Talanta 86 (2011) 233–240

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Blood matrix effects for male and female Wistar rats, in simultaneous HPLC-UV determination of riparin I and III from *Aniba riparia* (Nees) Mez. (Lauraceae)

Sócrates Golzio dos Santos, Karine Formiga Queiroga, Adriana Maria Fernandes de Oliveira, Josean Fechine Tavares, Stanley Juan Chavez Gutierrez, Margareth de Fátima Formiga Melo Diniz, José Maria Barbosa-Filho, Marcelo Sobral da Silva *

Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, 58051-900 João Pessoa, PB, Brazil

ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form 4 September 2011
Accepted 5 September 2011
Available online 10 September 2011

Keywords:
HPLC-UV
Matrix effect
Blood
Aniba riparia
Riparins

ABSTRACT

Aniba riparia (Nees) Mez. (Lauraceae) is popularly known as "louro", and is found in Amazonia and in the Guianas, its distribution extends to the Andes. Alkamide alkaloids were isolated from its green fruit; they were denominated riparin I (methyl ether of *N*-benzoyl tyramine), riparin II (methyl ether of *N*-2-hydroxy-benzoyl tyramine) and riparin III (methyl ether of *N*-2,6-dihydroxy-benzoyl tyramine) in tribute to the plant. When administered orally and intraperitoneally to mice, riparin I and III are anxiolytic, yet without any sedative or muscle relaxing effects. The present study shows that variables such as extraction solvent, centrifugation force, and centrifugation time, are important in the simultaneous liquid–liquid extraction of riparin I and III from male and female Wistar rat blood in HPLC-UV studies. The study confirms matrix influence on simultaneous recovery and detection of riparin I and III. The effect of rat blood matrix for riparin I was −13.86%, while for riparin III it was −10.94%. The recovery for riparin I was 82.14%, while for riparin III it was 87.42%. The efficiency of the process was 73.25% for riparin I and 77.81% for riparin III, demonstrating an optimal method for simultaneous recovery of riparins I and III from the blood of rats. The matrix effect for rat blood showed values of 10.25% for riparin I and −83.01% for riparin III. Recovery for riparin I was 113.11%, whereas for riparin III it was 13.65%. The process efficiency of this method for female rat blood was 125.88% for riparin I and 2.58% for riparin III. Simultaneous recovery of riparin I and III from the blood of male and female rats using acetonitrile as the precipitating solvent, while centrifuged at 10,000 × *g* for 10 min demonstrated the importance of the parameters chosen for the extraction/recovery process of different analytes.

1. Introduction

The family Lauraceae encompasses 52 genera and 3,000 species dispersed around the world. They are predominantly found in tropical and subtropical regions, especially in Central and South American forests, and usually in temperate zones as well [1]. In Brazil, we find the family represented by 22 genera which are generally arboreal [2].

Brazilian Neotropical Lauraceae are economically, and ecologically important [3], as many of species of the family are used in popular medicine for cutaneous lesions, gastric disturbances [4,5], as an anti-inflammatory, and for circulatory problems in both western and eastern cultures [4], they also have hypoglycemic [5] and anxiolytic properties [6].

Aniba riparia (Nees) Mez. (Lauraceae) is popularly known as "louro" and is found in Amazonia and the Guianas, and as far west as the Andes. Alkamide alkaloids were isolated from the green fruit of this plant, and were called riparin I (methyl ether of *N*-benzoyl tyramine) (Fig. 1A) and riparin III (methyl ether of *N*-2,6-dihydroxy-benzoyl tyramine) (Fig. 1B) [7]. Riparins I and III, when administered orally or intraperitoneally in mice showed anxiolytic effects, yet without any sedative or muscle relaxing effects, thus eliminating the common side effects associated with classic benzodiazepines [8,9].

Biological matrix effects are undesireable for analyte studies and can result in either suppression or increases in ionic signal, sensitivity variance over time in analyte detections, baseline increases, data imprecision, retention time alterations, peak shape distortions and/or chromatographic tails [10–12]. Signal diminuition in the matrix recovery is one of the effects of endogenous compound binding, and analyte metabolism in biological fluids [13,14].

Clean-up procedures for biological fluids, such as plasma, are used to separate endogenous material from the analyte. The sensitivity and selectivity of an assay are affected by the efficiency of the clean-up procedures [15–17].

* Corresponding author. Tel.: +55 83 3216.7427.
E-mail address: marcelosobral@ltf.ufpb.br (M.S. da Silva).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.09.008

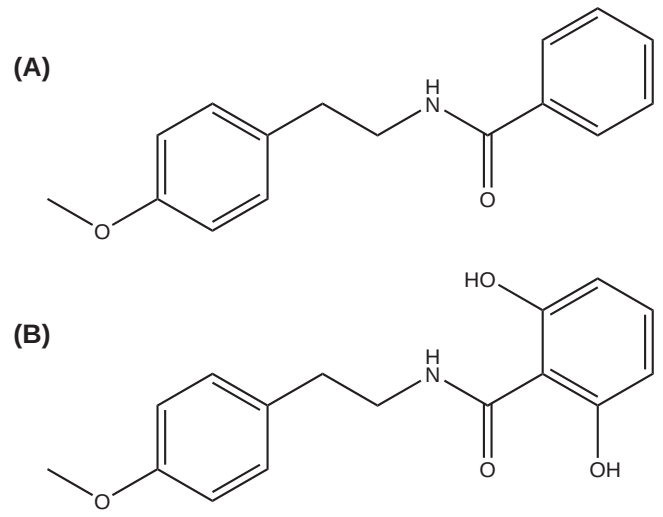


Fig. 1. (A) Structure of riparin I and (B) structure of riparin III.

No bioanalytical methods for riparin I and III determinations have been previously reported in the literature, and there are no published pharmacokinetic studies for these substances since these types of studies require delineation of blood matrix effects [18].

