

## Research Article

# Determination of Ranitidine in Human Plasma by SPE and ESI-LC-MS/MS for Use in Bioequivalence Studies

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A method for determining ranitidine in human plasma by ESI-LC-MS/MS was validated, using propranolol as internal standard. The extraction method used was solid phase extraction (SPE). Chromatographic separation was performed in a Chromolith C18 (50 mm × 4.6 mm i.d.) analytical column, which provided good separation of ranitidine and propranolol peaks with an analysis time of 2.5 minutes. Extraction yields of 94.4% for ranitidine and 89.4% for the internal standard were obtained. The lower limit of quantification (LLOQ) was 3.00 ng/mL, and limit of detection (LOD) was 0.05 ng/mL, with linearity ranging from 3.00 to 500 ng/mL. The results, thus, showed that this method is suitable for application in bioequivalence studies of ranitidine in human plasma.

## 1. Introduction

The chemical structure of ranitidine hydrochloride (Figure 1) consists of a five-membered furan heterocyclic ring and a nitroethenodiaminic group. The molecular mass of ranitidine hydrochloride is 350.9 daltons (g/mol), and its molecular formula is  $C_{13}H_{22}N_4O_3SHCl$  corresponding to hydrochloride  $N[2-[[[5-[(dimethylamino)methyl]-2-furan]methyl]thio]ethyl]-N'$ -methyl-nitro-2,1,1-ethenodiamine. Ranitidine hydrochloride appears as a crystalline powder, practically odorless and white to light yellow. It is very soluble in water, somewhat soluble in alcohol, and slightly soluble in chloroform. Furthermore, it should be stored in hermetically sealed containers and protected from light [1].

Ranitidine is widely used in the treatment of gastric pathologies, has a chemical structure that partially resembles histamine, and acts primarily as a competitive histamine inhibitor through the  $H_2$  receptors [2, 3].

Since around two thousand analyses are conducted before bioequivalence studies can be concluded, the search for quick

and simple methods to determine drugs in biological matrices has been intense. Thus, several methods for determining ranitidine in human plasma have been reported. Zendelovska and Stafilov [4] developed a method for determining ranitidine and cimetidine in human plasma using HPLC with a diode array detector. In this study, the analysis time was 9 minutes, with a 50.0 ng/mL limit of detection for ranitidine. Famotidine was used as internal standard. In another study using the same detector, an analysis time of 6.5 minutes was obtained in a C18 column (300 mm × 4.6 mm id, 5  $\mu$ m), with a limit of quantification of 20.0 ng/mL [5].

Methods for analyzing ranitidine in plasma by LC-MS/MS have been reported in some studies [6–8]. The limits of quantification ranged from 1.0 ng/mL to 12.2 ng/mL. However, these methods use the protein precipitation procedure. This has the disadvantage of reducing the useful life of the column due to the presence of compounds that are not precipitates or that use liquid-liquid extraction, which requires large volumes of sample and has a higher limit of quantification.

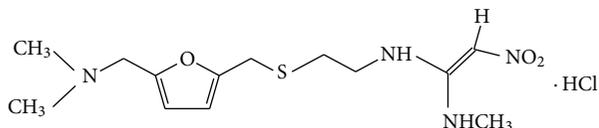


FIGURE 1: Chemical structure of ranitidine hydrochloride.

Thus, the object of this paper was to develop an analytical methodology for determining ranitidine in human plasma using the solid phase extraction (SPE) technique and high performance liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) in order to develop a fast, highly specific, and repeatable methodology for use in pharmaceutical bioequivalence studies.

## 2. Experimental

**2.1. Chemicals and Reagents.** Ultrapure water generated by the Millipore Milli-Q Gradient 10 ultrapurification system was used in all processes. HPLC-grade reagents were supplied by Merck. We used the (United States Pharmacopeia) USP reference standard for ranitidine hydrochloride (lot G) and the Brazilian Pharmacopoeia primary standard for propranolol hydrochloride (lot 1005).

