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Pharmacokinetic study of a new angiotensin-AT_1 antagonist by HPLC

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ABSTRACT

Recently an innovative novel class angiotensin-AT_1 antagonist has been developed by Rottapharm. In this study, we present a validated method for detecting CR 3834 in biological matrices using high-performance liquid chromatography (HPLC) with diode array detection. After oral administration (30 mg/kg) to Wistar rats, the plasma and urine concentrations of CR 3834 and its potential metabolic products were determined. Moreover, the plasmatic time course in rats has been determined after intravenous (IV) administration of CR 3834 (5 mg/kg). Biological samples (0.5 ml of plasma and 1 ml of urine) were purified using solid-phase extraction (SPE) of analytes and the internal standard Idebenone, 2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1-4-benzoquinone. A chromatographic separation was performed on an Adsorboshere C18 at 25 °C, with a pre-column of the same matrix; the eluent was made up of acetonitrile/acidified water with CF_3COOH (pH 2.01) in ratio of 75:25 (v/v); the flow rate was 1.0 ml/min and a 100 μl loop. The lower limit of detection (LOD) was taken as 25 ng/ml in plasma and 50 ng/ml in urine samples. The lower limit of quantification (LOQ) was taken as 0.1 and 0.2 μg/ml in plasma and urine samples, respectively. The procedures were validated according to international standards with a good reproducibility and linear response ($r = 0.9916$ in plasma; $r = 0.9997$ in urine). The coefficients of variation inter assay ranged between 2.579 and 4.951% in plasma, and between 0.813 and 2.460% in urine. Mean recovery for CR 3834 was 79% in plasma and 97% in urine samples. The experiments performed demonstrated that the method presented was suitable for determining this new angiotensin-AT_1 antagonist in rat plasma and urine.

1. Introduction

Angiotensin II (AII) is the biologically active component of the renin–angiotensin system (RAS) and is responsible for most of the peripheral effects of this system [1,2]. In fact, AII is a major regulator of blood pressure, aldosterone secretion, and fluid homeostasis, and is also an important etiological factor in hypertension and other cardiovascular disorders [3]. There are three commonly described classes of effective inhibitors of RAS: renin inhibitors, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor antagonists (ARA) [4,5]. ARA have been developed in sequence to ACE inhibitors as a further therapeutic action on the renin–angiotensin–aldosterone system.

Specifically, AII receptor antagonists have no effect on biologically active peptides of kallikrein–kinin system and it is expected that these agents create fewer clinical problems. Thus, the specific block of the AII actions, at the receptor level, represents a potentially advantageous approach in modulating the RAS. In humans, two main types of AII receptor subtypes have been characterized and called AT_1 and AT_2 [6]. The AT_1 receptor subtype mediated virtually all the known physiological actions of AII in cardiovascular, neuronal, endocrine, hepatic and other cells [7,8].

In order to develop new non-peptidic angiotensin AT_1 antagonist, we synthesized CR 3210 ($C_{27}H_{24}N_8$) 4-[4-[(2-ethyl-5,7-dimethylimidazo[4,5-*b*]pyridin-3-yl)methyl]phenyl]-3-(2*H*-tetrazol-5-yl)quinoline [9]. This structure is closely related to losartan (2-butyl-4-chloro-5-hydroxymethyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl]-4-yl]methyl-1H-imidazole), the first potent and orally active non-peptide AII antagonist that has been successfully identified by reversed-phase liquid chromatography [10–13].

Our previous pharmacokinetic studies reported the time profile of CR3210 determined after intravenous (IV), intraperitoneal and oral administration to Sprague–Dawley rats [14,15].