The present study demonstrates that centrifugation force and time, and the extraction solvent used for simultaneous liquid–liquid extraction of riparin I and III from male and female Wistar rat blood are important variables in determinating the extent of matrix effect when using high-performance liquid chromatography with UV detection (HPLC-UV).

2. Experimental

2.1. Chemical products, reagents and animais

Riparin I and III were obtained by organic synthesis and donated by PhD Stanley Juan Chavez Gutierrez, and PhD José Maria Barbosa Filho of the Paraiban Federal University of Brazil, in accordance with the methodology described in the literature [19].

For all experiments, ultra-pure water (type I) from an Option-Q Purelab labwater-system (Elga, São Paulo, Brazil), both HPLC-grade acetonitrile (MeCN) and HPLC-grade metanhol (MeOH) from TEDIA (São Paulo, Brazil), and EDTA supplied by HEMSTAB Laboratory (São Paulo, Brazil) were used.

The blood used was collected from male and female Wistar rats (8–10 weeks, weighing 250–300 g), in tubes with EDTA. The animals were from the Prof. Dr. Thomas George Animal House and kept under standard laboratory conditions: controlled temperature ($21 \pm 1\,^{\circ}C$), light/dark cycle (12 h), with pelleted feed and water *ad libitum*. The whole study was approved by the Committee on Ethics in Research Animals (CEPA) under protocol No. 0107/08, of the Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, João Pessoa/Paraíba, Brazil. The protocols conformed to the guidelines of the National Institute of Health (NIH Publication, Health Research Extension Act of 1985, Public Law 99-158, November 20, 1985 "Animals in Research") for the care and utilization of laboratory animals.

2.2. Instrumentation and chromatographic conditions

Chromatography was performed utilizing the HPLC-UV Shimadzu Serie 10A *vp* system, consisting of a Shimadzu LC-10 AD *vp* pump with FCV-10AL *vp* solenoid valve, SIL-10AD *vp* auto-injector, Shimadzu CTO-10AS *vp* oven, DGU-14h degasser, and a SPD-10AV *vp* detector. The separation was carried out with an ACE C18 (250 mm × 4.6 mm, 5 μm) column equipped with an ACE C18 (4.6 mm) guard column. The mobile phase used was (20:80, v/v, H_2O:MeCN), at a flow rate of 1 mL min^{-1}. The wavelength of the UV detector used for analysis was 225 nm, column temperature was controlled at 27 °C, and the auto-injector temperature controlled at 4 °C. The sample injection volume was 20 μl. Under these conditions, riparin I and riparin III have respective retention times of 7.89 ± 0.2 min and 10.03 ± 0.2 min.

The samples of rat blood were gently vortexed (AP-56-TECNAL/PHOENIX) and centrifuged utilizing a Sigma Laborventrisugen 2K15 centrifuge.

2.3. Preparation of standard solutions

The stock solutions of riparin I and III were prepared separately in H_2O:MeCN (50:50, v/v,) at a concentration of 1 mg mL^{-1} and stored at 4 °C.

2.4. Preparation of samples in blood, samples in solution and samples for quality control

Riparin I (1 mg mL^{-1}) and III (1 mg mL^{-1}) were added to samples of male rat blood (300 μl) to obtain a final concentration of 100 μg mL^{-1}, and the samples were then gently vortexed for 30 s. The blank rat blood samples were gently vortexed for 30 s. These procedures were repeated for the female rat blood samples.

Samples of a mixture solution of riparin I and III were prepared from stock solutions (1 mg mL^{-1}) in H_2O:MeCN (50:50, v/v) to obtain final concentrations of 100 μg mL^{-1} in 300 μl of solution, and were then gently vortexed for 30 s. The same solution samples of riparin I and III in H_2O:MeCN (50:50, v/v) were prepared for quality control (CQ).

2.5. Preparation of the calibration curve

Samples of riparin I and III in H_2O:MeCN (50:50, v/v) were prepared at concentrations of 20, 50, 80, 130 and 160 μg mL^{-1} to obtain a calibration curve of peak area (y) plotted against theoretical concentration in μg mL^{-1} (x) with a correlation coefficient of >0.99, which served to correlate, by linear regression, the peak areas obtained for the evaluation method at the concentration of the test substances.

2.6. Method of evaluation

Male rat blood samples were spiked with riparin I and III, and were prepared in triplicate. A volume of 600 μl of MeOH or MeCN was added, and the tubes were gently vortexed for 1 min and centrifuged at 10,000 × g, 12,000 × g and 14,000 × g, where each centrifugation was for 10, 15 and 20 min (Table 1). The same procedure was performed for female rat blood spiked with riparin I and III, and solution samples of riparin I and III (H_2O:MeCN, 50:50, v/v) (Table 1).

After centrifugation of the blood samples and solutions, 600 μl each of supernatant was transferred to another tube and then vacuum-dried at 40 °C. The dried samples were reconstituted with 300 μl solvent (50:50, v/v, H_2O:MeCN). The solution samples of riparin I and III, and the quality control (CQ), were not submitted to the extraction process, these groups were used for statistical comparison with the extracted solution groups of riparin I and III.

From the samples (blood, solutions of riparin I and III, and quality controls), a 20 μl aliquote was injected in duplicate into a HPLC-UV system and monitored at 225 nm. The areas of the peaks were recorded and replicates used to calculate the mean and standard deviation. The results were correlated by linear regression

Table 1
Samples of blood and solutions analyzed, varying the solvent type, centrifugal force and time of centrifugation.

