioavailability of 0.7%. Moreover, the plasmatic concentrations of oleanolic acid have been determined in healthy male volunteers by HPLC tandem mass spectrometry.[32] The pharmacokinetic study showed that, after the single oral administration of 40 mg of oleanolic acid, the maximum concentration was 26.5 ± 15.0 nM at 5.2 ± 2.9 h.[32]

In conclusion, a simple, precise, reproducible, and accurate method has been developed to determine maslinic acid in plasma. The subsequent oral administration of this pentacyclic triterpene to experimental animals and its presence in blood have provided evidence of its intestinal absorption. Further experiments should be carried out to establish which circulating levels of this compound have to be reached in plasma to reproduce the *in vitro* beneficial effects described for maslinic acid.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; GC, gas chromatography; RSD, relative standard deviation

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