RESEARCH ARTICLE

Chronopharmacokinetic and Metabolite Studies of the Cardiovascular Medication Sacubitril

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ABSTRACT

People with "heart failure with preserved ejection fraction" have heart failure, but their ejection fraction is still pretty normal (HFpEF). When it comes to heart failure, people with normal or nearly normal ejection fractions are more likely to get it than those with lower ejection fractions. In clinical trials with HFpEF, medications such as angiotensin-converting enzyme inhibitors (ACEIs), aldosterone antagonists, beta-blockers, calcium channel blockers, and angiotensin receptor blockers (ARBs) did not yield any beneficial results. Sacubitril is prescribed to peritoneal dialysis patients with end-stage renal disease in order to manage their hypertension and heart failure. The concentrations of sacubitril and its primary metabolite in plasma, urine, and peritoneal dialysis fluid were determined using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). This method is effective because it doesn't take much time but produces good results. Several samples of body fluids were taken with the help of protein precipitation. The positive ion mode of ionization from the UPLC-MS/electrospray MS was used to look at the extracts. With correlation values of 0.99991 for sacubitril, the suggested method was carefully tested. With this method, it is possible to find out exactly how much sacubitril are in plasma, urine, and peritoneal dialysis fluid. It is also honest, dependable, and nice. If the experiment works, we'll know that peritoneal dialysis changed how sacubitril were cleared from the body.

Keywords: Chronopharmacokinetic, Metabolites studies, Sacubitril, Cardiovascular drugs.

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INTRODUCTION

Chronopharmacology studies pharmacological effects on people and animals throughout time. In the early 1970s, chronopharmacology pioneered. It was revealed that the body's 24-hour clock influences pharmaceutical metabolism. The suprachiasmatic nucleus (SCN) is the mammalian clock. SCN neurons and other cells share chemicals that maintain a 24-hour circadian rhythm. Yet, there are major differences: Chemical zeitgebers like feeding-fasting signals govern peripheral oscillators, whereas the retina's sense of light and dark cycles regulates SCN neurons. Throughout the past decade, microarray research on several model animals has shown daily fluctuations in xenobiotic metabolism genes. The circadian clock governs xenobiotic detoxification like pharmacokinetics and pharmacodynamics influence drug effectiveness and safety. They may improve pharmaceutical effectiveness and reduce side effects in clinical practice due to their individualization. They also affect various conditions. Chronopharmacokinetics studies pharmacological effects across time. Daytime biological activities affect medication absorption, distribution, metabolism, and elimination.[1-4]

The discovery that medication distribution time affects kinetics led to the creation of chronokinetics. Not only the time of day, the patient or animal's activity level, or whether they went to sleep immediately after taking the drug impact its kinetics. The term "chrono" derives from biology and implies that metabolic activity occur at regular periods. Researchers found that all living organisms have their own rhythms, ranging from seconds to years. According to solar observations, it typically lines up with the 24-hour rotation of the Earth. Many studies link rhythmic changes to sickness and treatment response. Chronotherapeutic medicine administration matches the body's circadian cycles. Various medications' pharmacological sensitivity and pharmacokinetics are impacted by the progression from severe to moderate sickness symptoms and risk factors. Medications work differently

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depending on how long you take them. Depending on the patient's condition, medications should be given at different times. Although pharmacokinetics may stay constant, drug delivery systems must account for fluctuations in sickness status and medicine plasma concentration to treat a disease with the appropriate dose at the ideal time.[5-8]

In recent years, research and development into chronopharmaceutical drug delivery methods has grown. Body systems change every day. These alterations affect patient health and plasma drug concentration. The circadian rhythm, depending on the sleep-activity cycle and heredity, affects daily activities (24-hour period). After sickness or injury, the circadian cycle helps wound healing. Hormones are produced 24/7. Heart rate and blood pressure rise between 6 AM and 12 PM. High blood pressure, asthma, stomach ulcers, and arthritis are linked to circadian rhythm disturbances. Osteoarthritis pain, unlike rheumatoid arthritis pain, intensifies during the day and peaks at night.[9]

