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ISOLATION AND STRUCTURAL ELUCIDATION OF SACUBITRIL SODIUM DEGRADATION PRODUCTS BY MASS AND NMR SPECTROSCOPY

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ABSTRACT

As per ICH Q1A (R2), stress testing (i.e., acidic, basic, oxidative, thermolytic, hydrolytic, and photolytic) was carried out at room temperature with a single batch of Sacubitril Sodium of the drug substance. Though the drug is resistant to oxidation, thermal, and photolysis conditions, two degradation products were formed during base stress. By using gradient preparative HPLC and a normal C-18 column, the degradants were separated, and the obtained SAC D-1 and SAC D-2 were precisely characterized by LCMS and extensive NMR (including 2D) spectroscopic methods. Study of NMR spectra (1D, 2D) and Mass spectrometry confirmed the suggested structures for the products 5-([1,1'-biphenyl]-4-yl)-4-(3-carboxy propanamide)-2-methyl pentatonic acid (SAC D-1) and (3S)-5-([1,1'-biphenyl]-4-ylmethyl)-3-methyl pyrrolidin-2-one (SAC D-2).

Keywords: Sacubitril, Forced Degradation, Preparative HPLC, UPLC, LCMS, NMR Spectroscopy.
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INTRODUCTION

During the drug development process, stress tests are a fundamental requirement. In this stress testing, high levels of stress (above the accelerated stability testing) are applied for a short period of time on PAI/Drug products[1-3]. It helps to ascertain the expected degradation impurities and potential problems during storage/ transportation[4]. A pharmaceutical product may face the worst environmental condition such as uncontrolled humidity and temperature conditions which impacts the stability of PAI[5-8]. So stress testing is useful for developing the stability-indicating analytical method[9]. Several governing bodies like USFDA, CADTH, ICH, and EMA; encourage manufacturers to report impurities present beyond their limits[10-12]. Moreover, certain compendia like the Japanese Pharmacopeia (JP), the British Pharmacopeia (BP), and the Indian Pharmacopeia (IP) provide guidelines for limiting impurity levels in PAI and drug formulations. Sacubitril and valsartan are used together in adults with chronic heart failure. The sacubitril and valsartan help to reduce the likelihood of needing hospitalization if symptoms worsen, as well as reducing the chance of death from heart failure.[13-14] Few articles are found on the forced degradation study of Sacubitril PAI[15] with chromatographic techniques and these articles refer to the identification of new degradation impurity by using the LC-MS technique. Bahia *et al*. have published two degradation impurities namely Sacubitrilat(desethyl-sacubitril) (DPro-1) and the dimmer of Sacubitrilat(DPro- 2) with m/z 384.59 and 767.77 respectively[16] and other publication, Gopi Raju *et al*. has revealed two more degradation impurities at m/z values are 600, 620 which corresponds to Sacubitril Impurity-1,2[17-18]. However, no articles were published neither on isolation nor on the characterization of above degradation impurities. This study's objective is to identify the degradation compounds, as well as to characterize the impurities using various spectroscopic techniques like ^{1}H, ^{13}C NMR spectra, and 2D NMR.

EXPERIMENTAL

Chemical and Reagents

Sacubitril pharmaceutical active ingredient (PAI) was obtained from a manufacturing unit in Mumbai. Mobile phase prepared using high pure gradient grade Solvents and reagents were used for analysis and Methyl cyanide (Merck), TFA (Merck), deuterated solvent i.e. [D_6]-DMSO containing 0.03% (v/v), Tetra

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Methyl Silane which is used as a reference, and purified water (e.g. Milli-Q grade water) used for the preparation of mobile phase.

Preparative HPLC

The degraded Sacubitril sodium test sample was subjected to a preparative chromatography system to isolate a pure fraction of the degraded impurities. Preparative HPLC of Gilson make equipped Model GX-271 Liquid handler is selected and the Gilson DAD 171 detector module at 215 nm is set-upped. For better separation of impurities, Prontosil-C18 (250 × 20 mm & 10 µm) column is used & using the gradient programme with channel A: 0.1% TFA in water; channel B: Acetonitrile: 100% with flow ratio as B:0.0/30,6.0/55,10/55,10.2/98,12/98,12.1/30, 14/30. A combination of water and acetonitrile is used as a diluent, where acetonitrile is 50%.