Sample	Solvent	Centrifugal force (× g)	Centrifugtion time (min)
Blood female rats with riparin I and III	MeOH	10,000	10
Blood female rats with riparin I and III	MeOH	10,000	15
Blood female rats with riparin I and III	MeOH	10,000	20
Blood female rats with riparin I and III	MeOH	12,000	10
Blood female rats with riparin I and III	MeOH	12,000	15
Blood female rats with riparin I and III	MeOH	12,000	20
Blood female rats with riparin I and III	MeOH	14,000	10
Blood female rats with riparin I and III	MeOH	14,000	15
Blood female rats with riparin I and III	MeOH	14,000	20
Blood female rats with riparin I and III	MeCN	10,000	10
Blood female rats with riparin I and III	MeCN	10,000	15
Blood female rats with riparin I and III	MeCN	10,000	20
Blood female rats with riparin I and III	MeCN	12,000	10
Blood female rats with riparin I and III	MeCN	12,000	15
Blood female rats with riparin I and III	MeCN	12,000	20
Blood female rats with riparin I and III	MeCN	14,000	10
Blood female rats with riparin I and III	MeCN	14,000	15
Blood female rats with riparin I and III	MeCN	14,000	20
Blood male rats with riparin I and III	MeOH	10,000	10
Blood male rats with riparin I and III	MeOH	10,000	15
Blood male rats with riparin I and III	MeOH	10,000	20
Blood male rats with riparin I and III	MeOH	12,000	10
Blood male rats with riparin I and III	MeOH	12,000	15
Blood male rats with riparin I and III	MeOH	12,000	20
Blood male rats with riparin I and III	MeOH	14,000	10
Blood male rats with riparin I and III	MeOH	14,000	15
Blood male rats with riparin I and III	MeOH	14,000	20
Blood male rats with riparin I and III	MeCN	10,000	10
Blood male rats with riparin I and III	MeCN	10,000	15
Blood male rats with riparin I and III	MeCN	10,000	20
Blood male rats with riparin I and III	MeCN	12,000	10
Blood male rats with riparin I and III	MeCN	12,000	15
Blood male rats with riparin I and III	MeCN	12,000	20
Blood male rats with riparin I and III	MeCN	14,000	10
Blood male rats with riparin I and III	MeCN	14,000	15
Blood male rats with riparin I and III	MeCN	14,000	20
Solution of riparin I and III	MeOH	10,000	10
Solution of riparin I and III	MeOH	10,000	15
Solution of riparin I and III	MeOH	10,000	20
Solution of riparin I and III	MeOH	12,000	10
Solution of riparin I and III	MeOH	12,000	15
Solution of riparin I and III	MeOH	12,000	20
Solution of riparin I and III	MeOH	14,000	10
Solution of riparin I and III	MeOH	14,000	15
Solution of riparin I and III	MeOH	14,000	20
Solution of riparin I and III	MeCN	10,000	10
Solution of riparin I and III	MeCN	10,000	15
Solution of riparin I and III	MeCN	10,000	20
Solution of riparin I and III	MeCN	12,000	10
Solution of riparin I and III	MeCN	12,000	15
Solution of riparin I and III	MeCN	12,000	20
Solution of riparin I and III	MeCN	14,000	10
Solution of riparin I and III	MeCN	14,000	15
Solution of riparin I and III	MeCN	14,000	20

using calibration curves. A typical calibration curve was as follows: $y = 104579x - 127081$ ($r^2 = 0.9972$).

2.6.1. Evaluation of the matrix effect (ME%)

To evaluate the matrix effect (ME%), the peak areas for the blood samples containing the standards that underwent extraction (B) were compared with the areas of solution samples that also underwent extraction (A). Positive values (ME% > 0) demonstrate matrix interference, resulting in detection signal increases, and negative values (ME% < 0) result in decreasing detection signal, while a zero value (ME% = 0) indicates the absence of matrix effect [20,21]:

$$\mathrm{ME\%} = \left(\left(\frac{B}{A}\right) \times 100\right) - 100$$

2.6.2. Recovery in relation to extraction process (RE%)

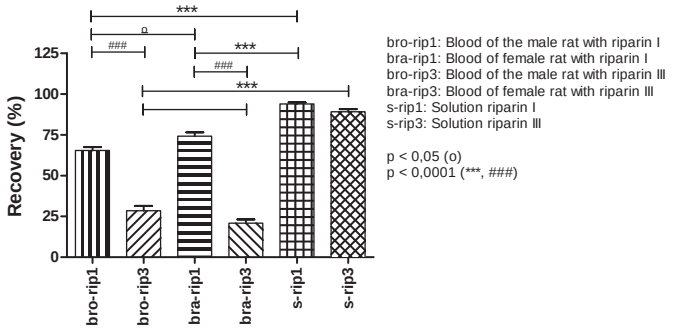
Recovery in relation to the extraction process in solution (RE%) can be calculated comparing the peak area of the sample that underwent extraction (B) to the peak area of the control (C) [20,22]:

$$\mathrm{RE\%} = \left(\frac{B}{C}\right) \times 100$$

2.6.3. Efficiency of the process (EP)

The evaluation of extraction process efficiency was determined by the adapted equation [20,22]:

$$\mathrm{EP} = \frac{((\mathrm{ME} + 100) \times \mathrm{RE})}{100}$$



Graph 1. Evaluation of riparin I and III recoveries from blood, in relation to sex ($n = 108$).

2.6.4. Statistical analysis

The results of this study were expressed as the mean ± standard error for the mean (SEM) of the percentage of recovery. The difference between the means were considered significant when $p < 0.05$. Statistical analyses were performed utilizing ANOVA one-way, with Bonferroni's post-test [23].

3. Results and discussion

3.1. The matrix effect on recovery

In bioanalyses, the biological components of the sample can affect detector/analyte response. These phenomena, generically called matrix effects degrade quantification, and these effects should be considered when developing and validating the bioanalytical method [24].