In order to develop a new non-peptidic angiotensin AT_1 antagonist, we recently synthesized CR 3834 ($C_{27}H_{31}N_5O$; 5-butyl-3-isobutyl-1-[2′-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-1H-pyrrole-2-carbaldehyde; Fig. 1) which proved to be a new angiotensin AT1 antagonist endowed with a peculiar pharmacological profile. CR 3834 showed, in fact, an *in vitro* affinity for AT1

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receptors slightly lower than that of the losartan, valsartan or eprosartan standards, coupled with a surprisingly higher efficacy in the hypertension models in rats (spontaneous or renal hypertension) [16]. This superior efficacy was tentatively explained by the superior absorption parameters obtained in *in vitro* pharmacokinetic studies performed in Caco-2 cells (i.e. A → B permeability about 100-fold higher than that of valsartan) [16]. The aim of this study was, therefore, to define the *in vivo* pharmacokinetic properties for CR 3834.

A highly sensitive and selective analytical method was needed, in order to define the pharmacokinetic profile of this new AT_1 antagonist, which allowed for the determination of CR 3834 in plasma samples. A reversed-phase high-performance liquid chromatography (HPLC) was chosen to quantify CR 3834 in the plasma and urine of Wistar rats. Moreover, a procedure based on the use of solid-phase extraction (SPE) was chosen to provide an efficient sample clean-up, that represents a crucial step especially for urine samples. The method, simple and rapid, has provided accurate and precise results that are illustrated and discussed.

2. Experimental

2.1. Chemicals and reagents

CR 3834 (MW = 441.56) was provided by Rottapharm; Ibedenone (MW = 338.44) (Takeda, Osaka, Japan) was selected as an internal standard (IS) in plasma and urine (Fig. 1). Purity analysis suggested that their purities were all above 99% by HPLC method. Chromatographic grade aceteonitrile and methanol were purchased from Carlo Erba (Milan, Italy) and deionized water was used to prepare buffer solutions. All other reagents were of analytical-reagent grade (Carlo Erba, Milan, Italy).

Oasis SPE cartridges HLB (1 ml/30 mg for plasma samples and 3 ml/60 mg for urine samples) from Waters (Milford, Massachusetts, U.S.A.), provided with a LiChrolut extraction unit (Merck), were chosen to isolate CR 3834 and IS from biological samples. Sample Concentrator Techne (Dry-block-3D, Bullington, NY, U.S.A.) was used to dry the sample under a nitrogen stream.

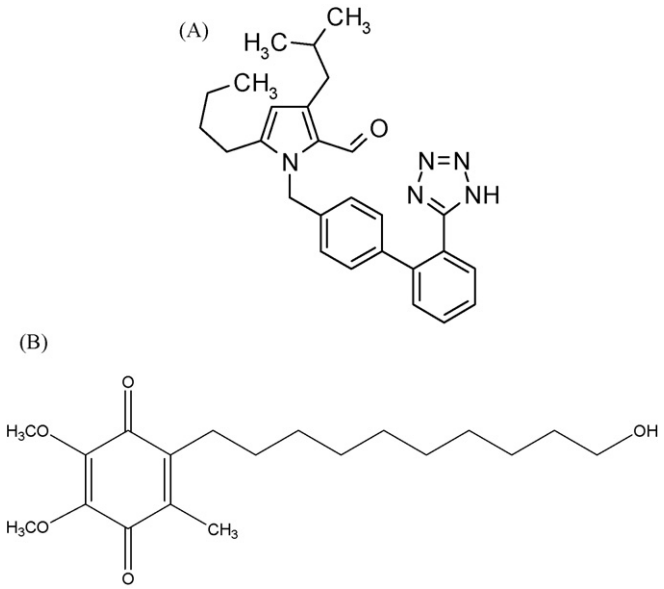

Fig. 1. Structures of CR 3834 (A) and the internal standard Idebenone (B).

2.2. Chromatography

A Jasco PU 1580 Intelligent HPLC pump (Tokyo, Japan) with a 100 μl loop injection valve was used. The chromatographic system was linked to a Jasco MD 1510 diode array detector (Tokyo, Japan), set at maximum absorption. The separation was performed on an Absorbosphere C18 (25 cm × 0.46 cm, 5 μm) reversed-phase column (Alltech, Deerfield, IL, U.S.A.), with a guard of the same matrix (7.5 mm × 4.6 mm). A block heater Gastorr GF 103 (Jones Chromatography, Colorado, U.S.A.) was used to maintain the analytical column at 25 °C. The mobile phase, formed by 75:25 of acetonitrile/H_2O acidified with CF_3COOH (pH 2.01), was delivered with a flow rate of 1.0 ml/min through the columns.