**2.2. Instrumentation and Chromatographic Conditions.** Chromatographic analyses were performed on a Shimadzu AD Vp HPLC (Shimadzu, Japan) coupled to a Quattro LC (MS-MS) triple quadrupole mass spectrometer (Micromass, UK) equipped with Z-spray operated in electrospray positive (ES +) mode, with argon as the collision gas. The system was controlled by MassLynx software version 3.4 for Windows NT. The chromatographic conditions were determined using a Merck Chromolith C18 analytical column (50 mm × 4.6 mm i.d.) with a flow rate of 0.5 mL/min for a mobile phase consisting of aqueous 0.1% formic acid solution: methanol (50:50 v/v pH = 3.8) pumped by a VDP-10A LC pump (Shimadzu, Japan), using a 1:2 postcolumn split. Solvents were first placed in ultrasound for 3 minutes and then put through a DGU-14A degassing system (Shimadzu, Japan). The system operated at pressures between 100 and 120 bar. The column and autosampler were kept at a temperature of 20°C, and the injection volume was 20 µL.

The Quattro LC mass spectrometer (MS-MS) operated in positive electrospray ionization mode (ES +) and MRM dual-channel mode. The following parameters were used for the electrospray source: capillary voltage 3.00 kV, cone voltage 25.00 V for ranitidine and 30.00 V for propranolol, extractor voltage 3.00 V, RF lens voltage 0.2 V, source temperature 150°C, cone gas flow 0.0 L/h, and desolvation gas flow 439 L/h. The following conditions were established for the quadrupoles of the Quattro LC mass spectrometer: LM1 resolution of 13.0, HM1 resolution of 13.0, ion 1 energy 0.2, second hexapole input 5.0 V, collision energy at 25.00 eV for ranitidine and 17.00 eV for propranolol, 5.0 output, LM2

resolution of 9.0, HM2 resolution 9.0, ion 2 energy 2.5, multiplier at 650 V, 0.5 s dwell time, and 0.1 s delay time.

**2.3. Preparation of the Stock and Standard Solutions.** The stock solutions of ranitidine and propranolol were prepared at a concentration of 0.5 mg/mL in methanol: water (50:50 v/v). These solutions were used to prepare working solutions of ranitidine at concentrations of 30.00, 50.00, 100.00, 500.00, 1000.00, 2000.00, 4000.00, and 5000.00 ng/mL. The working solutions of propranolol were also prepared from stock solutions (0.5 mg/mL) at a concentration of 2000 ng/mL. All solutions were placed in Falcon-type tubes. The tubes with the ranitidine solutions were protected from light with aluminum foil and stored in a freezer at -20°C. The other solutions were stored in a refrigerator at +4°C and replaced daily.

**2.4. Sample Preparation.** Plasma samples were thawed at room temperature, homogenized by vortexing and centrifuged at 3400 rpm for 4 minutes to precipitate materials suspended in the plasma. Waters Oasis 1-cc HLB solid phase extraction (SPE) cartridges and 30 mg of adsorbent were used. These cartridges were previously conditioned with 1 mL of methanol and 1 mL of ultrapure water for activation of the drug binding sites.

More over, 250 µL of human plasma, 100 µL of propranolol, and 500 µL of ultrapure water were added to the SPE. The cartridge was then placed inside a clean test tube, and the cartridge-test tube combination was centrifuged at 3400 rpm for 4 minutes for plasma elution and retention of the drug in the cartridge. Subsequently, the eluate was discarded, and the analytes were eluted with 1000 µL of water: methanol solution (20:80 v/v) with 0.1% of formic acid.

**2.5. Analytical Curve.** The analytical curve was prepared at concentration levels of 3.00, 5.00, 10.00, 50.00, 100.00, 200.00, 400.00, and 500.00 ng/mL of ranitidine. Also 250 µL of human plasma, 100 µL of each of the ranitidine solutions, 100 µL of propranolol, and 400 µL of ultrapure water were added to the SPE. Then, the sample preparation procedure was carried out. The sample quality controls (QCs) were prepared at concentrations of 3.00, 5.00, 200.0, and 400.00 ng/mL by spiking of blank plasma and the subsequent extraction process.

**2.6. Extraction Recovery.** The extraction method's recovery was determined by comparing ranitidine peak areas in samples that did not undergo the extraction process considering the extraction yield to be 100% and ranitidine peak areas in extracted samples at concentrations of 3.00, 5.00, 10.00, 50.00, 100.00, 200.00, 400.00, and 500.00 ng/mL. For the internal standard, the extraction yield of replicates at a concentration of 200.00 ng/mL was determined.