MATERIALS AND METHODS

Sacubitril was provided by Cipla in the city of Mumbai. Merck India Ltd. in Mumbai, India stocked HPLC-grade acetonitrile, analytical-grade HCl, methanol, NaOH pellets, and sodium dihydrogen phosphate. All of these chemicals were available for purchase. The TKA smart2pure water filtration system in Niederelbert, Germany was able to generate drinkable deionized water. In addition to that, a pH trainer manufactured by Eutech Instruments in Singapore, a Toshiba sonicator imported from New Delhi, and an electronic scale manufactured by Mettler-Toledo were used.[10]

Analytical and Bioanalytical Method Development and Validation of Sacubitril

Analytical and bioanalytical method development of sacubitril

- *Spectral study of sacubitril*

First, the candidate drug's highest absorbance, or Lambda max (λ_{max}) (Figure 1), was determined by creating an ultraviolet (U.V) spectrum using the appropriate U V spectrophotometer.[11]

- *Selection of chromatographic method*

The sample's composition (ionic/ionizable/neutral molecule, molecular weight, and solubility) determines the technique utilized. Due to the polar nature of the medication of interest, other chromatographic techniques, such as reverse phase, ion exchange, or ion pair chromatography, may be used. For primary separation, we opted for reverse-phase HPLC due to its user-friendliness, versatility, and reliability.[12]

- *Sample preparation for assay method*

From the information about the excipients and coating agents used in the tablet dosage form made by NDDS, Glenmark Generics Ltd, and Taloja, it has been found that the coating used is not water-soluble. Based on the solubility data, it was found that it dissolves very well in acetonitrile. This means that when the whole tablet is used for testing, a solvent is needed to dissolve the coating so that the drug can be completely extracted from the excipients. For diluent optimization, buffer and ACN were mixed in different proportions, such as 80:20, 60:40, and 50:50. The ratio of 50/50 was then set by trying it out several times.[13]

Analytical and bioanalytical method validation of sacubitril

Mixing 5 mg of racemic sacubitril with 5 mL of methanol in a volumetric flask yielded a main stock solution of 1 mg/mL Sacubitril racemic combination. The working standard solutions of 1, 5, 25, 50, 250, 500, and 750 µg/mL sacubitril were prepared by sequentially adding the mobile phase to the main stock solutions. There were created sacubitril plasma standards at values of 0.02, 0.10, 0.50, 1.05, 10.5, and 15 µg/mL. Four quality control (QC) standards were also prepared, one for each concentration point along the calibration curve: low, medium, high, and low (LoQQC = 0.05 µg/mL, MQC = 4 µg/mL, and HQC = 12 µg/mL, respectively) concentrations. Similar standards were developed for the central nervous system, respiratory system, hepatic system, and cardiovascular system using Sacubitril concentrations ranging from 0.05 to 5 µg/mL. Bio samples were processed and analyzed in accordance with procedures outlined in the sample preparation section.[14,15]

Pharmacokinetics and bio-distribution of sacubitril

Sacubitril, like many other compounds, has several possible etymologies. An ultrasonic cell disruptor (Microson TM) and a tissue tearer (Sorvall) were utilized to achieve homogeneity in the tissue. Glass syringes, forceps, and scissors were among the surgical implements used only after they had been thoroughly sterilized.[16]

- *Animals*

In the plasma and tissues of healthy male Wistar rats, bio-distribution and pharmacokinetic studies of sacubitril were done. Two hundred rats, each of which weighed 20 g, were used in the experiment. The animals were given the usual lab pellets to eat, and they could drink as much water as they wanted. In the rat facilities, temperature, humidity, and the amount of artificial light and dark were all carefully controlled (12 hours).