3.1.1. Effect of sexual blood type

The recovery percentages of riparin I and III were grouped according to the type of blood utilized (with respect to sex), and the results are shown in Graph 1. After statistical analysis, it was seen that the bro-rip1 group (male rat blood spiked with riparin I that underwent the extraction process) had a mean recovery of 65.44%, significantly different than group sr-rip1 (riparin I solution that also underwent extraction) which had a mean recovery of 93.96%. The study with riparin III in male rat blood showed that the groups bro-rip3 and sr-rip3 differed significantly being 28.50% and 89.25%, respectively. The bra-rip1 group (female rat blood spiked with riparin I that underwent extraction) showed a mean recovery of 74.26%, significantly less than 93.96% for the sr-rip1 group (riparin I solution that also underwent extraction). For riparin III, the bra-rip3 group showed a significant difference from solution (sr-rip3), with values of 20.88% and 89.25%, respectively. On comparing the recovery rates of the solutions that underwent extraction (sr-rip1 and sr-rip1) in relation to the control group (not extracted), there was no significant difference. The recovery of riparin I from female rat blood (bra-rip1) was 74.26%, significantly different from 65.44% in male rat blood (bro-rip1), and for riparin III there was no difference in the recovery between male and female rat blood. These results demonstrated recovery differences for riparin I and III with respect to the sex of the animal. The differences can be explained by a diversity of interfering substances, and their quantity between the sexes [25,26]. Fischette et al. [27] also demonstrated that sex and hormonal conditions are important variables for experimental determinations, and perhaps clinical responses to drugs as well.

3.1.2. Effect of the solvent

The data obtained for recovery percentages of riparin I and III were grouped according to the type of solvent used and separated by animal sex, for type of blood. To precipite the large macromolecules and blood cells we used (methanol or acetonitrile). The results are shown in Graph 2 (A, male rats; B, female rats).

The results for male rat blood (Graph 2A); the group boh-r1 had a mean recovery of 53.89%, which was markedly different from that for the group soh-r1 (81.28%). For riparin III, the group boh-r3 had a mean recovery of 3.78%, which was much lower than that for soh-r3 (87.32%). For the group bcn-r1, the mean recovery value was 77.04%, not significantly different from the 84.46%, observed for group scn-r1. Riparin III recovery utilizing acetonitrile (bcn-r3) was 54.58%, significantly different than that of scn-r3 (92.47%).

The groups boh-r1 and bcn-r1 (riparin I) were significantly different than boh-r3 and bcn-r3 (riparin III). Methanol as precipitating solvent showed a lower mean recovery when compared to the use of acetonitrile. The significant recovery difference between riparin I and riparin III in male rat blood using acetonitrile was 22.46%. They differ structurally by two hydroxyls in the *N*-benzoylamide ring of riparin III (Fig. 1B).

The boh-r1 group had a mean recovery of 51.89% for female rat blood (Graph 2B), a value significantly different from the soh-r1 group which had a value of 92.75%. For the bcn-r1 group, mean recovery was of 98.00%, which was not significantly different from that of 94.37% for the scn-r1 group. For riparin III (boh-r3), mean recovery was 2.10% with the use of methanol, which was significantly lower than soh-r3 at 79.77%, while the use of acetonitrile showed a mean recovery of 39.55% for bcn-r3, significantly lower than the solution scn-r3, at 86.10%. Comparing the groups (boh-r1 and bcn-r1) of female rat blood, they showed a difference which was similar to that observed between boh-r3 and bcn-r3, with both being significant.

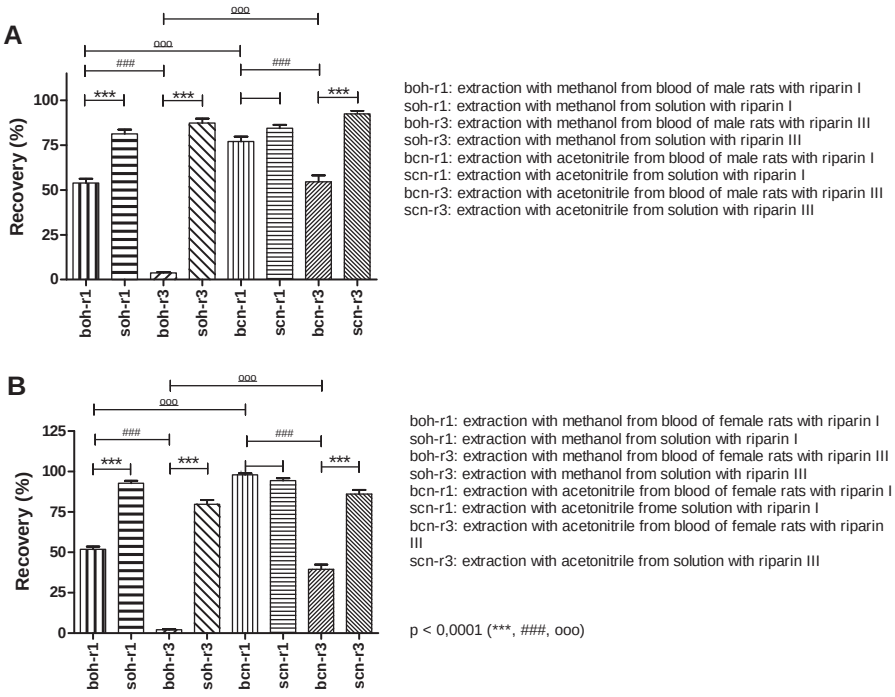
Statistical comparison of solvent effect between the blood of female rats and male rats showed significant differences only when using acetonitrile. A review of the literature between 1980 and 2010, indicates that for each class of molecule, different types of organic solvents are utilized for extractions from plasma or blood. Hoellinger et al. [28] used acetonitrile as the extraction solvent for alkaloids in blood because of the greater rate of recovery. The same has been shown for alkamides riparin I and III.

