

Development and Validation of a Sensitive UFLC–MS/MS Method for Quantification of Quercitrin in Plasma: Application to a Tissue Distribution Study

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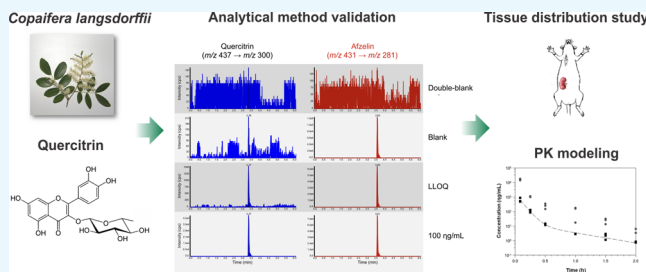
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ABSTRACT: Quercitrin, a glycosylated form of the flavonoid quercetin, is one of the major constituents of *Copaifera langsdorffii* leaves and potentially contributes to the medicinal properties reported for this plant species, including the treatment and prevention of kidney stones. To better understand the pharmacokinetics of quercitrin, a simple, rapid, and sensitive ultra fast liquid chromatography–tandem mass spectrometry method was developed and validated for the quantification of quercitrin in rat plasma and applied to a tissue distribution study. Sample preparation involved simple liquid–liquid extraction by ethyl acetate with high efficiency, using afzelin as internal standard. The chromatographic separation was performed on a Phenomenex Synergi Polar-RP (100 × 3.0 mm², 2.5 μm), with a gradient elution of acetonitrile and 0.5% formic acid in water. The mass spectrometry analysis was conducted in negative ionization mode with multiple reaction monitoring transitions at m/z 447 → 300 for quercitrin and m/z 431 → 281 for afzelin. The method showed linearity in the concentration range of 5–100 ng/mL ($r^2 > 0.9959$) and the lower limit of quantification was 5 ng/mL. The intraday and interday precision (relative standard deviation) were less than 10.73%, whereas the accuracy ranged from 81.4 to 111.0%. The extraction recovery, stability, matrix effect, and integrity dilution involved in the method were also validated. In addition, tissue distribution was assessed after an intravenous administration of 1 mg/kg quercitrin. This is the first report quantifying quercitrin in kidneys, demonstrating that the free tissue/plasma ratio was 23.7.



1. INTRODUCTION

Quercitrin (quercetin-3-*O*- α -L-rhamnopyranoside) is one of the most common glycosylated forms of the flavonoid quercetin,¹ displaying many pharmacological activities, such as antioxidant,² chemopreventive,³ and anti-inflammatory.⁴ It can be extracted from several plant species,^{5,6} including *Copaifera langsdorffii* Desf., in whose leaves is one of the major metabolites.^{7,8} *C. langsdorffii* is a large tree popularly known as “copaiba” and distributed in many Brazilian states.⁹ This plant species leaf extracts and isolated compounds display gastroprotective^{10,11} and antilithiatic^{12,13} activities.

Kidney stones disease, urolithiasis, occurs due to many etiologic factors, including genetic predisposition,¹⁴ diet, lifestyle, and metabolic syndromes, which together make the incidence and prevalence of this disease significantly variable among different cultures.¹⁵ Although kidney stones can be asymptomatic, they are often associated with debilitating pain as it passes through ureter and bladder, becoming worst when causing obstruction and infection.¹⁶ The three main procedures currently used to remove upper urinary tract stones are shockwave lithotripsy, ureteroscopy, and percuta-

neous nephrolithotomy.¹⁷ Though these techniques are minimally invasive, they tend to be expensive and generally not accessible to the needy portion of the population. Therefore, research for a safe, efficacious technique with an affordable noninvasive treatment made us consider that quercitrin, which is present in *C. langsdorffii* leaves, might be related to the antilithiatic activity of *C. langsdorffii*. For a better understanding of its mechanism of action, we first investigated its pharmacokinetics.