**2.7. Precision and Accuracy.** The accuracy and precision of the method were determined by the extraction of eight samples containing the internal standard and the ranitidine analyte at concentrations 3.00, 5.00, 200.00, and 400.00 ng/mL,

which were analyzed in replicates. This procedure was carried out intraday and between-day. The approval criterion for relative standard deviation (RSD) was 15%.

**2.8. Stability.** Stability studies of ranitidine in plasma were performed under three conditions. First, stability was evaluated in three freezing cycles over 24 h at  $-20^{\circ}\text{C}$ , followed by thawing. Subsequently, we evaluated the stability of ranitidine which was kept at room temperature for eight hours. Long-term stability was evaluated over a forty-day storage period. The acceptance criteria were a deviation from nominal concentration that was less than or equal to 15% and precision and accuracy less than or equal to 15%.

The long-term stability of the standard drug solutions and the internal standard in the biological liquid at room temperature was evaluated after 12 h of preparation. These solutions were kept frozen at  $-20^{\circ}\text{C}$  for seven days before the analyses were carried out.

**2.9. Biological Samples.** The samples for bioequivalence tests were collected at the Cardiology Institute of Anápolis in the state of Goiás, Brazil. The study involved 24 volunteers and was conducted after approval of the study protocol by the Research Ethics Committee of the Universidade Federal de Goiás. Ranitidine was assessed for bioequivalence in 150 mg tablet form.

Blood samples were collected with a total volume of 10 mL. The blood was centrifuged and the plasma removed. Plasma samples were frozen at  $-20^{\circ}\text{C}$ . The collection times were 15 min, 30 min, 45 min, 1:00 h, 1:15 h, 1:30 h, 2:00 h, 2:30 h, 3:00 h, 4:00 h, 5:00 h, 6:00 h, 8:00 h, and 12:00 h. All samples collected were placed in test tubes containing 2 drops of heparin and then sent to the hospital analysis laboratory for plasma extraction.

### 3. Results and Discussion

**3.1. Analyses by HPLC-MS-MS.** The chromatographic method which was used allowed the ranitidine and the internal standard (propranolol) to elute with a resolution of 1.23, with a retention time of 1.66 min for ranitidine and 2.13 min for the internal standard (Figure 2). The retention time obtained was similar to that found in a study by Zhang et al. [7] and was lower than that reported by Sun et al. [8] who obtained an elution time of 4.5 minutes for ranitidine.

Ranitidine and propranolol were identified through analysis of mass spectra obtained during the first and second ionizations of these analytes, as illustrated in Figure 3. Ranitidine underwent protonation in the first mass spectrometer, generating a quasimolecular ion  $[M+H]^+$   $m/z$  of 315.1. Through collision with argon gas, this generates the fragment (daughter ion)  $m/z$  129.9 determined in the second mass spectrometer. These mass spectra coincide with those obtained by Schellen et al. [9] and Kataoka et al. [10].

The quasimolecular ion generated by the internal standard (propranolol)  $[M+H]^+$  has  $m/z$  of 260.3 which produces the fragment (daughter ion)  $m/z$  116.1 (Figure 3). These

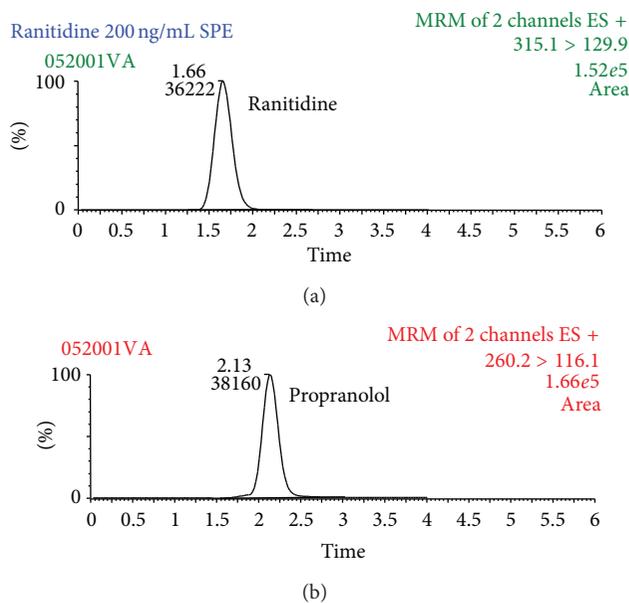


FIGURE 2: Total ion chromatogram (TIC) for ranitidine and propranolol.

spectra coincide with those obtained by Schellen et al. [9] and Xia et al. [11].