Data was processed using Borwin chromatography software (Version 1.5) from Jasco (Tokyo, Japan).

2.3. Preparation of standard solutions

Pure solutions of CR 3834 and Idebenone compounds were prepared with methanol at concentrations of 1 mg/ml.

CR 3834 stock solution at a concentration of 100 μg/ml was prepared with the same solvent. Working solutions (for preparing plasma standards at known concentrations) were produced by diluting the stock solution in methanol at concentrations of 0.1-0.38-0.5 and 1 μg/ml.

Working solutions containing 0.2-1-2-5 and 20 μg/ml, were produced to prepare urine standards at known concentrations.

The internal standard working solution was prepared at a concentration of 100 μg/ml. All solutions were stored at 4 °C and were identified as being stable for at least 1 month.

2.4. Treatment of animals and collection of samples

Wistar rats, with a body weight ranging between 175 and 180 g, were housed, three per cage, in stable conditions of humidity (60 ± 5%) and temperature (22 ± 2 °C). The rats had free access to water but were deprived of food 12 h before the experiment. The animals were maintained on 12 h light, 12 h dark cycles (lights on 7:00 a.m.–7:00 p.m., off 7.00:p.m.–7:00 a.m.).

CR 3834 was dissolved in a solution containing 50% distilled water and 50% sterile saline. Blood samples were taken from the animals by intraventricular catheter. Blood cells were removed by centrifugation and separated plasma was stored at −20 °C until assay.

The animals (n = 3 each time analyzed) were treated intravenously (5 mg/kg body mass) and the blood was collected, by indwelling catheter, after 0.083-0.25-0.5-1-2-3-4-6-8-12 and 24 h of drug administration.

Wistar rats (n = 3 each time analyzed) were treated orally (30 mg/kg body mass) and the blood was collected by indwelling catheter after 0.25-0.5-1-2-3-4-6-8-12-24 and 32 h of drug administration.

Urine samples were collected in metabolic cages and stored at 4 °C, before the assay. To be precise, eight different aliquots of urine were collected after drug administration, in the following sequence: after each hour from 0 to 2 h; every 2 h from 2 to 8 h; after 4 h from 8 to 12 h, and finally after 12 h from 12 to 36 h.

2.5. Extraction

The solid-phase extraction procedure was applied to alkalinized plasma and urine samples. Samples stored at −20 °C were defrosted using a water bath at 30 °C and centrifuged at 4000 × g for 10 min.

Each 500 μl aliquot of rat plasma and 1000 μl of urine were mixed, respectively, with 500 and 1000 μl of 0.01 M phosphate buffer (pH 11).

Plasma and the collected aliquots of urine, taken from the rats administered with the drug for research purposes, were added 100 μl of methanol containing the internal standard: 5 μg used in 0.5 ml of plasma and 15 μg in 1 ml of urine have been used to calculate the concentration of CR 3834.

Plasma and urine samples derived from untreated rats, were added to 100 μl of methanol containing the appropriate calibration standard concentrations of CR 3834 and the same quantity of IS used for the standard samples.

To isolate CR 3834 and IS the from samples, cartridges of 1 ml/30 mg for plasma and 3 ml/60 mg for urine were chosen. After cartridge washing with 2 ml of water containing 5% methanol, the analyte and the IS were eluted twice with 2 ml of methanol. A pressure was maintained at 11 inHg for 3 min for all the dry steps The eluate was dried under nitrogen stream at 40 °C and the residue was dissolved in 300 μl of aceteonitrile, centrifuged with a Mini Spin (Eppendorf, Milan, Italy), and the supernatant was filtered with Anotop 10 LC (0.2 μl/10 mm, Whatman, Maidstone, England). A third part of this extract underwent chromatographic separation.

2.6. Calibration curve and method validation

CR 3834 and Idebenone compounds were injected into the analytical column and identified by their relative retention time.

