



ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SACUBITRIL IN DRUG SUBSTANCE BY RP-HPLC METHOD

Bhavani, D. Lalitha, D. Kumari, R. Venkamma, G. Vijay Kumar, Dr. B. Tirumaleswara Rao and Dr. Challa Sudheer*

Department of Chemistry, Vikas PG College, Vissannapeta, Krishna Dist, Andhrapradesh, India- 521215.

*Author for Correspondence: Dr. Challa Sudheer

Department of Chemistry, Vikas PG College, Vissannapeta, Krishna Dist, Andhrapradesh, India- 521215.

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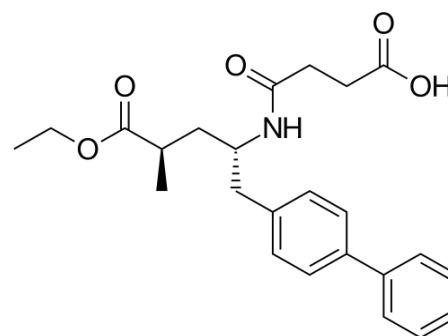
ABSTRACT

Analytical method was developed for the estimation of Sacubitril drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 150*4.6, 5μm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water : Acetonitrile (25:75). The flow rate was 1.0 ml/ minute and ultra violet detector at 254nm. The average retention time for Sacubitril found to be 2.71 min the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 50-150μg/ml for Sacubitril.

KEY WORDS: Sacubitril, Isocratic, HPLC, C18, Formic acid, Inertsil ODS, methanol and validation.

Sacubitril is an antihypertensive drug used in combination with valsartan. The combination drug valsartan/sacubitril, known during trials as LCZ696 and marketed under the brand name *Entresto*, is a treatment for heart failure. It was approved under the FDA's priority review process for use in heart failure on July 7, 2015. Sacubitril is a prodrug that is activated to sacubitrilat (LBQ657) by de-ethylation via esterases. Sacubitril at inhibits the enzyme neprilysin, which is responsible for the degradation of atrial and brain natriuretic peptide, two blood pressure-lowering peptides that work mainly by reducing blood volume. In addition, neprilysin degrades a variety of peptides including bradykinin, an inflammatory mediator exerting potent vasodilatory action.

Sacubitril is chemically designated as 4-[(2S,4R)-1-(4-Biphenyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl]amino}-4-oxobutanoic acid. Its molecular formula is C₂₄H₂₉NO₅, and its molecular weight is 411.49 g/mol. Sacubitril is a white-to-off white powder. It is freely soluble in methanol and practically insoluble in water.



Structure of Sacubitril

EXPERIMENTAL

Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Inertsil 5μ, 150 mm × 4.6 mm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vacuum micro filtration unit with 0.45μ membrane filter was used in the study.

Materials: Pharmaceutically pure sample of Sacubitril were obtained as gift samples from Fortune pharma training institute, sri sai nagar, KPHB and Hyderabad, India.

HPLC-grade Acetonitrile and methanol was from qualigens reagents pvt ltd. Formic acid (AR grade) was from sd fine chem.

Chromatographic conditions The sample separation was achieved on a C18 (5 μ , 15 cm X 4.6 mm i.d.) INERTSIL column, aided by mobile phase mixture of 0.1%v/v Formic acid in water : Acetonitrile (25:75). The flow rate was 1.0 ml/ minute and ultra violet detector at 254nm, that was filtered and degassed prior to use, Injection volume is 10 μ l and ambient temperatures.

Preparation of mobile phase:

Buffer Preparation: Take accurately 1ml of formic acid in 1000mL of water

Mobile phase: Then add 25 volumes of buffer and 75 volumes of ACN mixed well and sonicated for 5 min.
Diluent: water: methanol: 50:50 v/v

Preparation of standard stock solution: A 50mg of pure Sacubitril were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 1000 μ g/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100 μ g/ml of Sacubitril.

Preparation of sample solution: A 50mg of Sacubitril sample were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 1000 μ g/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 100 μ g/ml of Sacubitril.