- *Pharmacokinetic and bio-distribution studies*

The research used pure racemic drug solutions prepared for intravenous and oral administration of single doses. The medication concentrations in various tissues, including plasma, were tested at regular intervals. The primary objective of the research was to examine the drug's enantioselective behavior following administration.[17-19]

- *Dosing and plasma sample collection*

After heating the vein with hot water, a 1-mL syringe containing 1.6 mg/kg of Sacubitril solution was inserted into the caudal vein of the rat and filled. Three animals were employed at each of the following times after drug administration: 4, 8, 16, 32, 46, 1, 2, 4, and 8 hours. One blood sample was extracted from the vein behind the eye of each cat. At each time point while the

patient was under anesthesia, 1-mL of blood was drawn. The blood samples were spun at 12000 rpm for 30 minutes at -4°C in tubes that already contained 100 mL of 10% w/v sodium EDTA to stop the blood from clotting. Plasma samples were taken and kept at -20°C until they could be examined further.[20]

- *Oral dosing and plasma sample collection*

An oral feeding tube containing a sacubitril solution (3.2 mg/kg) was used to provide the drug to rats. In 5, 10, 15, 30, 1, 2, 4, 6, 9, and 12 hours following medication administration, each animal had blood samples drawn by retro-orbital puncture. An overnight blood sample (1-mL) was obtained at each time point. We spun 100 mL of blood samples at 12000 rpm for 30 minutes at -40°C in tubes containing 10% w/v sodium EDTA. Before being analyzed, the plasma samples were frozen to a temperature of -20°C.[21]

- *Tissue sample collection*

As part of an oral pharmacokinetic study, surgery was done on the heart, kidney, liver, lungs, and brain 15, 30, 2, and 6 hours after blood was drawn from the inferior vena cava with a 10 mL syringe. To do this, an ether anesthetic was used to cut open the abdomen and show all the important organs. Tissues were washed with salt water to get rid of any blood. The organs inside the body were put into a petri dish with ice-cold saline. In 2 to 5 mL of saline was used to wash the tissues. After the tissue had been dried on filter paper and weighed, it was mixed in a salt solution to make it all the same size. The homogenates were spun in a centrifuge in the same way that the plasma samples were, and the liquid that came out on top was saved. Before being looked at, biosamples were frozen and kept at -20°C.

- *Analysis of biological samples*

In 200 µL of mobile phase and 100 mL of formic acid (5% v/v) were added to 500 µL of plasma and tissue samples. Afterward, solid-phase extraction was used to elute the medication using methanol. The eluate was dried off by evaporation at 400°C using N2 gas. To recombine residues, mobile phase 500 µL was added. Standardized bioanalytical procedures were used to determine the drug concentration in a biological sample.[22]

- *Pharmacokinetic data analysis*

The non-compartmental analysis method was used with the Win Nonlinver 2.1 software to figure out how much drug was in different plasma and tissue at different times. C_{max}, AUC0-∞, MRT, VD, Ke, t1/2 and Cl are some of the pharmacokinetic parameters observed for sacubitril and its enantiomers. Finally, the correct statistical tests were used with a significance level of $p < 0.05$ to examine the in vivo pharmacokinetics of enantiomers at various doses.[23]

RESULT AND DISCUSSION

Analytical and Bioanalytical Method Development and Validation of Sacubitril

Analytical and bioanalytical method development of sacubitril

- *Spectral study of sacubitril*

The following spectra were obtained using a sacubitril in methanol (10 ppm) solution, which is crucial since HPLC detection is mostly UV-based.

- *Selection of chromatographic method*

Chromatograms were constructed by varying one or two parameters in each experiment to find best conditions for separating, eluting, and measuring Sacubitril by chromatography.

With an area of 270649 and a tailing factor of 1.21, retention time was found to be 7.671 minutes. Peak reached was the best of all the ones that had been tried before (Figure 2).

- *Final method of assay method*

A HPLC method for measuring the sacubitril content of sacubitril tablets has been developed. This process is used to validate if sacubitril tablets have the correct concentration of the drug (Table 1).