Calibration curves were obtained by plotting the peak area ratio of the CR 3834 to the IS, versus the theoretical concentration of the analyte added to drug-free rat plasma and urine. The curves were constructed from replicate measurements: in plasma of four concentrations of CR 3834 over a range of 0.1–1 μg/ml; in urine of five concentrations of CR 3834 over a range 0.2–20 μg/ml. The data was subjected to the least squares regression analysis (weighted as appropriate), to provide information on correlation coefficients and the back-calculated standard concentrations.

Plasma and urine samples containing a known amount of CR 3834 and IS were prepared and stored frozen at −20 °C until use. These samples were used as quality control specimens, to compare the measured to the theoretical concentrations of CR 3834.

The coefficients of variation occurring within (intra-) and between (inter-) batch analysis were evaluated to determine the precision of the bio-analytical method. The accuracy was expressed in terms of relative measurement error.

The intra-batch precision and accuracy were calculated for plasma and urine by three replicate analysis at each chosen concentration, and repeated twice (batches 1 and 2). The inter-batch precision and accuracy were determined by considering the coefficients of variation and the mean relative errors of the combined measurements over a period of 6 weeks ($n = 6$ at each concentration).

Samples spiked with internal standard alone, as well as those spiked with low and high concentration of CR 3834 and internal standard, were examined.

Moreover, the presence of interfering peaks were assessed in chromatograms obtained from plasma and urine analysis to verify the specificity of the analytical method.

2.7. Extraction efficiency and stability

The mean recovery of CR 3834 and IS from spiked rat plasma and urine was evaluated to test the efficiency and reproducibility of the extraction procedure. The determination of the extraction efficiency in biological samples was made by adding different amounts of the studied compound: 0.5–1 and 5 μg/ml in plasma and 0.1–2 and 10 μg/ml in urine ($n = 3$). The extraction was conducted as described above and 100 μl of internal standard working solution was added prior to the extraction. The responses of these standards, taken by means of the extraction procedures, have been compared with those of standard solution at the same concentration injected directly into the liquid chromatographic apparatus. The peak-area ratios were compared to the ratio of the standard aqueous samples without extraction.

The stability of CR 3834 was assessed in rat heparinised plasma and urine by analysing 500 μl stability samples at concentrations of 1 μg/ml. Samples were analyzed immediately after preparation and after storage at room temperature (*ca.* +22 °C) for 2 h, frozen (*ca.* −20 °C) after 14-28-70 and 90 days of storage.

Three repeated determinations were made in each case and at each time for each biological matrix. The concentration of CR 3834 measured in the stability samples were compared to the theoretical spiked concentration.

3. Results and discussion

The HPLC method proposed provides a simple procedure for the determination of a innovative angiotensin-AT_1 antagonist in biological samples.

CR 3834 is a molecule characterized by a pyrrole moiety substituted with a carboxaldehyde in 2-position. A benzoquinone moiety bearing a hydroxyldecyl chain is present in the structure of Idebenon. Therefore, a solid-phase extraction, based on a polymeric matrix with a high hydrophilic–lypophilic balance was chosen. It gives high and reproducible recovery for different compounds, even if the cartridge runs dry.

In preliminary studies, the removal of interfering plasma and urine components was attempted by SPE, washing the column with eluent at different pH values. The largest number of recoveries and the cleanest extraction procedure were achieved by washing the column with water containing 5% methanol, with a dry step of 3 min.

Chromatographic condition, based on the isocratic separation, gave a good profile in both plasma and urine samples, using a reverse phase column. Preliminary studies with different mobile phase combinations of polar and non-polar solvents have been performed: using acetonitrile and water; a strong acid pH needed to obtain a high sensitivity.

The method was validated with regard to sensitivity, linearity, limits of detection and quantification, precision, accuracy, extraction efficiency and stability.

3.1. Detection, sensitivity and linearity

Compounds CR 3834 and IS were identified by their relative retention time (RT), i.e. 7.7 ± 0.3 and 5.4 ± 0.3 min, respectively.