### 4. Method Validation

**4.1. Selectivity/Specificity.** Figure 4 shows chromatograms of normal blank plasma for ranitidine and the internal standard (propranolol) after the blank plasma samples were tested using the same procedure and under the same conditions as for sample analysis. As can be seen, no metabolites or interferences significant for the retention time of the drug or of the internal standard were found.

**4.2. Analytical Curve.** The mean determination coefficient of the calibration curve for ranitidine was 0.9989. Linearity varied from 3.00 to 500.00 ng/mL. Relative error values ranged from  $-6.60$  to  $8.66\%$ , which indicates an analytical curve of excellent linearity, broad linear dynamic range and adequate accuracy. This provides the quantification process with analytical reliability.

The lower limit of quantification (LLOQ) was 3.00 ng/mL, with precision of 4.61% and accuracy of 100.62%. The limit of detection (LOD) was 0.05 ng/mL, a concentration whose signal-to-noise ratio was 3.98. The limit of quantification was lower than the LLOQ obtained by Zhang et al. [7] and Sun et al. [8] and near that obtained by Wang et al. [6] who used the protein precipitation method and obtained an LLOQ of 1 ng/mL. Thus, the precision values are adequate and provide analytical reliability for LLOQ determination and validation.

**4.3. Method Recovery.** The absolute recovery of ranitidine was evaluated for eight concentrations ( $n = 6$ ). The recoveries were  $90.74 \pm 3.22\%$  for a nominal concentration of 3.00 ng/mL;  $87.49 \pm 1.34\%$  for 5.00 ng/mL;  $86.18 \pm 2.88\%$  for

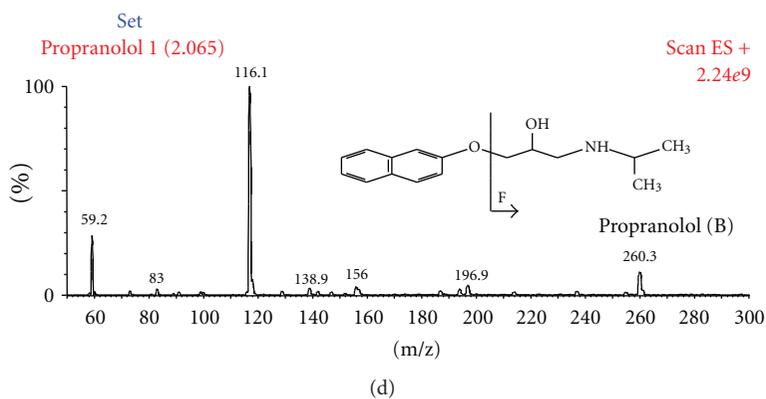
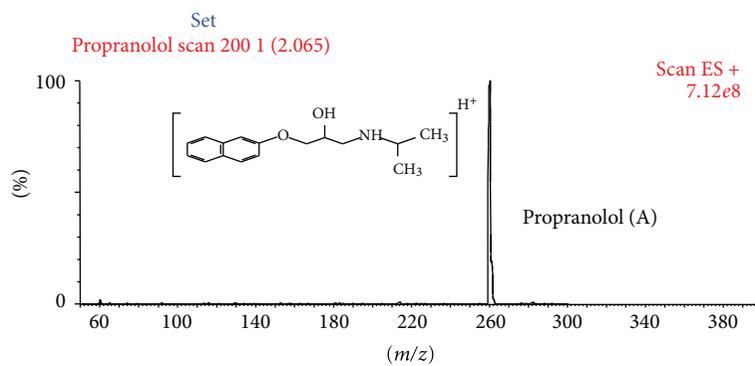
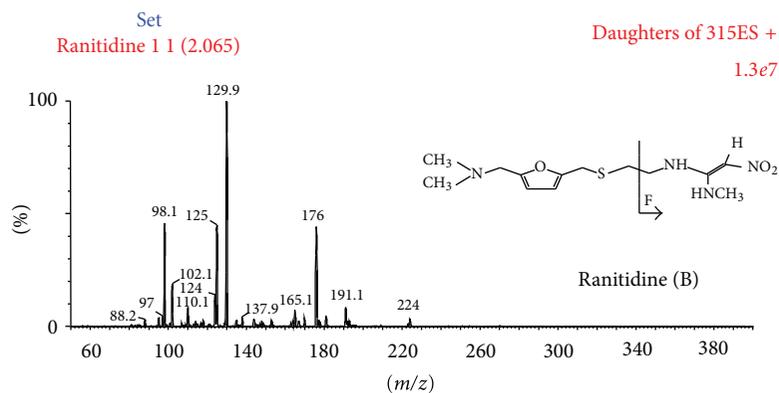
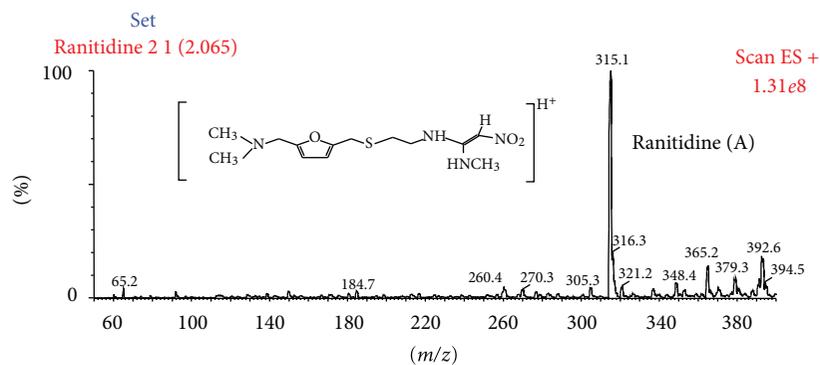