Table 1: Final method of assay method

Parameter	*Condition*
Stationary phase	Phenomenex Luna C18 (250 × 4.6 mm, 5 µm)
Mobile phase	Buffer: ACN (80: 20)
Flow rate	1.5 mL/min
Detection	250 nm
Pump mode	Isocratic
Injection volume	20 µL
Run time	12 minutes
Column temperature	Ambient
Retention time	About 7–9 minutes
Needle wash	Water : ACN (50:50)

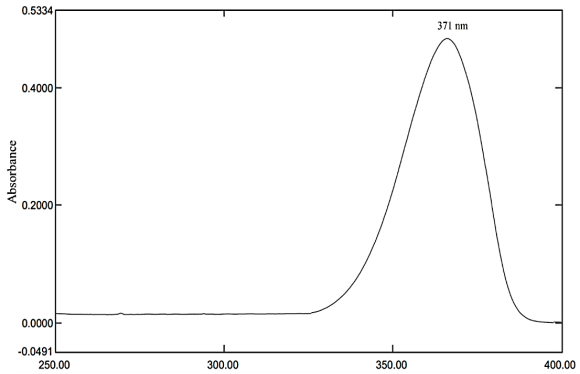

Figure 1: UV spectrum for sacubitril obtained from UV spectrophotometer

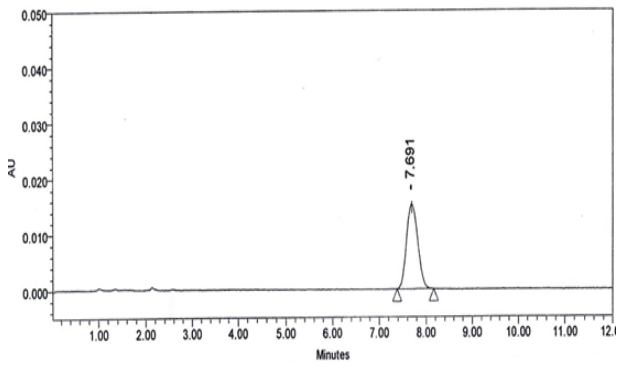

Figure 2: Chromatogram obtained from trial

Table 2: Validation report of a method for assay of Sacubitril HCl in tablet dosage form by HPLC

S. No.	*Validation parameter*	*Acceptance criteria*	*Result*
1. Specificity			
1.1	Identification	When it comes to retention time, the results should be comparable.	Standard solution has an R.T. of 7.671 minutes. 7.714 min. R.T. of sample solution
1.2	Placebo interference	At the same time that Sacubitril Peak peaks, neither Mobile Phase nor Placebo should show any peaks.	There is no interference. In both the Standard solution and the Sample solution, the sacubitril peak is pure.
1.3	Known impurity interference	Assays of spiked and unspiked (control) samples shouldn't differ by more than 1% in terms of their averages. Both the control sample and the sample that has been tampered with should pass the peak purity test.	Complies. Peak purity passes.
1.4	Forced degradation studies	Sacubitril peak should be uniform, and there shouldn't be any other peaks that come out at the same time. The analyte peak purity should pass.	Complies. Peak purity passes.
2	Linearity & range	The correlation coefficient must be at least 0.999.	Correlation coefficient is 0.99991.
3.	Accuracy (Recovery)	The average rate of recovery should be between 98.0% and 102.0%. RSD shouldn't be higher than 2%.	RSD is 0.72% and mean recovery is 100%.
4. Precision			
4.1	System precision	RSD should > 2.0%.	The RSD is 0.10%.
4.2	Method precision	RSD should >2.0%.	The RSD is 0.50%.
4.3	Intermediate precision (Ruggedness)	RSD should > 2.0%.	The RSD is 0.74%.
5	Stability of solution	Standard Solution: The difference between the old standard and a freshly made one is between 98.0 and 102.0%. Sample Solution: The relationship between the old sample solution and the first Assay is between 98 and 102%.	At room temperature, standard and sample solutions are stable for 72 hours.
6	Robustness	Each conditional variable test must be satisfied by system suitability. Relative standard deviations (RSDs) for the control and variable conditions combined shouldn't exceed 2.0%.	The test method works well in all different situations.
7	Filter equivalency	If the correlation is between 98.0 and 102.0%, the filter will be deemed appropriate.	Nylon 0.45 µ and glass filters are suitable.