A series of different diluted plasma and urine standard samples were used to determine the limit of detection (signal/noise ratio >3:1) and quantification (signal/noise ratio of 10:1). The LOD of CR 3834 was taken as 25 ng/ml in plasma and 50 ng/ml in urine samples, whereas LOQ was taken as 0.1 and 0.2 μg/ml in plasma and urine samples, respectively.

A series of different diluted plasma and urine standard samples were examined.

A linear response was observed in the examined concentration of CR 3834 added to plasma (0.1-0.38-0.5 and 1 μg/ml) and urine (0.2-1-2-5 and 20 μg/ml). The mean regression coefficient was 0.99166 ($y = 2.3935x - 0.0404$; S.E. = 0.07) in plasma and 0.9997($y = 0.3428x + 0.0638$; S.E. = 0.0354) in urine.

Table 1
Precision and accuracy measurements of CR 3834 in plasma ($n = 6$)

Concentration spiked (μg/ml)	Mean concentration found (μg/ml)	S.D.	CV (%)	RE (%)
Batch 1				
0.5	0.361	0.010	2.791	−27.9
1	0.685	0.014	2.007	−31.5
2	1.408	0.070	4.995	−29.6
3	2.412	0.1752	7.265	−19.6
Batch 2				
0.5	0.335	0.010	2.871	−33.1
1	0.711	0.023	3.210	−28.9
2	1.482	0.068	4.582	−25.9
3	2.279	0.057	2.497	−24.0
Inter-batch measurements ($n = 6$)				
0.5	0.348	0.015	4.310	−30.4
1	0.698	0.018	2.579	−30.2
2	1.445	0.069	4.775	−27.8
3	2.345	0.1161	4.951	−21.8

S.D.: standard deviation, CV: coefficient of variation, RE: relative error.

The lowest concentrations of the linear range of calibration curves were tested with five replicate tests as the limit of quantification for both biological samples.

3.2. Precision, accuracy, recovery and stability

The CR 3834 precision and accuracy measurements of intra-batch and inter-batch assay in plasma and in urine are reported in Tables 1 and 2, respectively.

In intra-batch assay, the precision was determined on two different occasions. It was indicated by the coefficient of variation (CV) of the measured concentrations of three replicate control samples, for the four chosen concentrations (0.5-1-2 and 3 μg/ml). It ranged between 2.497 and 7.265% in plasma and 0.233 and 2.997% in urine. The inter-batch precision values were determined combining the measurements of batch 1 and 2, performed in plasma, and batch 3 and 4, performed in urine. On both occasions ($n = 6$ for each concentration), the CV ranged between 2.579 and 4.951% in plasma, and 0.813 and 2.460% in urine. The accuracy of measurements was expressed in terms of mean relative error. In plasma it was −27.5% whereas in urine it was −2.2%.

The extraction efficiency, expressed as mean recovery of CR 3834 from rat plasma and urine, was determined at low, medium and high concentration: 0.5–1 and 5 μg/ml in plasma and 0.1–2 and 10 μg/ml in urine (Table 3). The recovery in plasma was 72%(±2.5), 77% (±1.5) and 89.4% (±0.9), respectively. The recovery in urine was 113% (±1.7), 77.4 (±0.8) and 101.2 (±0.5). The use of SPE column could effectively eliminate the interfering material: it was found to be satisfactory only in plasma samples, but excellent in urine ones. There were no interfering peaks present in chromatograms corresponding to the retention times of CR 3834 or IS, which affected the precision and accuracy of measurements at the lower LOQ, in the extracts taken from three separate batches of blank rat plasma and urine.

Table 2
Precision and accuracy measurements of CR 3834 in urine ($n = 6$)

Concentration spiked (μg/ml)	Mean concentration found (μg/ml)	S.D.	CV (%)	RE (%)
Batch 3				
0.5	0.502	0.011	2.147	0.5
1	0.964	0.022	2.258	−3.6
2	1.966	0.005	0.233	−1.7
3	2.911	0.030	1.016	−3.0
Batch 4				
0.5	0.492	0.014	2.773	−1.5
1	0.955	0.016	1.623	−4.5
2	1.968	0.027	1.375	−1.6
3	2.878	0.086	2.997	−4.1
Inter-batch measurements ($n = 6$)				
0.5	0.497	0.012	2.460	0.5
1	0.959	0.019	1.981	−4.1
2	1.967	0.016	0.813	−1.7
3	2.894	0.058	2.004	−3.5

S.D.: standard deviation, CV: coefficient of variation, RE: relative error.