Pharmacokinetics and tissue distribution studies are required during the development of novel drugs and, to analyze complex biosamples, liquid chromatography tandem–mass spectrometry (LC–MS/MS) has been frequently used due to its high sensitivity and selectivity.¹⁸ In the present study, a reliable, precise, and accurate ultra fast (UF) LC–MS/MS method was developed and validated for the quantification of quercitrin in rat plasma. The method was then successfully

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applied to plasma pharmacokinetics profile and kidney tissue distribution of quercitrin in rats after tail intravenous administration.

2. RESULTS AND DISCUSSION

2.1. Plasma Extraction. One of the most important steps that directly influence the quality of a LC–MS/MS bioanalytical method is the sample preparation.¹⁹ Choosing the best extraction method will effectively reduce matrix components in the samples and eliminate ion suppression in LC–MS/MS analyses.²⁰ The three main plasma extraction procedures are protein precipitation (PP), liquid/liquid extraction (LLE), and solid-phase extraction.¹⁹ Sometimes these methods also need some adjustments, with the incorporation of antioxidants and/or acids to extract substances from plasma.^{21,22}

Even though a complex extraction method is necessary in some cases,²² a simple procedure that requires very few steps and removes a great amount of proteins from plasma is preferable, mainly when executing pharmacokinetics studies.¹⁹ In this manner, a simple LLE with ethyl acetate was done to efficiently extract quercitrin from rat plasma (109.5%) (Table 1). This is predictable because during the partitioning of C.

Table 1. Plasma Extraction Efficiency of Quercitrin (%)

extraction method	quercitrin plasma extraction efficiency (%)
1st – Sun et al. ^a	44.30
2nd – Sun et al., with modifications ^b	65.75
3rd – LLE ^c with ethyl acetate	109.5
4th – PP ^d with acetonitrile	44.55
5th – PP with methanol	2.07

^aTo the plasma was added 20 μ L of vitamin C (100 mg/mL), 20 μ L of internal standard afzelin (400 ng/mL), and 20 μ L of hydrochloric acid (1 M) and the mixture was incubated at 80 °C for 30 min and extracted with 1 mL of ethyl acetate. ^bThe same procedure as describe in 1st, but without the incubation of plasma at 80 °C for 30 min. ^cLLE = liquid–liquid extraction. ^dPP = protein precipitation.

langsdorffii crude extract with different organic solvents, the ethyl acetate fraction is the richest one in quercitrin.^{7,8}

2.2. Analytical Method Validation for Plasma Samples. Mass spectrometry is one of the most employed techniques for the investigation of a potential drug, especially in pharmacokinetic studies.²³ Ideally, a bioanalytical method should provide high selectivity, resulting in rapid sample analysis and, consequently, rapid method development.¹⁹ Following these recommendations and FDA guidelines, this study developed and validated a LC–MS/MS method to investigate the pharmacokinetics of quercitrin in rat plasma. Specific method parameters were evaluated: selectivity, linearity, accuracy, precision, and matrix effects.

The selectivity of the method was determined by comparing the double blank (unspiked plasma) with blank plasma (spiked with internal standard), lower limit of quantification (LLOQ) (5 ng/mL), quality control low (QCL) (15 ng/mL), and quality control high (QCH) (80 ng/mL) and no interfering peaks were observed in the chromatograms (Figure 1). The retention time of quercitrin and the internal standard afzelin were 3.36 and 3.60 min, respectively. Afzelin was chosen as the internal standard of this bioanalytical method due to the physicochemical similarities with quercitrin.⁷ The same LC–

MS/MS parameters were set to both compounds, differing only on the monitored transitions that were m/z 447 \rightarrow m/z 300 for quercitrin and m/z 431 \rightarrow m/z 281 for afzelin. In agreement with Huang et al., the addition of formic acid in the aqueous mobile phase helped the achievement of peak symmetry and resolution and increased the sensitivity of the method.