FIGURE 3: Mass spectrum of ranitidine and propranolol. (a) The first mass spectrometer. (b) The second mass spectrometer.

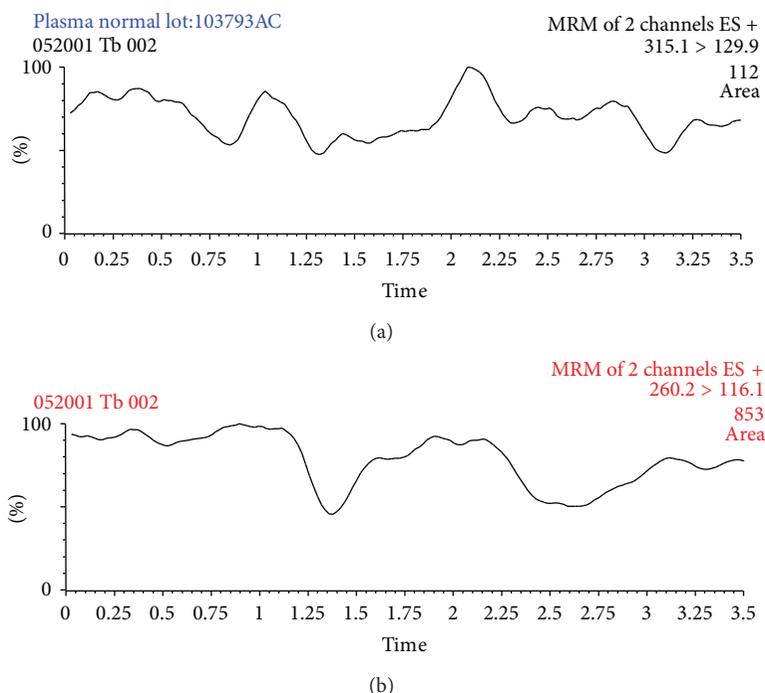


FIGURE 4: Chromatograms of normal blank plasma monitoring ranitidine and propranolol.

TABLE 1: Intraday and between-day precision and accuracy for the determination of ranitidine in plasma.

Nominal	Concentration ( $\mu\text{g/mL}$ ) $n = 8$					
	Determined	Intraday RSD (%)	Accuracy	Determined	Between-days RSD (%)	Accuracy
3.00	2.79	9.75	93.2	2.92	4.04	97.3
5.00	4.67	2.99	93.5	4.64	0.99	92.8
200.00	197.19	3.29	98.6	202.43	2.41	101.2
400.00	406.26	3.37	101.6	419.38	2.73	104.8

10.00 ng/mL;  $95.28 \pm 3.9\%$  for 50.00 ng/mL;  $93.79 \pm 5.81\%$  for 100.00 ng/mL;  $96.20 \pm 2.10\%$  for 200.00 ng/mL;  $100.23 \pm 3.38\%$  for 400.00 ng/mL;  $105.44 \pm 10.1\%$  for 500 ng/mL. The recovery of the internal standard was  $89.4 \pm 4.37\%$ , and the mean recovery of ranitidine in plasma by SPE was 94.41%, a result that demonstrates the effectiveness of the method in bioequivalence studies.