Table 3: Pharmacokinetic parameters of sacubitril

Parameters	*Sacubitril*
$AUC_{0-\infty}$(µg.h/mL)	7.55 ± 0.77
MRT(h)	2.57 ± 0.10
V_d(L/kg)	0.53 ± 0.04
$K_e(h^{-1})$	0.30 ± 0.02
$t_{1/2}$(h)	1.70 ± 0.08
Cl(L/h/kg)	0.20 ± 0.02

*If the probability value is less than 0.05 ($p < 0.05$), there exists a significant difference between the values at 95% confidence interval between enantiomers

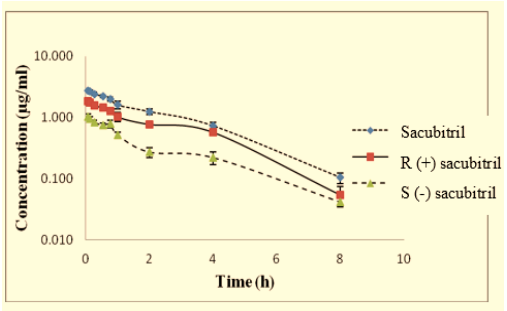

Figure 3: Log plasma concentration-time profiles of sacubitril after IV administration in rat

Analytical and bioanalytical method validation results of sacubitril

The selected method was analysed bioanalytically and results obtained are mentioned in Table 2.

Pharmacokinetics and bio-distribution of sacubitril

- *Administration*

Intravenous administration of sacubitril resulted in the time-concentration curve seen in Figure 3. It means sacubitril AUC0- was significantly increased. Table 3 displays the pharmacokinetic characteristics of both enantiomers as well as the probability values for the differences between the two enantiomers as determined by the non-compartmental method paired t-test.

- *Oral route of administration*

How sacubitril levels increase in the body following oral administration is shown in Figure 4. Blood levels of sacubitril

Table 4: Pharmacokinetic parameters of sacubitril and its enantiomers

Parameters	*Sacubitril*
T_{max}(h)	0.5
C_{max}(µg/mL)	4.82 ± 0.15
$AUC_{0-\infty}$(µg.h/mL)	12.25 ± 0.74
MRT(h)	2.71 ± 0.03
$K_e(h^{-1})$	0.38 ± 0.01
V_d(L/kg)	0.69 ± 0.04
$t_{1/2}$(h)	1.81 ± 0.01
Cl(L/h/kg)	0.26 ± 0.02
F	0.81 ± 0.019

*If probability value less than 0.05 ($p < 0.05$), there exists a significant difference between the values at 95% confidence interval between the enantiomers.

Table 5: Pharmacokinetic parameters of sacubitril in tissues of rat

Biological sample	*Parameters*	*Sacubitril*
	T_{max} (h)	0.5
	C_{max} (µg/g)	0.86 ± 0.06
	AUC 0-∞ (µg.h/g)	1.71 ± 0.12
Heart	MRT (h)	2.50 ± 0.01
	Ke (h-1)	0.36 ± 0.001
	t1/2 (h)	1.95 ± 0.01
	Cl (L/h/kg)	1.88 ± 0.13
	T_{max} (h)	0.5
	C_{max} (µg/g)	6.95 ± 0.48
	AUC 0-∞ (µg.h/g)	14.17 ± 1.64
Kidney	MRT (h)	1.93 ± 0.03
	Ke (h-1)	0.46 ± 0.001
	t1/2 (h)	1.52 ± 0.03
	Cl (L/h/kg)	0.23 ± 0.03
	T_{max} (h)	0.5
	C_{max} (µg/g)	2.08 ± 0.24
	AUC 0-∞ (µg.h/g)	3.06 ± 0.39
Liver	MRT (h)	4.51 ± 0.44
	Ke (h-1)	0.90 ± 0.01
	t1/2 (h)	0.77 ± 0.01
	Cl (L/h/kg)	1.06 ± 0.13
	T_{max} (h)	0.5
	C_{max} (µg/g)	1.73 ± 0.02
	AUC0-∞ (µg.h/g)	5.48 ± 0.48
Lungs	MRT(h)	0.92 ± 0.02
	Ke (h-1)	0.22 ± 0.02
	t1/2 (h)	3.13 ± 0.30
	Cl (L/h/kg)	0.42 ± 0.6

*If probability value less than 0.05 ($p < 0.05$), there exists a significant difference between the values at 95% confidence interval between the enantiomers.

were same between oral dosing and intravenous administration. There was evidence that sacubitril had a higher rate of systemic uptake. In Table 4, we can see the pharmacokinetic characteristics for both enantiomers, as well as the paired t-test probability that reveals how significant the differences are.