The calibration curves were linear and had at least the correlation coefficient (r^2) of 0.9959 or higher to quercitrin (Table 2). The curves were constructed using five calibration standards. The lowest concentration in the standard curve was defined as the LLOQ, which presented an acceptable accuracy and precision at 5 ng/mL.

Percentage accuracy and precision values for the interday and intraday variations are shown in Tables 3 and 4, respectively. The results indicated that both inter- and intraday values were all within the acceptable range (relative standard deviation (RSD) < 20% for LLOQ and < 15% for QC samples).

Quercitrin was stable under all testing conditions, including short-term storage (benchtop 4 h), freeze–thaw cycling, and long-term storage (30 days at -80 °C) (Table 5), which is in agreement with previous studies.²² The RSD calculated from the QC samples under all testing conditions ranged from ± 2.0 to 5.5%.

The matrix effect was considered negligible, in which six QC replicates presented a coefficient of variance smaller than 9.54%. The obtained values corroborate the acceptable limits for the validation of bioanalytical methods.²⁴

During sample analyses, two out of six collected plasma samples exceeded the higher upper limit concentration of the validated method, and that is why they were diluted to be adjusted to the calibration curve. This procedure was also validated, presenting an accuracy of $97.4 \pm 2.1\%$ on 10-fold dilutions.

All of the obtained results for the validation of bioanalytical method were in accordance with the FDA and European Medicines Agency criteria of validation,^{24,25} and it is appropriate to quantify quercitrin in plasma samples.

2.3. Determination of Plasma Protein Binding. Measuring the protein binding of drugs is extremely important, since it strongly affects the distribution, elimination, and pharmacological action of drugs.²⁶ Free fractions of quercitrin were calculated using ultrafiltration device. For that, concentrations of 50, 100, 150, 250, and 500 ng/mL of quercitrin were prepared in 1 mL of plasma. After being incubated for 30 min at 37 °C, the samples were centrifuged in an appropriate device that separated the unbound fraction of compounds. It was also verified that quercitrin did not present binding to the ultrafiltration system.

The protein binding of quercitrin to rat plasma was determined to be $91.4 \pm 5.1\%$ in the range of 50–500 ng/mL, which corroborates the published data reporting a high protein binding around 85% in human plasma.²⁶

2.4. Applicability of the Analytical Method: Tissue Distribution. Concentration of quercitrin in plasma and rat's kidneys was determined at 5, 15, 30, 60, 90, and 120 min after intravenous administration of 1 mg/kg of quercitrin (Figure 2). Quercitrin was highly distributed to kidney in comparison to plasma after intravenous administration. This characteristic might be attributed to the high blood flow in certain organs¹⁸ and also to the moderate lipophilicity of quercitrin ($\log P = 1.31$), which might be responsible for the ability to cross