Table 3
Recovery measurements of CR 3834

Concentration spiked (μg/ml)	Mean concentration found (μg/ml)	CV (%)	Recovery (%)
In plasma ($n = 3$)			
0.5	0.363	5.510	73
1	0.770	2.597	77
5	4.473	0.846	89
In urine ($n = 3$)			
0.1	0.113	1.770	113
2	1.538	2.163	77
10	10.097	1.040	101

CV: coefficient of variation.

The results of stability tests for CR 3834 in biological samples are reported in Table 4. The concentration of 1 mg/ml of CR 3834 was added to drug-free plasma and urine. The studied compound was shown to be stable in rat plasma at ambient temperature (*ca.* 22 °C) for up to 2 h, stored frozen at *ca.* −22 °C for 0, 2 h, 14, 28 and 70 days. The percentage of spiked concentrations found in plasma samples ranged from 93 to 105%whereas in urine ranged from 93.5 to 105.5%.

3.3. Application to a pharmacokinetic study

The method described here was applied to the pharmacokinetic study of CR 3834 after intravenous (5 mg/kg) and oral (OS; 30 mg/kg) administration to Wistar rats.

The time profile of urine concentration for animals treated orally with CR 3834 was determined over the time, and related to the aliquots of urine collected in the metabolic cage. Moreover, the total concentration of the investigated drug, detected over 36 h, was reported.

Chromatograms obtained from drug-free rat plasma and urine are shown in Fig. 2(A and B); no interfering peaks are present at the retention times of CR 3834 and IS, i.e. 7.4 ± 0.3 and 5.2 ± 0.3 min, respectively.

Chromatograms from rat plasma sample spiked with IS, obtained 240 min after IV and OS administration of CR 3834, are shown in Fig. 3(A and B).

The peak referred to the studied compound and the IS are the only two peaks present. This feature suggested that the studied compound is not subjected to extensive bio-transformation *in vivo*.

Chromatogram from rat urine sample spiked with IS, obtained 240 min after OS administration of CR 3834 is shown in Fig. 3C.

Also in this chromatogram, there is the presence of a peak due to the studied compound and another corresponding to the IS. This feature suggested that CR 3834 was excreted, unchanged, in urine.

The time profiles of plasma concentrations of Wistar rats ($n = 3$ for each time), treated intravenously and orally with CR 3834, are shown in Fig. 4. The curves represent the variation of the concentrations during the 32 h after drug administration.

Table 4
Stability measurements of CR 3834 in rat plasma and urine during storage

Time of storage	Concentration spiked (μg/ml)	Mean concentration founded (μg/ml)	S.D.[a]	CV (%)[b]	Calculated value (%)
In plasma (n = 3).					
Day 0	1	0.985	0.008	−1.5	98.5
2 h	1	1.050	0.017	5.0	105.0
Day 14	1	0.985	0.012	−1.5	98.5
Day 28	1	1.020	0.009	12.0	102.0
Day 70	1	0.930	0.013	−7.0	93.0
Day 90	1	0.937	0.016	−7.0	93.7
In urine (n = 3)					
Day 0	1	0.975	0.013	−8.0	97.5
2 h	1	1.020	0.015	8.0	102.0
Day 14	1	0.93.5	0.011	−10.5	93.5
Day 28	1	0.940	0.012	−6.0	94.0
Day 70	1	0.955	0.008	−4.5	95.5
Day 90	1	1.055	0.018	8.5	105.5

[a] S.D.: standard deviation.
[b] CV: coefficient of variation.