Liquid Chromatography-Mass Spectrometry (LC-MS)

Thermo 3000 LC with PDA detector (wavelength of detection: 200 – 400nm) Coupled with MS LCQ FLEET. Source used as an ESI positive mode spray voltage is 2.99(kv), spray current 30.0µA, Sheath gas, and sweep gas flow rates 45 and 1.99 respectively, the capillary temperature is 35 ^{0}C and Aux gas flow rate is 12.0. LCMS is used to identify the new degradation impurities of Sacubitril. Column: Aquity UPLC, C-18, (100mm X 2.1 mm with micron size of 1.7) is selected and the mobile phase consists of channel A with 0.05% v/v Tri fluoro acetic acid in purified water; channel B with the combination of tri fluoro acetic acid in acetonitrile (0.05%v/v) using gradient elution with the programme as %B: 0/10, 7/98, 12/98, 12.1/10, 13/10, Combination of water and acetonitrile are used as a diluent, where acetonitrile is 50% for the LC-MS study.

Nuclear Magnetic Resonance Spectroscopy

Both degradation impurities were dissolved individually in DMSO-d6 solvent and recorded the spectra of ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC of these degradant products from 400 MHz w.r.t. ^{1}H NMR spectrometer equipped with the multinuclear probe. Methylated silanes like $SiMe_4$ (TMS) [(Me = CH_3)] were taken as an internal reference compound and relatively this, ^{1}H and ^{13}C nuclear chemical shifts are reported using ppm units with a reference $SiMe_4$ (TMS). The spectra were recorded by referencing Tetra Methyl Silane to δ 0.0 ppm in both ^{1}H and ^{13}C NMR, and δ 2.5 ppm, δ 39.50 ppm in ^{1}H, ^{13}C NMR respectively.

Degradation Study Plan: Stress Methods:

The degradation study was planned by degrading the Sacubitril sodium sample in solid-state as well as in the liquid state (i.e., Acid hydrolysis, Base hydrolysis, oxidative degradation, thermal degradation, hydrolytic, and photolytic degradation) with a single batch of Sacubitril Sodium of the drug substance. Stressing agent concentration and conditions were given in the below table.

Table–1: Stressing Agent Concentration and Conditions

Degradation condition	Details of the Plan
Acid hydrolysis	Sacubitril sodium was subjected to 2N Hydrochloric acid for Twenty four hours (~25° Centigrade)
Base Hydrolysis	Sacubitril sodium was subjected to 2N Sodium Hydroxide for Twenty four hours (~25° Centigrade)
Oxidation	Sacubitril sodium was subjected to 30% H_2O_2 for Twenty four hours (~25° Centigrade)
Thermal degradation	Sacubitril sodium exposed at 100°C for 48 hours
Photolytic degradation	Sacubitril sodium exposed for 200 Wh/m^2 at 40°C for a duration of 48 hours

Isolation of Degradation Products

Two base degradation products (SAC D-1 and SAC D-2) have been found in the base degradation study and the same were identified with the LC-MS technique (as mentioned above section) and found the molecular ion peak at 383.17 and 265.15 corresponding to SAC D-1 & SAC D-2 respectively. After many injections, a few litters of organic solvents were collected by using an optimized condition in preparative HPLC (as mentioned above section). The peaks are almost baseline-separated, which leads to high purity

and yield. At the end of a sequence of purification runs, the collected fractions evaporated from the fraction container into the laboratory air. Refer to the chromatograms of base degradants product in (Fig.-1 and 2).