3.1.3. Effect of centrifugation

Published studies have shown that the influence of endogenous compounds, and different centrifugal forces make it possible to obtain a greater precipitation of proteins [29].

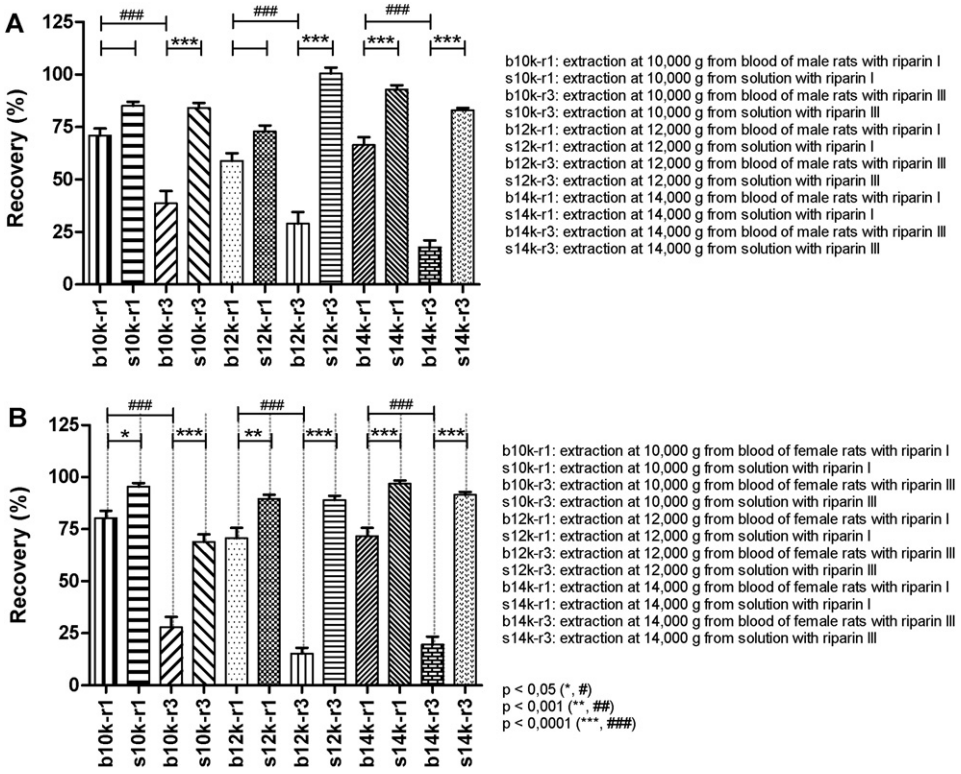
The data obtained for the recovery percentages of riparin I and III were grouped in accordance with centrifugal force, and separated by sexual blood type. The results are presented in Graph 3 (A, male rats; B, females rats).

The b10k-r1 (male rat blood) group showed a mean recovery of 70.99%, which was not statistically different from the 85.14% of the s10k-r1 group (Graph 3A). Extracted riparin III with a recovery from blood of 38.72% was significantly lower than that from solution (84.16%). With centrifugation at 12,000 × *g*, the b12k-r1 group (riparin I in blood) showed a recovery of 58.93%, which was not significantly different from that of solution s12k-r1 (73.00%). Extraction of riparin III from blood (b12k-r3) was 29.00%, significantly less than that from the solution s12k-r3 (100.50%). For the highest centrifugation force of 14,000 × *g*, recovery of riparin I from the blood (b14k-r1) was 66.39%, significantly less than the 92.84%, obtained from solution (s14k-r1), and for riparin III the corresponding values obtained were 17.76% in the b14k-r3 group and 83.08% for the s14k-r3 group, markedly different. Graph 3A shows that there was no significant difference in recovery of riparin I from blood using different centrifugation forces, this was unlike the recovery of riparin III from blood where there was a decrease of recovery for an increase in centrifugal force, especially between 10,000 × *g* and 14,000 × *g*.

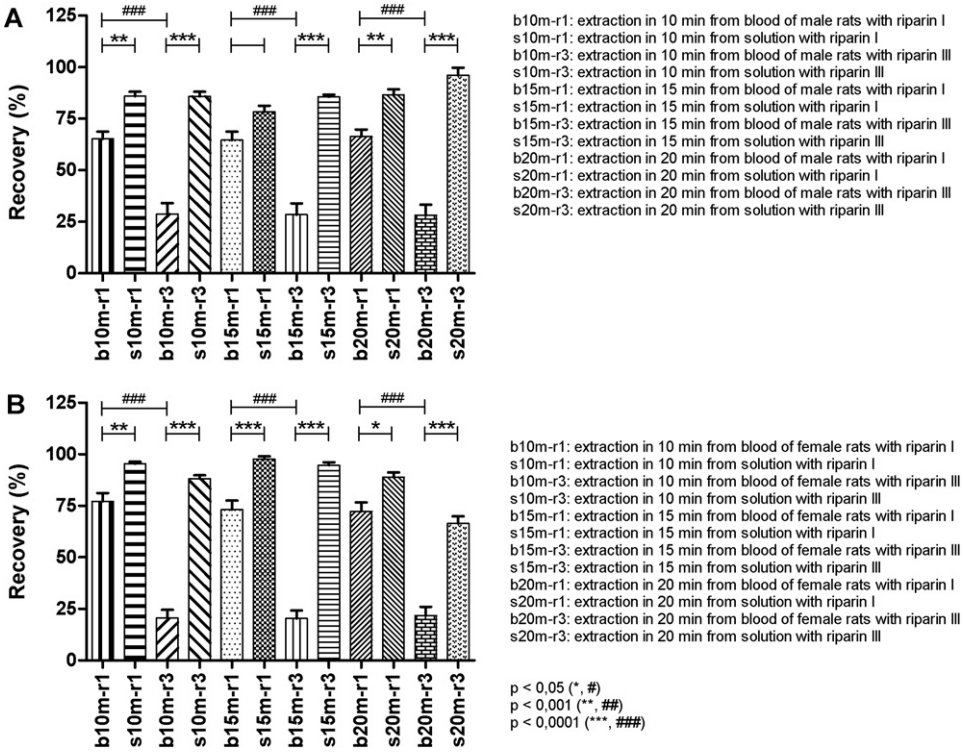


Graph 2. Evaluation of riparin I and III recoveries from blood, in relation to solvent ($n = 52$; A, males; B, females).