- *Biodistribution studies: oral route of administration*

At an oral dose of 3.2 mg/kg, racemic drug levels in various organs vary with time. Table 5 lists all pharmacokinetic parameters determined. Figures 5–8 illustrate the time-course drug dosages in the heart, kidneys, liver, and lungs as a percentage of tissue weight. The brain showed no drug before 30 minutes. This implies no presentation and a non-permeable brain. The kidneys, heart, and liver had the greatest drug concentrations after 30 minutes. C_{max}, AUC tissue, MRT, Ke, t1/2, and Cl were calculated by plotting drug concentration vs. time for the four organs (heart, kidney, liver, and lungs). Both enantiomers had similar cardiac pharmacokinetics, despite sacubitril's larger C_{max}. Despite S sacubitril being the enantiomer, the two isomers were identical. Sacubitril was found in the kidney, liver, lungs, and heart. Each sacubitril enantiomer has distinct features, according to researchers. Sacubitril and its enantiomers have poor brain penetration, according to tissue distribution studies. Enantioselective protein binding may explain the two enantiomers' drastically differing distributions.

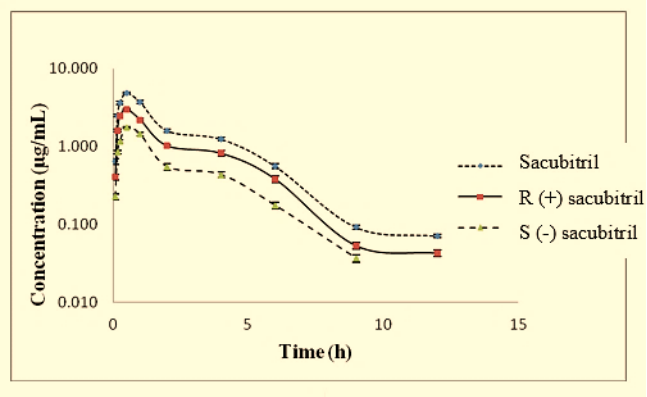

Figure 4: Log plasma concentration-time profiles of sacubitril after oral administration in rat