The IV administration of CR 3834 (5 mg/kg) determined a peak plasma concentration of 2.866 μg/ml (C_{max}) that was achieved after 0.083 h (T_{max}); the plasma levels decreased significantly after 3 h and the tested compound totally disappeared 24 h after administration.

The OS administration of CR 3834 (30 mg/kg) determined a peak plasma concentration of 2.386 μg/ml (C_{max}) that was achieved after 4 h (T_{max}); the plasma levels decreased after 6 h and the tested compound totally disappeared 32 h after administration.

Table 5 reported the urinary excretion of CR 3834 in Wistar rats.

The substance was excreted unmodified and started to be detected in urine samples 1 h after the oral administration. Nevertheless the concentration of the tested substance remained very low from 1 to 2 h after oral administration, showing an increased concentration from 4 to 8 h after the drug intake.

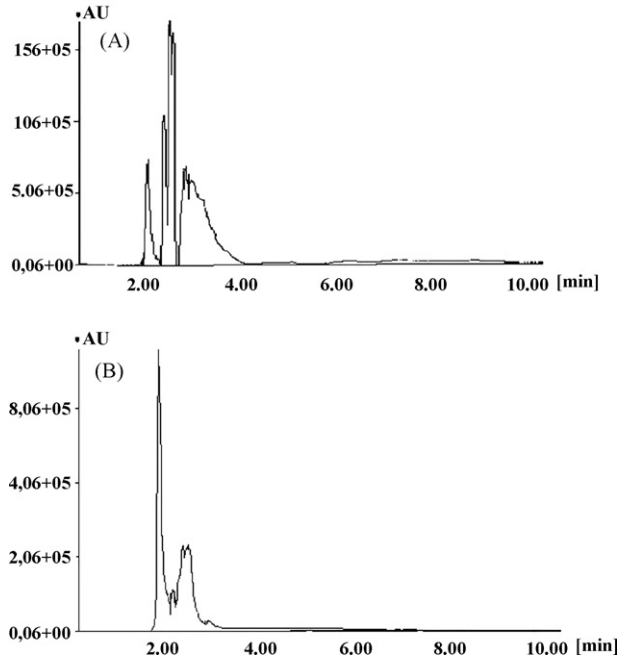

Fig. 2. Representative chromatograms showing: (A) extract of drug-free rat plasma; (B) extract of drug-free rat urine.

The oral administration of CR 3834 gave a peak urine concentration of 5.373 μg, determined in the urine aliquot of 3 ml collected from 8 to 12 h after the drug intake.

The excretion drastically decreased after 12 h and CR 3834 disappeared from urine 24 h after the oral administration.