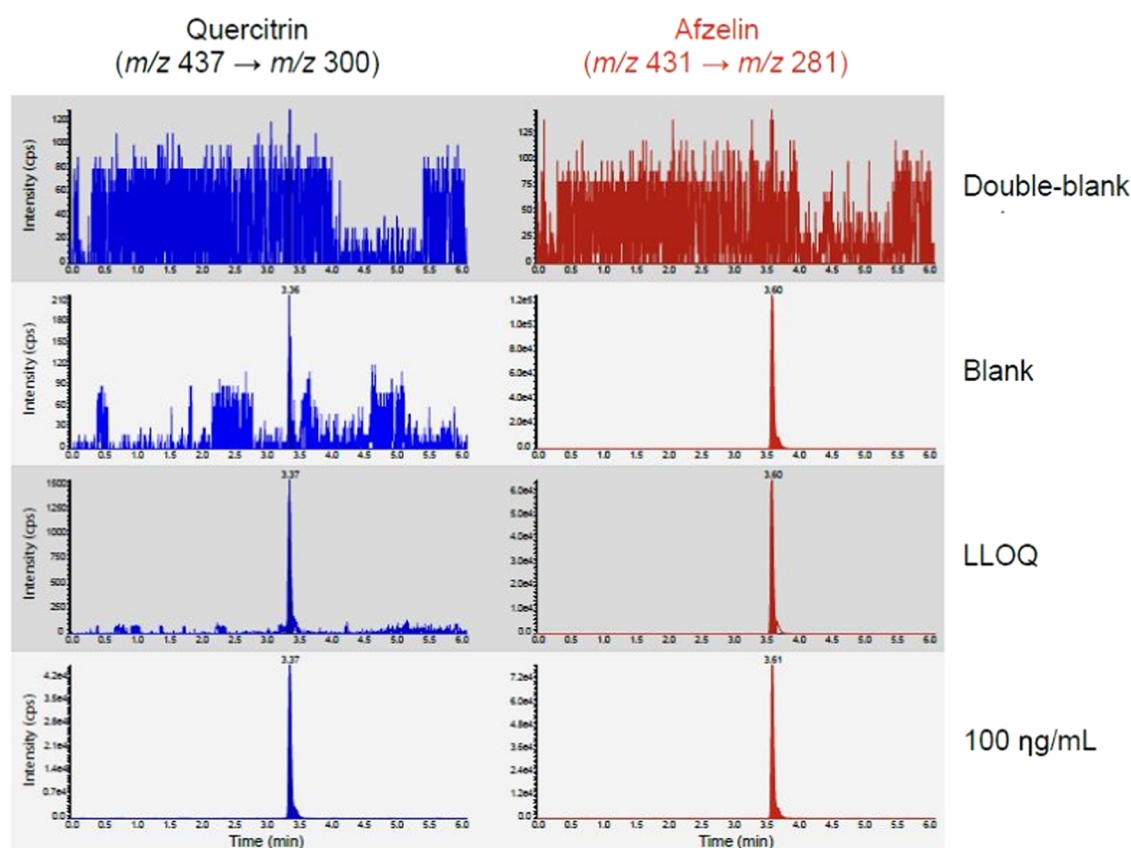


Figure 1. Chromatograms of quercitrin and the internal standard afzelin extracted from plasma samples. Double blank (plasma), blank (plasma spiked with internal standard afzelin), LLOQ (5 ng/mL), QCL (15 ng/mL), and QCH (80 ng/mL).

Table 2. Parameters of the Calibration Curves of Quercitrin in Plasma Samples

curve	slope	y-intercept	correlation coefficient
day 1			
1	1.0745	0.3431	0.9997
2	0.9622	0.5603	0.9981
3	1.0655	0.8627	0.9959
day 2			
1	1.0327	0.2634	0.9997
2	1.0121	0.5645	0.9965
3	1.0103	0.2414	0.9998
mean ($n = 6$)	1.0224		
SD	0.0412		
RSD	4.0250		

biological membranes. This ability was also observed for a moderately lipophilic compound in other study.²⁷

For the pharmacokinetic parameters in renal tissue and plasma, it was observed that the highest quantified concentration in the tissue was as early as 5 min after intravenous administration of quercitrin (Table 6). The peak tissue concentration was higher in the kidney (15.84 $\mu\text{g/mL}$) in comparison with plasma (0.61 $\mu\text{g/mL}$), corroborating the fact that the ratio of AUC_{0-t} tissue and AUC_{0-t} plasma was 23.7.