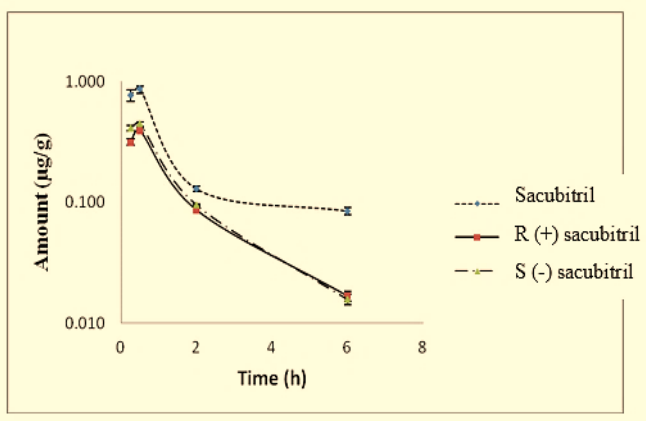

Figure 5: Log amount - time profiles of sacubitril in rat heart

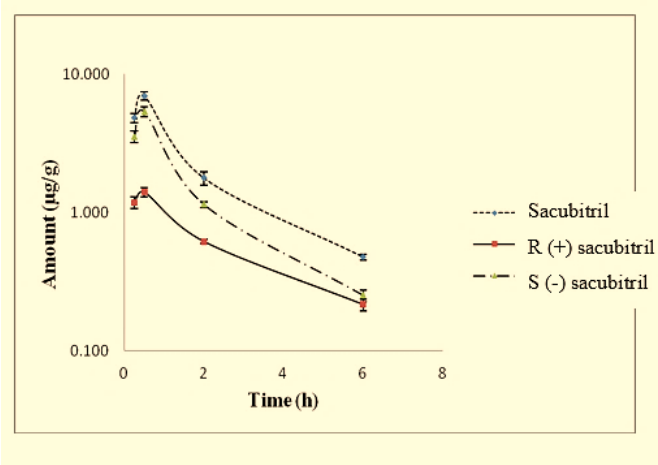

Figure 6: Log amount - time profiles of sacubitril in rat kidney

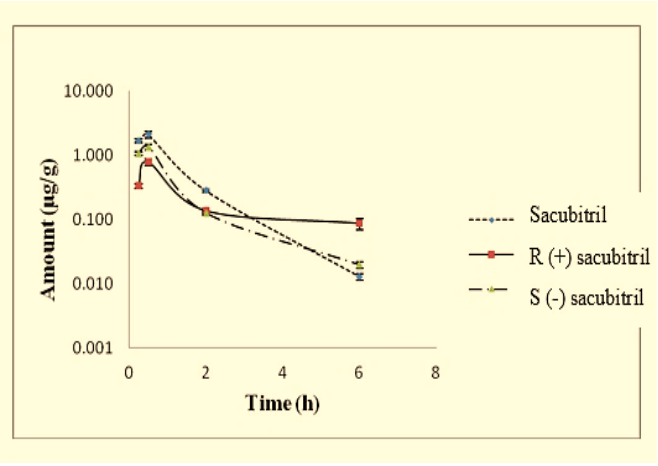

Figure 7: Log amount - time profiles of sacubitril in rat liver

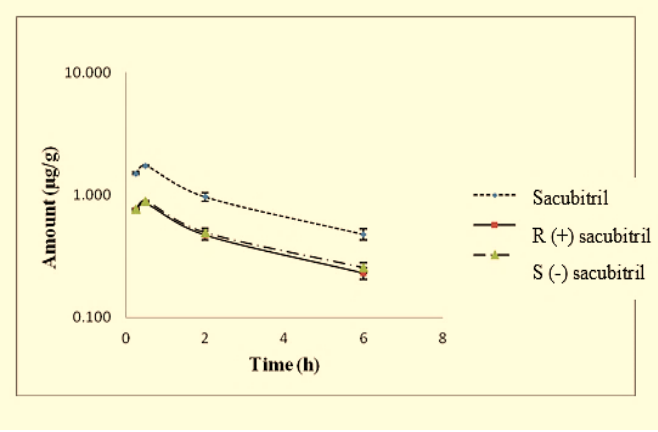

Figure 8: Log amount - time profiles of sacubitril and its enantiomers in rat lungs

SUMMARY AND CONCLUSION

Clinical pharmacists can enhance therapeutic drug monitoring, side effects, and patient care *via* chronopharmacokinetics. This requires correct method utilization. Chirality in natural and synthetic drugs has several medicinal benefits. Remember this in chemistry. A single-stereogenic chiral molecule may be enantiopure or include both enantiomers. Enantiomers only have identical physicochemical qualities in an achiral context. They change drastically in chiral environments. Each isomer's metabolic, toxicological, and pharmacological actions may vary or even conflict. Hence, one member of an enantiomeric pair may have quite different pharmacokinetic and pharmacodynamic effects from the other. Pharmacologists cannot overlook chirality. They must clearly identify stereoisomers. Further research is needed to solve racemization during synthesis and generate stereochemically specified peptide products, especially for medicinal and industrial purposes. This thesis's goals include drawing the chemical community's attention to an intriguing topic. After a single dose or an overdose. Acute overdoses are usually obvious, but many drugs may take time to affect the cardiovascular system. Chronic impacts may take longer to show and may be hard to pinpoint. The cardiovascular system, especially the heart and blood arteries, is essentially unknown since much of what is known about the potentially detrimental effects of these new drugs comes from long-term clinical investigation or clinical experience. A higher risk of mortality from cardiovascular disease has been associated with even small changes in arterial blood pressure and heart rate; thus, it is critical to determine if the patient is taking any medications that could induce these issues.

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