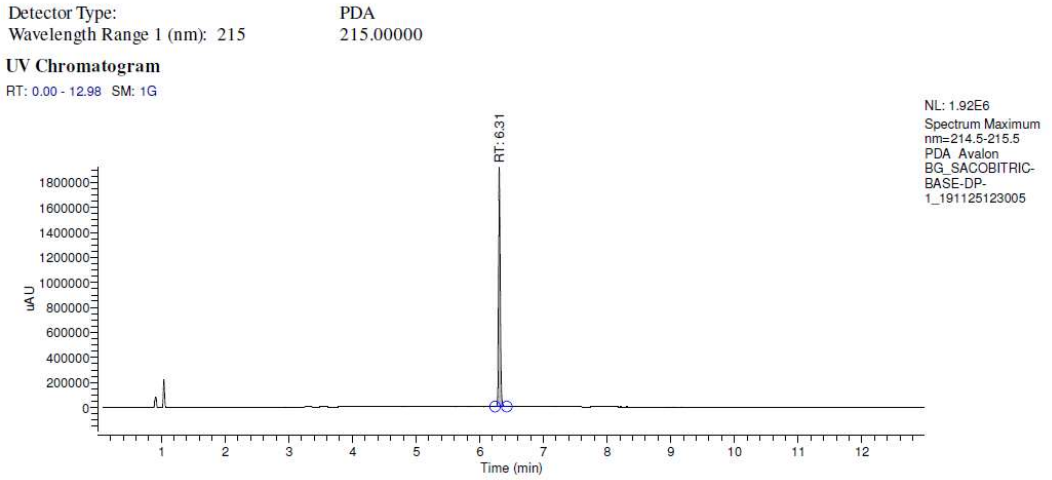


Fig.-1: Chromatograms of Base Degradants Product (SAC D-1)

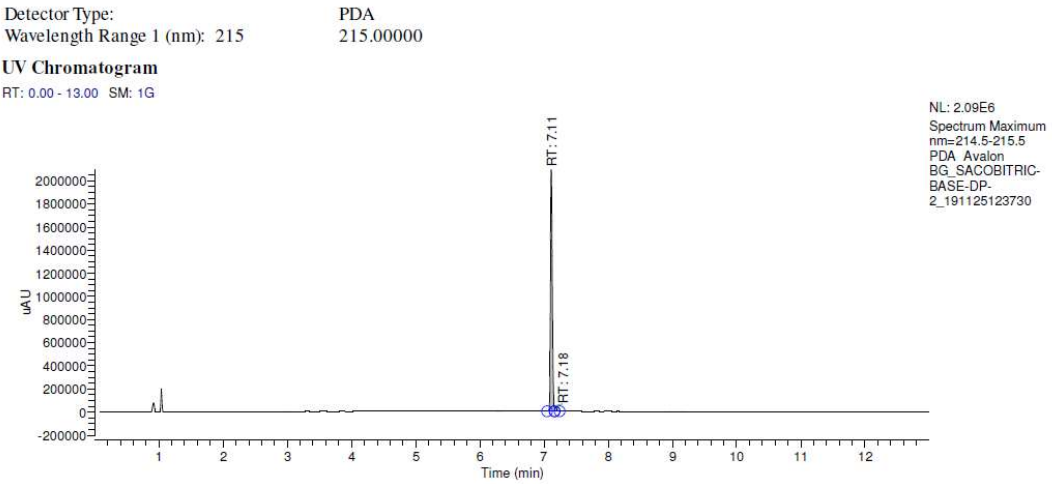


Fig.-2: Chromatograms of Base Degradants Product (SAC D-2)

RESULTS AND DISCUSSION

The Sacubitril sodium PAI was subjected to stress degradation as mentioned in Table-1, viz., acid hydrolysis, base hydrolysis, thermal, oxidative, and photolytic degradation. Based on the chromatographic data, it was observed that the Sacubitril sodium was degraded more extensively in Base hydrolysis. The degraded Sacubitril sodium impurities were isolated using preparative chromatography, and the isolated impurities were again injected to confirm the retention time. These two degradation products are tagged as SAC D-1 and SAC D-2. The Sacubitril sodium eluting at a retention time of 4.4 min and the degradants SAC D-1 and SAC D-2 elute at retention times of 6.3 min and 7.1 min, respectively. These impurities were isolated using preparative chromatography and then subjected to structural elucidation using mass spectrometry and NMR spectroscopy to assign the structures. Refer to below (table-2) for the δ values in ppm of NMR of Sacubitril sodium, SAC D-1 & SAC D-2.