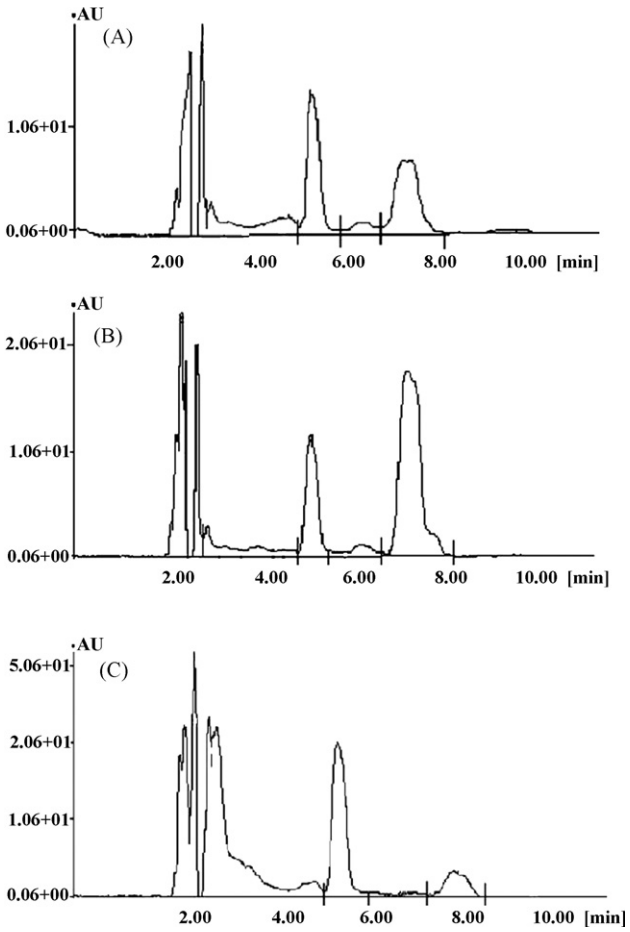

Fig. 3. Representative chromatograms showing: (A) extract of rat plasma obtained 240 min after IV administration of CR 3834 (RT = 7.493) spiked with 5 μg of IS (RT = 5.267); (B) extract of rat plasma obtained 240 min after OS administration of CR 3834 (RT = 7.453) spiked with 5 μg of IS (RT = 5.293); (C) extract of rat urine obtained 240 min after OS administration of CR 3834 (RT = 7.772) spiked with 15 μg of IS (RT = 5.400).

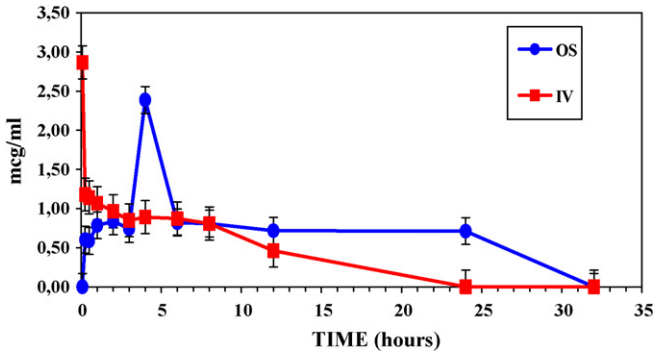

Fig. 4. Time profiles of plasma concentrations after IV and OS administration of CR 3834. The ordinate shows the plasma level, abscissa shows the time after the administrations of the drug ($n = 3$; error bars = S.D.).

Table 5
Urinary excretion of CR 3834 in Wistar rats ($n = 3$)

Time of collection (h)	Mean volume collected (ml)	Mean total excreted amount (μg)
0–1	0.3	0.805
1–2	0.5	1.082
2–4	0.6	1.601
4–6	1.5	1.732
6–8	2.3	2.069
8–12	3	5.373
12–24	5.4	2.05
24–36	4.6	0.0
0–36	18.2	14.712

The total amount of CR 3834 detected was 14.712 μg in a 18.2 ml of total volume of urine, collected over 36 h.

4. Conclusions

The study presented a simple, reliable and validated SPE-HPLC method for the determination of a new non-peptidic angiotensin AT_1 antagonist in rat plasma and urine was developed. The method was used for the plasma determination of CR 3834 concentration after intravenous and oral administration to Wistar rats. Moreover, it has been used to describe urinary excretion-time profile and cumulative excretion of the studied compound after oral administration.

The assay procedure was fully validated. The extraction procedures demonstrated a good efficiency. Solid-phase extraction allowed isolating CR 3834 from rat plasma and urine with a good recovery. The accuracy determined in the inter-assay, in both plasma and urine, was adequate for biological samples. The sensitivity of the method allowed the pharmacokinetic study of CR 3834 in Wistar rats.

The plasmatic time course showed that this drug was detected within fifteen minutes after the oral intake indicating an adequate absorption; moreover, it reached the maximum concentration in 4 h, maintaining a moderate concentration over the examined time; the plasma level decreased very slowly and CR 3834 disappeared from plasma 32 h after administration. No other metabolites were detected in the plasma and urine samples analyzed, indicating that the substance did not undergo bio-transformation. Moreover, considering the total amount of urinary excretion over the 36 h, we observed that the concentration of CR 3834 detected in urine was low compared to intake dosage, suggesting the possibility of alternative ways of excreting the tested substance.

In conclusion, the high-performance liquid chromatographic method described here proved to be sensitive and specific for the determination of CR 3834, allowing the pharmacokinetic study in Wistar rats of this new AT_1 receptor antagonist.

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