For the female rat blood study (Graph 3B), the b10k-r1 group showed a recovery of 80.37%, significantly less than that for s10k-r1 at (95.47%), riparin III in the b10k-r3 group had a 27.88% recovery, which was significantly less than that for group s10k-43 (68.93%). With greater centrifugal force ($12{,}000 \times g$), the b12k-41 group showed a recovery of 70.67%, which is significantly less than that for s12k-r1 (89.60%). Riparin III showed a 15.24% mean recovery rate from blood of the b12k-r3 group, which was significantly less than that from the solution (s12k-r3; 89.04%). With the highest centrifugal force used, i.e., $14{,}000 \times g$, riparin I recovery from blood in the b14k-r1 group was 71.72%, significantly less than that from solution (s14k-r1; 96.82%). Riparin III in group b14k-r3, showed a 19.50% recovery, which is significantly less than that of s14k-r3 (91.48%). There were no significant differences between riparin I



Graph 3. Evaluation of riparin I and III recoveries from blood, in relation to centrifugal force ($n = 36$; A, males; B, females).



Graph 4. Evaluation of riparin I and III recoveries from blood, in relation to centrifugation time ($n = 36$; A, males; B, females).

groups; b10k-r1, b12k-r1 and b14k-r1, nor between the riparin III groups; b10k-r3, b12k-r3 and b14k-r3, however, these latter groups did show lower recovery rates than the riparin I groups.

The statistical analysis focused on the spin/force variable showed no significant difference between the blood of male and female rats in the recovery of analytes. For both, the blood of male and female rats, a low recovery value of riparin III was obtained. Centrifugation speed influences the precipitation of proteins, and the substituents in riparin III increase the affinity to albumin decreasing recovery [30].

3.1.4. Effect of the time

In general, centrifugation time does not affect the formation of micelles, but accelerates the separation of phases, just as in conventional separations of a precipitate from its originally aqueous medium [25].

The data obtained in relation to the mean recovery of riparin I and III were grouped according to; time of centrifugation utilized for precipitation of proteins in the blood, and separation by the type of blood with regard to sex of the animal. The results are shown in Graph 4 (A, male rats; B, female rats).

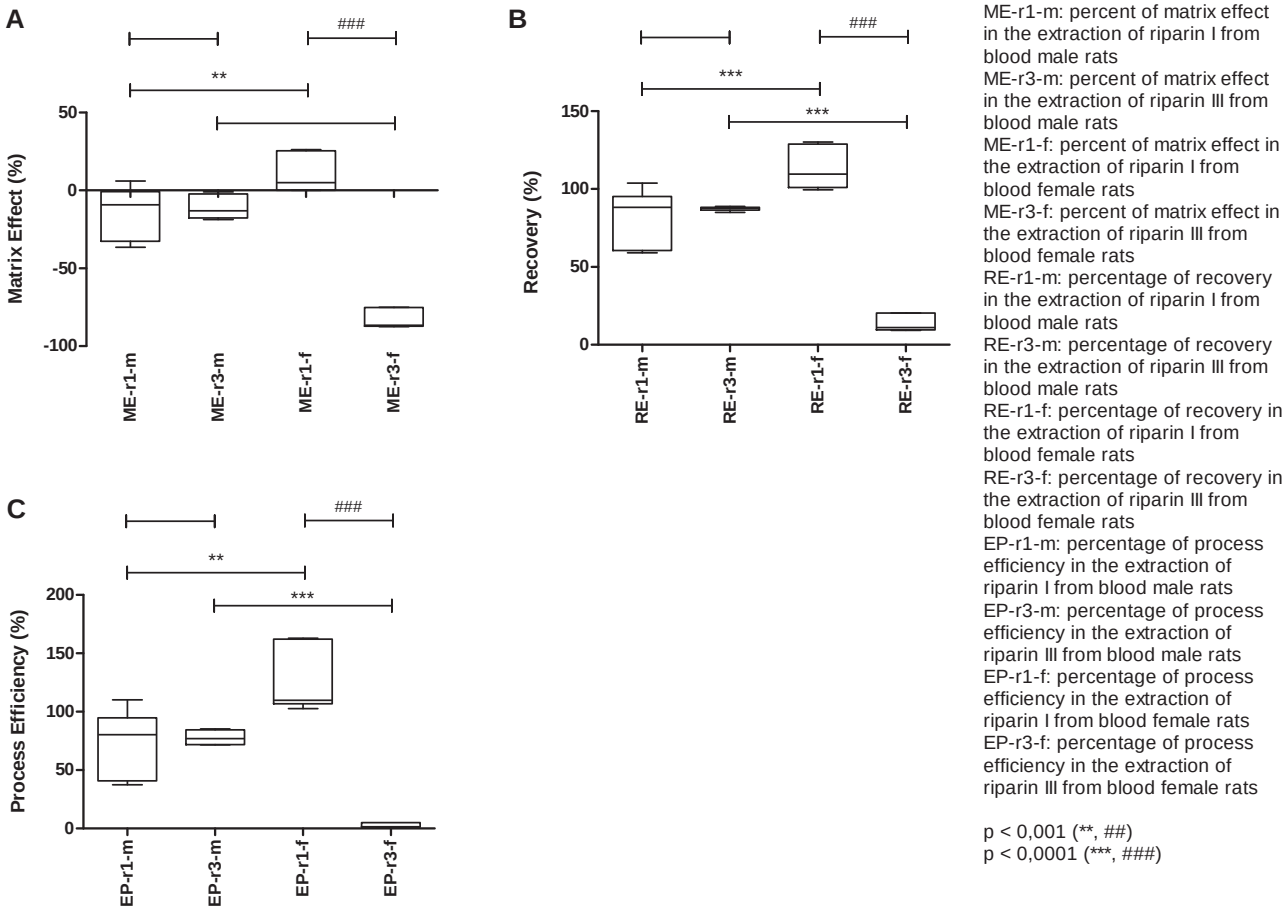
In the study using male rat blood (Graph 4A), the b10m-r1 group (blood spiked with riparin I centrifuged for 10 min and put through extraction) had a mean recovery of 65.22%. This was significantly different from the 85.97% of the s10m-r1 group (solution of riparin I centrifuged for 10 min and put through extraction). For riparin III, the b10m-r3 group showed a recovery of 28.79%, which was significantly less than that for the drug solution (s10m-r3 group; 85.93%). With a centrifugation time of 15 min, the b15m-r1 group had a recovery of 64.66%, which was not significantly different than s20m-r1. For riparin III in blood (b15m-r3), the mean recovery was 28.53%, significantly less than that in solution (s15m-r3; 85.73%). With the longest time of 20 min the recovery of b20m-r1 was 66.43%, significantly less than that for s20m-r1 at (86.65%), for b20m-r3, the mean recovery was 28.16%, significantly less than that for s20m-r3 (96.08%). Comparison using ANOVA showed no significant difference between different centrifugation times for recovery of riparin I from blood for (b10m-r1, b15m-r1 and b20m-r1), and riparin III (b10m-r3, b15m-r3 and b20m-r3). These results corroborate findings demonstrating that spontaneous equilibrium in the precipitation of proteins occurs only after long centrifugation times [31].