Structure Elucidation of Sacubitril

The Mass spectroscopic analysis using positive polarity with Electro Spray Ionization technique showed the molecular mass as 412.01 Daltons (as M+H). Based on this mass data, the molecular mass of Sacubitril sodium was confirmed as 411 Daltons. The ^{1}H NMR spectrum exhibited 27 protons, of which 18 protons were from aliphatic chains and 9 protons are from the aromatic regions. Similarly, the ^{13}C

NMR showed 24 carbons of which 9 carbons were from the aliphatic region and 15 carbons from the aromatic region. The ^{1}H-^{13}C correlation spectrum confirmed that there are 11 methyne, 5 methylene and 2 methyl groups present. Further confirmation of structure and assignments of ^{1}H and ^{13}C signals were established by NMR studies by correlating ^{1}H-^{1}H and ^{1}H-^{13}C data. Refer to table-2 below for details.

Structure Elucidation of SAC D-1

The Mass spectroscopic analysis using positive polarity with Electro Spray Ionization technique showed the molecular mass as 383.99 Daltons (as M+H). Based on this mass data the molecular mass of SAC D-1 was confirmed as 383 Daltons. The ^{1}H NMR spectrum of SAC D-1 exhibited 22 protons of which 13 protons are from the aliphatic region and 9 protons are from the aromatic region, one labile proton with broad signal arising from –NH group, and two labile protons with broad signal due to acidic protons are observed. Ethyl ester protons which were observed in Sacubitril proton NMR were absent in ^{1}H NMR of SAC D-1. ^{13}C NMR is complimenting the ^{1}H NMR in that 2 aliphatic carbons are absent in its spectrum when compared to Sacubitril PAI ^{13}C NMR spectrum. This confirms that Ethyl ester in Sacubitril was converted into acid during base degradation. The ^{13}C Spectra of SAC D-1 exhibited 7 aliphatic carbons and 15 aromatic carbons. The ^{1}H-^{13}C correlation NMR data confirmed that SAC D-1 had 11 methyne, and 4 methylene, one methyl group present in the 2D-NMR spectrum. Further, the SAC D-1 subjected to Hetero nuclear Multi Bond correlation NMR spectroscopy (i.e. ^{1}H-^{13}C multi bond correlation) and interpretation from this study confirmed the assigned structure as 5-([1,1'-biphenyl]-4-yl)-4-(3-carboxypropanamido)-2-methylpentanoic acid.

Structure Elucidation of SAC D-2

The Mass spectroscopic analysis using positive polarity with Electro Spray Ionization technique showed the molecular mass as 266.09 Daltons (as M+H). Based on this mass data, the molecular mass of SAC D-1 was confirmed as 265 Daltons. The ^{1}H NMR spectrum of SAC D-2 exhibited 18 protons of which 9 protons are from the aliphatic region and 9 protons are from the aromatic region, and one labile proton with a broad signal arising from the –NH group was observed. ^{13}C NMR is complimenting to the ^{1}H NMR that 5 carbons detected in the aliphatic region and 13 carbons detected in the aromatic region. The ^{1}H-^{13}C single bond and multi bond correlation NMR study show that SAC D-2 had 11 methyne groups, 2 methylene groups, and one methyl group in the spectrum. The interpretation using Mass data and NMR data confirms that the isolated degradation product SAC D-2 is named to be as (3S)-5-([1,1'-biphenyl]-4-ylmethyl)-3-methylpyrrolidin-2-one.

Table - 2: Molecular Masses, ^{1}H-NMR, and ^{13}C NMR, and Heteronuclear NMR Correlation Data of Sacubitril Sodium and Degradation Products (SAC D-1 and SAC D-2)

Assignment	SAC PAI		EM-383.99 SAC D-1		EM-265.09 SAC D-2	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	7.44	128.9	7.45	128.9	7.46	128.9
2	7.33	127.2	7.34	127.2	7.35	127.2
3	7.44	128.9	7.45	128.9	7.46	128.9
4	7.65	126.5	7.65	126.5	7.66	126.5
5		140		140.1		139.9
6	7.65	126.5	7.65	126.5	7.66	126.5
7		137.8		137.8		138
8	7.57	126.3	7.58	126.3	7.6	126.47
9	7.26	129.8	7.27	129.9	7.32	130
10		138.2		138.1		137.3
11	7.26	129.8	7.27	129.9	7.32	130
12	7.57	126.3	7.58	126.3	7.6	126.47
13	2.64,2.73	40.7	2.73	40.3	2.68,2.82	41
14	3.92	48	3.99	48.5	3.74	52.1