**4.4. Accuracy and Precision.** The intra-day and between-day accuracy and precision results for the determination of ranitidine in human plasma are presented in Table 1. The results indicate that the proposed method provides intraday and between-day accuracy and precision within the parameters desirable for an analytical method for the study of drug bioequivalence.

**4.5. Stability.** Table 2 presents data on the stability of ranitidine and propranolol under different conditions. The acceptance criteria were a deviation from the nominal concentration and precision less than or equal to 15%. Neither

ranitidine nor propranolol was degraded by the influence of various storage temperature fluctuations, which ensures that these samples may undergo several cycles of freezing and thawing with no loss in analyte concentration. In addition, the drugs are not degraded during the period in which the sample remains in the equipment to be analyzed. The long-term stability assessment has shown that the samples remain stable over the forty-day period.

**4.6. Pharmacokinetic Study.** The method was effectively applied in the bioequivalence study. Figure 5 displays mean plasma concentration of ranitidine versus time, comparing the test drug (T) to the reference (R), for the 24 volunteers who participated in the study. The test ranitidine showed a profile similar to that of the reference drug, with a maximum plasma concentration ( $C_{\text{max}}$ ) of 90.0 ng/mL occurring at 1.8 h.

For some volunteers, there were two plasma concentration peaks for ranitidine. Several other studies have also reported this [12–14]. Some authors attribute this finding

TABLE 2: Stability of ranitidine and propranolol under different conditions ( $n = 5$ ).

Conditions	Drug	Concentration (ng/mL)	
		Nominal	Determined mean
Freezing and thawing cycles	Ranitidine	5.06	$5.46 \pm 1.39\%$
		407.13	$429.99 \pm 1.17\%$
Room temperature for 8 h	Ranitidine	4.97	$5.05 \pm 2.63\%$
	Propranolol	214	$195 \pm 7.06\%$
Storage at $-20^{\circ}\text{C}$ for 40 days	Ranitidine	4.8	$5.28 \pm 3.23\%$
		390.79	$400.33 \pm 4.05\%$
Standard solutions for 12 h	Ranitidine	5.00	$5.15 \pm 0.87\%$
	Propranolol	185.2	$181.2 \pm 7.06$

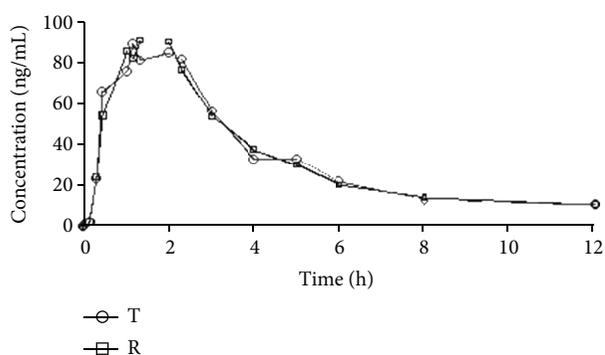


FIGURE 5: Mean plasma concentration of ranitidine versus time for test (T) and reference (R) products.

to the mechanism of enterohepatic recirculation [15], while others attribute it to the existence of multiple drug binding sites along the gastrointestinal tract [16].

## 5. Conclusions

The analytical method for determining ranitidine in human plasma using SPE and LC-MS/MS offers high recovery, excellent linearity, accuracy, and precision, and a lower limit of quantification that is adequate for checking the remaining plasma concentration of ranitidine. In addition, the procedure offers fast execution and chromatographic analysis, with excellent resolution.

This methodology is appropriate for assessing the stability of ranitidine under various conditions. Ranitidine was stable in all the processes used to assess the stability of these drugs in biological matrices. Analysis of these drugs is therefore possible after long periods of adequate storage at  $-20^{\circ}\text{C}$ .

Thus, the method is fully applicable to bioequivalence and bioavailability studies of ranitidine.

## Conflict of Interests

The authors declare that they do not have a direct financial relation with Merck and Millipore (Milli-Q).

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