Using female rat blood (Graph 4B), the b10m-r1 group was found to have a mean recovery of 77.18%, significantly less than that for the s10m-r1 group (95.42%). The recovery from blood was also less than that of solution where riparin III showed a value of 20.54% for the b10m-r3 group, which is significantly less than the 88.31% found for the s10m-r3 group. For 15 min of centrifugation, the mean recovery of riparin I from blood in b15m-r1 was 73.20%, significantly less than that in s15m-r1 (97.61%). For riparin III, b15m-r3 showed a recovery rate of 20.38%, significantly less than that in s15m-r3 (94.71%). The recovery values for the longest centrifugation time of 20 min, were 72.39% for the b20m-r1 group which was significantly less than that for s20m-r1 (88.87%), and 21.70% for the b20m-r3 group, which was significantly less than with s20m-r3.

According to a one-way ANOVA with Bonferroni's post test, the group comparisons in relation to centrifugation time, and drug recovery from male and female rat blood, for riparin I as well as riparin III, did not show significant differences. Also we observed for the blood of both male and female rats a lower recovery value for riparin III.

3.1.5. Matrix effect

The exact mechanisms of matrix effects are unknown [26], yet the reproducibility of assay results in bioanalysis is influenced by many components of the biological matrix [24]. It was observed in the preceding experiments that the recovery of riparin III, limited the study, of the simultaneous recovery of the two analytes. Matrix effect can be seen as an undesireable effect of a biological medium. Graph 5 demonstrates the measurement of this effect in blood using acetonitrile, and centrifugation at 10,000 × g for 10 min. The matrix effect (ME%) in male rat blood (Graph 5A) for riparin I



Graph 5. Comparative statistical study of riparin I and III recoveries in blood, in relation to solvent ($n = 52$; A, males; B, females).

was −13.86%, while for riparin III it was −10.94%; there is interference by the matrix in the simultaneous recovery of riparin I and III, diminishing the signal, and as a consequence making the recovery values lower. The recovery value (RE%) in Graph 5B for riparin I was 82.14%, while for riparin III it was 87.42%, where both recoveries showed values over 80%, and a process efficiency (EP%), (Graph 5C), of 73.25% for riparin I and 77.81% for riparin III. This demonstrates a good method for simultaneous recovery of riparins I and III from male rat blood. The matrix effect (ME%) for female rat blood (Graph 5A) showed values of 10.25% for riparin I and −83.01% for riparin III; the biological matrix caused an increase in the signal for riparin I and markedly diminished the signal for riparin III resulting in simultaneous recovery disparity. The recovery value (RE%), Graph 5B for riparin I was 113.11%, while for riparin III it was 13.65%. The process efficiency (EP%) for this method in female rat blood (Graph 5C) was 125.88% for riparin I, and (EP%) 2.58% for riparin III. Components of the blood caused signal suppression for riparin I and III, yet increased the signal for riparin I in female rat blood.

4. Conclusion

In the development of a bioanalytical method, it is important to remove matrix effects [20]. Process efficiency is determined by the combination of these effects on the recovery and detection of the extracted analyte. Our studies confirm the critical influence of matrix effects on the simultaneous recovery and detection of riparin I and III. If the interferents in the matrix could be minimized, the method would provide greater recovery/detection values [32]. Zhao and Ren [33] demonstated that native BSA contains hydrophobic groups in the interior of the tertiary structure, and polar groups on the surface which allow binding of flavonoid OH groups to the polar groups on the surface proteins. They also demonstated that the binding between flavonoids and albumin is influenced by flavonoid hydroxylation. Xiao et al. [34] also studied the influence of flavonoid hydroxylation on the interaction with BSA and found the same result. This would suggest that due to matrix effect, the recovery of riparin III from rats bloods would be less than that of riparin I. Our method gave simultaneous recovery of riparin I and III from blood of male and female rats with the use of acetonitrile as the precipitating solvent, and centrifugation at 10,000 × g for 10 min.