For many years, it was assumed that quercetin glycosides had to be hydrolyzed to aglycone form prior to absorption.²⁸ However, many studies have demonstrated the pharmacokinetic profile of these flavonoids after being administered

Table 3. Intra- and Interday Variation of Quercitrin in Microdialysates Samples

nominal concentration	day	experimental concentrations		
		mean (ng/mL)	SD	RSD (%)
intra-day variation				
5 ng/mL (LLOQ)	1	4.65	0.47	10.11
	2	4.50	0.387	8.58
15 ng/mL	1	14.73	0.69	4.70
	2	14.78	0.54	3.66
40 ng/mL	1	37.25	2.58	6.92
	2	38.36	1.59	4.15
80 ng/mL	1	78.93	6.46	8.19
	2	77.63	6.45	8.31
inter-day variation				
5 ng/mL (LLOQ)		5.57	0.46	8.24
15 ng/mL		14.77	0.64	4.34
40 ng/mL		37.18	3.99	10.73
80 ng/mL		77.97	7.80	10.01

Table 4. Accuracy of the Analysis of Quercitrin in Microdialysate Samples

concentration (ng/mL)	range (ng/mL)	accuracy (RE %)
5 (LLOQ)	4.9–5.8	81.4–91.7
15	14.4–15.8	95.9–105.0
40	35.7–42.0	89.3–105.0
80	71.1–88.4	88.9–111.0

orally^{22,29} and intravenously.²¹ Moreover, quercitrin is better absorbed than quercetin itself.¹

Table 5. Stability of Quercitrin in Plasma Samples ($n = 3$)

spiked conc. (ng/mL)	conditions	mean measured conc. \pm SD (ng/mL)	RSD (%)	accuracy (RE %)
15 (QC low)	benchtop (4 h)	16.70 \pm 0.44	2.61	111.33
	3 \times freeze–thaw	14.00 \pm 0.46	3.27	93.33
	30 days at -80°C	14.40 \pm 0.36	2.50	96.00
80 (QC high)	benchtop (4 h)	78.83 \pm 3.19	4.05	98.54
	3 \times freeze–thaw	73.80 \pm 1.47	2.00	92.25
	30 days at -80°C	73.73 \pm 4.05	5.50	92.17

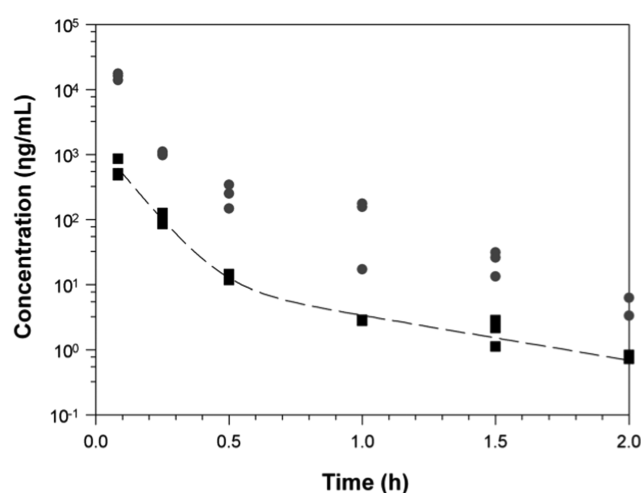


Figure 2. Concentration profiles versus time of quercitrin in plasma (■) and renal tissue (●). The line represents the concentration predicted by the two-compartment model in plasma (----).

Table 6. Estimated Pharmacokinetic Parameters in Renal Tissue and Plasma after Intravenous Administration of 1 mg/kg Quercitrin in Wistar Rats ($n = 3$ Animals/Time)

sample	C_{\max} ($\mu\text{g/g}$)	t_{\max} (min)	AUC_{0-t} ($\mu\text{g}\cdot\text{h/g}$)	ratio tissue/plasma
kidney	15.84	5.0	3.32	23.7
plasma	0.61	5.0	0.14	

It is known that pharmacokinetics/pharmacodynamics studies promote a pivotal role in the development of a new drug, being efficient on selecting potential drug candidates.^{18,30} The present study investigated for the first time the affinity of quercitrin to kidneys after intravenous administration. As the results demonstrated a high affinity, it can be inferred that *C. langsdorffii* leaf extract, which contains great amount of quercitrin and low acute toxicity,¹⁰ can be a potential noninvasive treatment for urolithiasis.