RESULTS AND DISCUSSIONS

Determination Of Working Wavelength (λ max): 10 mg of the Sacubitril standard drug is taken in a 10 ml volumetric flask and dissolved in water:methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the water:methanol to give a concentration of 10 μ g/ml. The above prepared solution is scanned in uv between 200-400 nm using water:methanol as blank. The λ max was found to be 254nm.

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: ACN (25:75). The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.

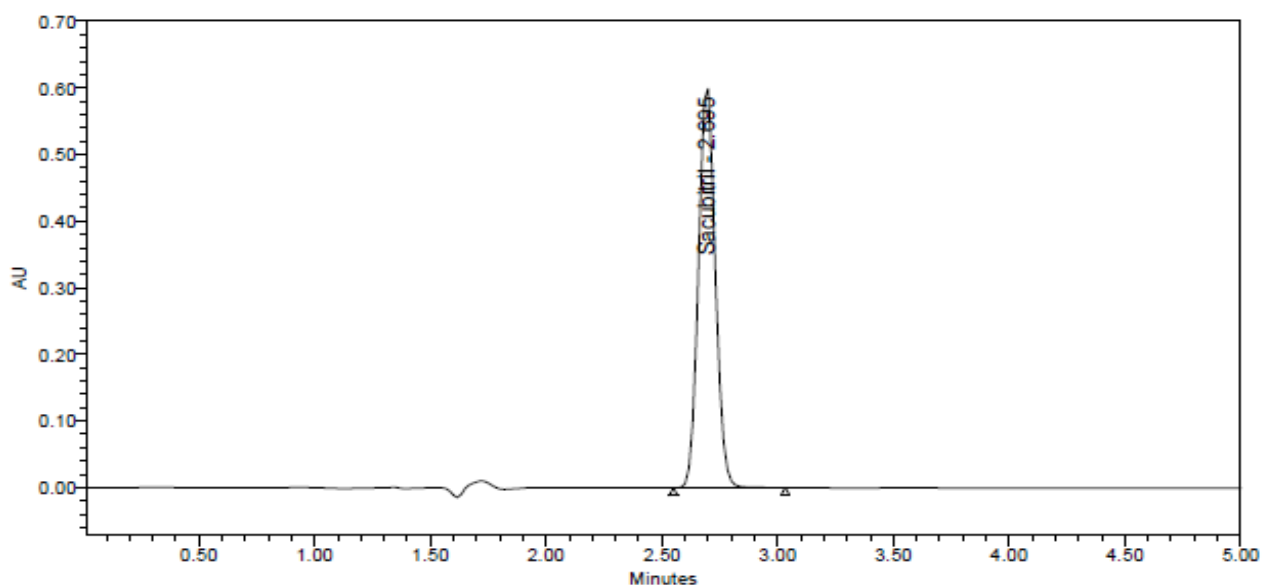


Figure: 1 Chromatogram of Sacubitril

METHOD VALIDATION

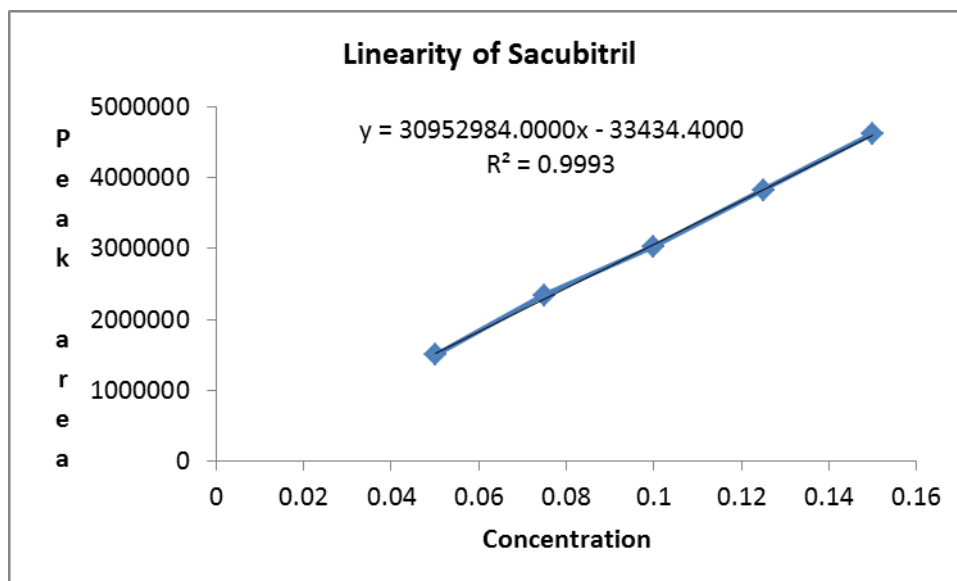
Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50-150 μ g/ml of Sacubitril. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark

with the mobile phase to give a concentrations of 50 μ g/mL, 75 μ g/mL, 100 μ g/mL, 125 μ g/mL and 150 μ g/mL of Sacubitril. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Table No: 1

Level	Concentration (mg/mL)	Peak area
50%	0.05	1495525
75%	0.075	2337708
100%	0.10	3030108
125%	0.125	3824954
150%	0.150	4621025



FigureNo.2: Linearity (calibration) curve of Sacubitril

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$$\text{LOD} = 3.3 \delta/S \dots\dots\dots (1)$$

$$\text{LOQ} = 10 \delta/S \dots\dots\dots (2)$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table no.2: LOD and LOQ values Calculated from calibration curve

	mg
LOD	0.004
LOQ	0.012

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100 µg/ml of SACUBITRIL without changing the parameter of the proposed chromatographic method.

Table.3: Summary of peak areas for method precision

Sample No	Retention time	Peak area	% Assay
1	2.691	3021169	100.2
2	2.693	3025717	100.5
3	2.694	3032616	100.4
4	2.694	3027920	100.6
5	2.696	3005163	99.5
6	2.696	3029104	100.7
Mean	2.694	3023615	100.3
%RSD	0.07	0.32	0.45

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of sacubitril by analyzing

solutions containing approximately 50%, 100% and 150% of the working strength of sacubitril. The percentage recovery results obtained are listed in Table.

Table No.4: Recovery data

LEVEL	S.No	%Recovery of Sacubitril	Average
50	1	100.9	100.2%
	2	100.4	
	3	99.2	
100	1	100.2	100.4%
	2	100.5	
	3	100.4	
150	1	100.9	100.8%
	2	100.9	
	3	100.7	

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ± 2 nm and flow rate was varied ± 0.1 ml/min. The results were shown in (Table no.5)

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e., (limit <2%).

This indicates the method was rugged. The results were shown in Table no.6.

Table No.5: Results of Robustness study

parameter	Rt of Sacubitril	Theoretical plates	Asymmetry
Decreased flow rate (0.9ml/min)	2.999	6532	1.09
Increased flow rate (1.1ml/min)	2.464	5759	1.05
Wave Length 252 nm	2.695	6602	1.06
256 nm	2.690	6558	1.07

Table No.6: Results of Ruggedness

		%Assay	%RSD
Analyst-1	Sacubitril	100.2	0.21%
Analyst-2		100.5	

Table No.7: Validation parameters of evaluated method:

S. No	Parameter	Limit	Value Obtained
1	Linearity concentrations Range (mg/mL)	NLT 0.990	0.05 to 0.15 mg\ml
	Correlation coefficient		0.9997
2	Method precision (Repeatability) (%RSD, n = 6)	98.0 to 102.0 %	99.5 to 100.7 %
3	ACCURACY(%Recovery)	98-102%	100.2 to 100.8%
4.	Robustness	It should be meet System suitability criteria	Complies
	Flow Variation(0.9mL to 1.1 mL/min)		
	Wavelength Variation (252nm to 256nm)		
5.	Ruggedness (Intermediate Precision) (%RSD analyst to analyst variation)	NMT2%	0.21%

*RSD = Relative standard deviation

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of SACUBITRIL was found to be simple, precise, accurate and high resolution and

shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

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