Acknowledgments

This work is partly supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico, and PRONEX/FAPESQ-PB Brazil.

References

[1] F.R. Garcez, W.S. Garcez, L. Hamerski, C.H. Miguita, Quim. Nova 32 (2009) 407–411.
[2] J.G. Rohwer, K. Kubitzki, J.G. Rohwer, V. Bittrich, The Families and Genera of Vascular Plants, Springer-Verlag, Berlin, 1993.
[3] P.L.R. Moraes, Biota Neotrop. 5 (2005) 253–270.
[4] H. Yu, S. Lee, C. Jang, Pharmcol. Biochem. Behav. 87 (2007) 164–170.
[5] J.M. Barbosa-Filho, T.H.C. Vasconcelos, A.A. Alencar, L.M. Batista, R.A.G. Oliveira, D.N. Guedes, H.S. Falcão, M.D. Moura, M.F.F.M. Diniz, J. Modesto-Filho, Rev. Bras. Farmacogn. 15 (2005) 392–413.
[6] F.C.F. Sousa, C.T.V. Melo, M.C.O. Citó, F.H.C. Félix, S.M.M. Vasconcelos, M.M.F. Fonteles, J.M. Barbosa Filho, G.S.B. Viana, Rev. Bras. Farmacogn. 18 (2008) 642–654.

[7] G. Thomas, U.J.C. Castelo Branco, J.M. Barbosa Filho, M. Bachelet, B.B. Vargaftig, J. Pharm. Pharmacol. 46 (1994) 103–107.
[8] C.T.V. Melo, A.P. Monteiro, C.P. Leite, F.L.O. Araujo, V.T.M. Lima, J.M. Barbosa-Filho, M.M.F. Fonteles, S.M.M. Vasconcelos, G.S.B. Viana, F.C.F. Sousa, Biol. Pharm. Bull. 29 (2006) 451–454.
[9] F.C.F. Sousa, C.T.V. Melo, A.P. Monteiro, V.T.M. Lima, S.J.C. Gutierrez, B.A. Pereira, J.M. Barbosa-Filho, S.M.M. Vasconcelos, M.F. Fonteles, G.S.B. Viana, Pharmacol. Biochem. Behav. 78 (2004) 27–33.
[10] B.N. Patel, N. Sharma, M. Sanyal, P.S. Shrivastav, J. Pharm. Biomed. Anal. 49 (2009) 354–366.
[11] C.D. Bernardes, R.J. Poppi, M.M. Sena, Talanta 82 (2010) 640–645.
[12] G.-W. Cheng, H.-L. Wu, Y.-L. Huang, Talanta 79 (2009) 1071–1075.
[13] S. Ahuja, H. Rasmussen, HPLC Method Development of Pharmaceuticals, 1st ed., Academic Press, New York, USA, 2007.
[14] H.-F. Hsieh, W.-S. Chang, Y.-K. Hsieh, C.-F. Wang, Talanta 79 (2009) 183–188.
[15] E. Katz, R. Eksteen, P. Schoenmakers, N. Miller, Handbook of HPLC Illustrated Edition, CRC Press, Gaithersburg, MD, USA, 1998.
[16] W. Lv, Talanta 80 (2010) 1406–1412.
[17] Y.-K. Liu, X.-Y. Jia, X. Liu, Z.-Q. Zhang, Talanta 82 (2010) 1212–1217.
[18] J. Chamberlain, The Analysis of Drugs in Biological Fluids, 2nd ed., CRC Press, New York, USA, 1995.
[19] J.M. Barbosa-Filho, E.C. Silva, J. Bhattacharyya, Quim. Nova 13 (1990) 332–334.
[20] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019–3030.
[21] J.C. Van De Steene, W.E. Lambert, J. Am. Soc. Mass Spectrom. 19 (2008) 713–718.
[22] K.M. Matar, J. Pharm. Biomed. Anal. 48 (2008) 822–828.
[23] I. Šperlingová, L. Dabrowská, V. Stránský, Š. Dušková, J. Kučera, M. Tvrdíková, M. Tichy, Anal. Bioanal. Chem. 397 (2010) 433–438.
[24] M.L. Chiu, W. Lawi, S.T. Snyder, P.K. Wong, J.C. Liao, V. Gau, J. Assoc. Lab. Automat. 15 (2010) 233–242.
[25] F. Han, R. Yin, X.-l. Shi, Q.A. Jia, H.-Z. Liu, H.-M. Yao, L. Xu, S.-M. Li, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 868 (2008) 64–69.
[26] D.L. Buhrman, P.I. Price, P.J. Rudewicz, J. Am. Soc. Mass Spectrom. 7 (1996) 1099–1105.
[27] C.T. Fischette, A. Biegon, B.S. McEwen, Life Sci. 35 (1984) 1197–1206.
[28] H. Hoellinger, M. Re, A. Deroussent, R.P. Singh, T. Cresteil, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 799 (2004) 195–200.
[29] A. Weber, Biochim. Biophys. Acta: Gen. Subj. 19 (1956) 345–351.
[30] W. Kramer, Biochim. Biophys. Acta: Mol. Cell Biol. Lipids 1257 (1995) 230–238.
[31] A. Hüttermann, U. Guntermann, Anal. Biochem. 64 (1975) 360–366.
[32] P.J. Taylor, Clin. Biochem. 38 (2005) 328–334.
[33] J. Zhao, F. Ren, Spectrochim. Acta A 72 (2009) 682–685.
[34] J. Xiao, M. Suzuki, X. Jiang, X. Chen, K. Yamamoto, F. Ren, M. Xu, J. Agric. Food Chem. 56 (2008) 2350–2356.