3. CONCLUSIONS

In this study, we developed and validated a reliable, precise, and accurate ultra fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS) method for the quantification of quercitrin in rat plasma, which was successfully applied to the pharmacokinetics and tissue distribution study of quercitrin in rats after intravenous administration. To the best of our knowledge, this is the first report quantifying quercitrin in

kidney, which should be helpful for investigating the prevention and treatment of kidney stones by *C. langsdorffii* leaves. The results indicate that quercitrin has high penetration in kidney, culminating in an elevated tissue/plasma ratio.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents. Quercitrin and the internal standard afzelin (kaempferol-3-*O*- α -L-rhamnopyranoside) were isolated from *C. langsdorffii* ethyl acetate fraction using high-speed counter current chromatography followed by semipreparative reverse-phase high-performance liquid chromatography purification, as described by Nogueira, Furtado, and Bastos.⁷ Analytical grade formic acid and HPLC grade acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). Water was purified using the Milli-Q-plus filter system (Millipore, Bedford, MA).

4.2. Instrumentation and Chromatographic Conditions. The instrument consisted of an ultrafast liquid chromatography (UFLC) Shimadzu binary pump and an autosampler (Shimadzu, Columbia, MD) attached in a quadrupole AB Sciex QTRAP 5500 (AB Sciex, Framingham, MA) with electrospray ionization operated in a negative mode. The chromatographic separation was achieved in an ether-linked phenyl column with polar end capping, Phenomenex Synergi Polar-RP (100 \times 3.0 mm², 2.5 μm) with a guard column containing the same pack material as the analytical column.

The mobile phase consisted of 0.5% formic acid in water (v/v) as reservoir A and acetonitrile as reservoir B. The mobile phase was filtered and degassed ultrasonically for 20 min before use, and it was delivered as a gradient that consisted of 15% of reservoir B at 0 min, 40% at 2.0 min, 40% at 4.0 min, 15% at 4.1 min, and 15% at 6.0 min. The flow rate was set as 0.4 $\mu\text{L}/\text{min}$. To preserve the detector from the presence of salt in the samples, the elute flow from the column was diverted to waste from 0–2.2 and 5.0–6.0 min. The autosampler was conditioned at 4 $^\circ\text{C}$ and the injection volume was set as 10 μL .

Samples were introduced into the interface through a heated nebulizer probe set at 350 $^\circ\text{C}$. Nitrogen was the nebulizer and desolvation gas and helium was used as the collision gas. The other operating conditions such as declustering potential, collision energy, entrance potential, and collision cell exit potential were -56 , -28 , -8 , and -11 V, respectively. The ion spray voltage, curtain gas, collision-activated dissociation gas, nebulizer gas, and turboheater gas were set at -3500 V, 30 psi, medium, 60 psi, and 60 psi, respectively.

The spectrometer was programmed in multiple reaction monitoring mode to allow the specific transition of precursor ion to fragment for each compound. The detection of ion species was performed by monitoring the decay of the mass-to-charge (m/z) ratio 447 and 431 corresponding to the precursor ion of quercitrin and afzelin, respectively. The daughter ion of quercitrin was m/z 300 and afzelin was m/z 281, which correspond to the loss of sugar moiety for both compounds.

Analyst software version 1.6.2 (AB Sciex) was used to obtain the data acquisition and quantification.

4.3. Animals. The experimental protocol was approved by the Animal Ethical Committee of Federal University of Rio Grande do Sul, Porto Alegre, Brazil (#29547). Eighteen male Wistar rats were kept in acclimatized ($22 \pm 1^\circ\text{C}$, 65% humidity) and ventilated cages with food and water ad libitum. The circadian cycles (12 h light and 12 h darkness) were also

respected. Before executing the tissue distribution experiment, the animals were fasted for 12 h.

A pool of male Wistar rats plasma (Sodium heparin, lot 16063; Innovative Research, MI) was used to perform all method development and validation procedures.