15	1.40,1.75	37.7	1.37,1.80	37.8	1.63,1.99	33.7
16	8.28		7.79		7.76	
17		172.8		170.7	2.17	34
18	2.22	33.6	2.42	29.5		178.7
19	2.11	34.3	2.32	30.4	0.98	16
20						
21		176.6		174		
22						
23						
24	2.5	36	2.46	35.9		
25		175.5	1.07	18.1		
26	1.05	18		177.2		
27						
28						
29	4	59.7				
30	1.11	14				

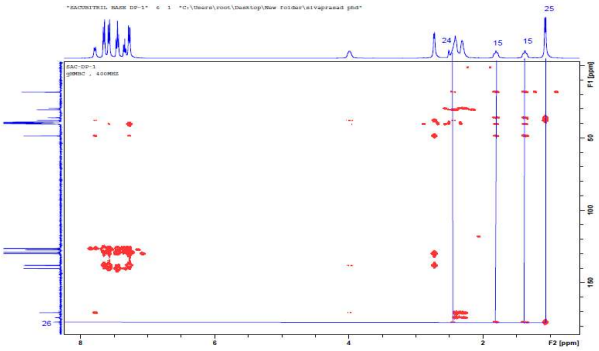


Fig.-3: HMBC Information of SAC D-1

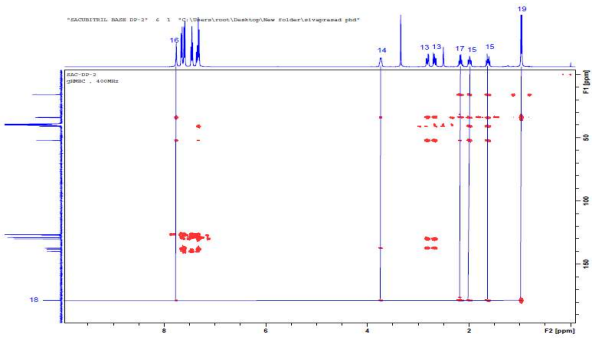
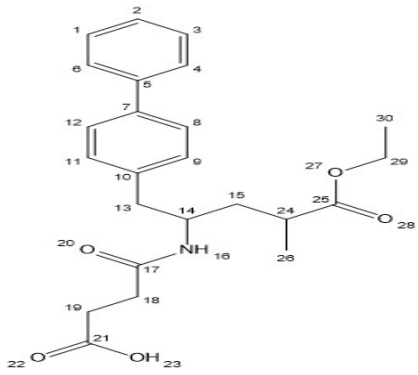
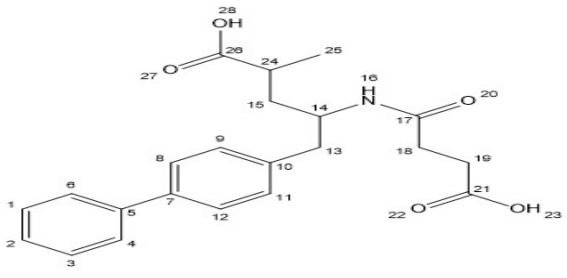


Fig.-4: HMBC Information of SAC D-2.



Sacubitril
Chemical Formula: $C_{24}H_{29}NO_5$
Exact Mass: 411.20
Fig.-5a



SAC D-1
Chemical Formula: $C_{22}H_{25}NO_5$
Exact Mass: 383.17
Fig.-5b

SAC D-2
Chemical Formula: $C_{18}H_{19}NO$
Exact Mass: 265.15
Fig.-5c

Fig.-5. Structure of Sacubitril (5a) and Sacubitril degradation compounds (5b, 5c)

CONCLUSION

Two degradation products, SAC D-1, and SAC D-2 were formed during base hydrolysis of the Sacubitril drug substance. All the degradants were isolated & unambiguously characterized by LCMS and NMR techniques. Structure elucidation of the degradation product SAC D-1 and SAC D-2 were carried out by Mass spectrometry and 1H, 13C, and 2D-NMR techniques and found the structures have molecular masses of m/z 383 and 265 respectively. As far as we know, no literature has been published on the degradation product SAC D-2 and it is a new impurity. Here, we have deduced the structure of SAC D-2 for the first time.

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