4.4. Plasma Extraction. Different methods were applied to define the best one to extract quercitrin and the internal standard afzelin from rat plasma. The following procedures were tested with 180 μL of rat plasma spiked with 20 μL quercitrin (1 $\mu\text{g}/\text{mL}$):

1st: To the spiked plasma was added 20 μL of vitamin C (100 mg/mL) and 20 μL of internal standard afzelin (400 ng/mL). After mixing for 30 s, 20 μL of hydrochloric acid (1 M) was added and then mixed for another 30 s. This plasma mixture was incubated at 80 $^{\circ}\text{C}$ for 30 min and a liquid–liquid extraction (LLE) was performed with 1 mL of ethyl acetate by mixing during 3 min.²²

2nd: The same procedure as describe in 1st, but without the incubation of plasma at 80 $^{\circ}\text{C}$ for 30 min.

3rd: To the spiked plasma was added 20 μL of internal standard afzelin (400 ng/mL) and a LLE was performed with 2 mL of ethyl acetate.

4th: To the spiked plasma was added 20 μL of internal standard afzelin (400 ng/mL) and a protein precipitation (PP) was performed with 2 mL of acetonitrile.

5th: To the spiked plasma was added 20 μL of internal standard afzelin (400 ng/mL) and a PP was performed with 2 mL of methanol.

In all procedures, the supernatant was collected after centrifugation at 4000 rpm for 10 min and evaporated until dryness in a vacuum centrifugal evaporator at 37 $^{\circ}\text{C}$. The residues were dissolved in 200 μL of acetonitrile (15%, v/v) and injected into the UFLC–MS/MS system as described in Section 2.2.

4.5. Preparation of Stock Solutions, Calibration Standards and Quality Controls. On a methanol/water (1:1, v/v) stock solution of 0.1 mg/mL quercitrin, dilutions with water were performed to obtain solutions in the concentration range of 50–1000 ng/mL . To these solutions, 10 μL of aliquots was added to 90 μL of rat plasma to obtain the final concentrations of the standard curve: 5, 10, 25, 50, and 100 ng/mL . Quality control samples were prepared at 15, 40, and 80 ng/mL , corresponding to the quality control low (QCL), medium (QCM), and high (QCH), respectively.

Likewise, internal standard stock solution of afzelin was prepared at 0.1 mg/mL in methanol/water (1:1, v/v). Successive dilutions in water resulted in a concentration of 400 ng/mL . An aliquot of 10 μL of this solution was added to all plasma samples, resulting in a final concentration of 40 ng/mL .

4.6. Sample Preparation and Analytical Method Validation. The sample preparation comprised of 100 μL of collected plasma mixed with 10 μL of internal standard afzelin (400 ng/mL) for 30 s. A LLE was undertaken with 2 mL of ethyl acetate by mixing for 30 s. This plasma was centrifuged at 4000 rpm for 10 min and 1700 μL of the supernatant was collected and evaporated until dryness in a vacuum centrifugal evaporator at 37 $^{\circ}\text{C}$ for 2 h. The residue was reconstituted in 85 μL of acetonitrile (15%, v/v).

The analytical method was validated in compliance with the bioanalytical guidelines of the US Food and Drug Administration.²⁴ The parameters evaluated included selectivity, linearity, lower limit of quantification, precision (intra- and

interday), accuracy, and stability. Moreover, matrix effect and dilution integrity were also evaluated.²⁵

The selectivity of the method was evaluated by comparing the chromatograms of the blank plasma samples with those of the corresponding spiked samples to determine if endogenous matrix compounds were interfering in quercitrin and internal standard peaks.

Calibration standards were daily prepared during the ongoing analysis. The linear curves were obtained by plotting the ratio between the peak area of quercitrin and the internal standard afzelin against the nominal concentrations (5, 10, 25, 50, and 100 ng/mL). The results were fitted to the linear regression analysis without using weighing factor.

The lower limit of quantification (LLOQ) was determined by the lowest amount of quercitrin in plasma with acceptable accuracy and precision.

The precision was evaluated by intra- and interday analyses on two consecutive days by processing six replicates of LLOQ (5 ng/mL) and QC samples (15, 40, and 80 ng/mL). Accuracy was determined by comparing the measured concentration to the nominal concentration spiked in plasma. The results were obtained as relative standard deviation (RSD%) for precision and as relative error (RE%) for accuracy. The method was precise and accurate when the values of LLOQ and QC samples were less than 20% and 15%, respectively.

The stability of quercitrin in plasma was assessed by analyzing QCL (15 ng/mL) and QCH (80 ng/mL) under different conditions of storage such as at room temperature for 4 h, after 30 days stocking in -80°C , and after three freeze–thaw cycles. The samples were considered stable for deviation from the original concentration within $\pm 15\%$.

To determine the matrix effect, the blank plasma was processed and then spiked with the internal standard afzelin and quercitrin at lower and higher QC concentrations, with six replicates for each QC. The obtained value was compared to that of the pure analyte solution (in the absence of matrix). The coefficient of variation must not be higher than 15%.

The integrity dilution was carried out by 10 times dilution of plasma samples containing 500 ng/mL of quercitrin with blank rat plasma to obtain samples containing 50 ng/mL of quercitrin. Dilution was performed in triplicate and accuracy was determined.

4.7. Protein Binding. The protein-binding ability of quercitrin in rat plasma was determined using the ultrafiltration method. Concentrations of 50, 100, 150, 250, and 500 ng/mL of quercitrin were tested in Wistar rat plasma (Na Heparin, lot 16063; Innovative Research, MI). In summary, quercitrin was spiked in plasma in a proportion of 1:9 to a final volume of 1 mL. Then, the plasma was mixed in a vortex and incubated at 37 $^{\circ}\text{C}$ for 30 min, added into the upper part of the centrifugal filter device (Centrifree, cutoff value: 30 kDa; Millipore Corp.), and centrifuged at 1000g for 20 min at room temperature. To the ultrafiltrate collected was added the internal standard and analyses were performed by UFLC–MS/MS as described in Section 2.2. The free quercitrin fraction was determined by the ratio between ultrafiltrates and pure analyte solution. It was also determined if quercitrin binds to the ultrafiltrate device by solubilizing it in water instead of plasma at the same concentrations. Duplicates were performed for each concentration.

4.8. Tissue Distribution. The tissue distribution of quercitrin was determined after a single intravenous dose of 1 mg/kg injected into lateral tail vein of Wistar rats.

Subsequently, the animals were sacrificed by decapitation at times 5, 15, 30, 60, 90, and 120 min ($n = 3$ animals/time point). After removing, the kidney was placed in an absorbent paper for removal of the surface blood. At the time of decapitation, blood samples were also collected and plasma was separated by centrifugation. Tissues and plasmas were immediately stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

For the quantification of quercitrin in kidney, tissue homogenates were prepared by weighing 1 g of tissue for each 2.5 mL of saline. The samples were then homogenized for 2 min using ultraturrax apparatus (Marconi). The homogenates were then transferred to microtubes and centrifuged at 3500g for 15 min at $4 \pm 1\text{ }^{\circ}\text{C}$. Then, 100 μL of the supernatants was treated as described for plasma samples. Calibration curves were prepared using kidneys obtained from untreated animals. Blank tissue analysis was also performed to ensure the absence of any contaminating compounds. The amounts of quercitrin in the tissue were expressed in terms of ng/g.

The pharmacokinetic parameters were determined using noncompartmental analysis and tissue penetration was determined by the ratio of AUC_{0-t} tissue, and AUC_{0-t} plasma.

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Notes

The authors declare no competing financial interest.

The data used to support the findings of this study are included within the article.

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