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AQbD Approach to Stability Indicating Method Development for Valsartan Sacubitril Drug Product

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Introduction

Valsartan and Sacubitril tablets are a combination medication used in the treatment of hypertension and heart failure. Valsartan is an angiotensin II receptor blocker (ARB) that relaxes blood vessels, while Sacubitril inhibits Neprilysin, an enzyme that can increase blood pressure.

The complex nature of the formulation, coupled with the need for accurate identification and quantification of impurities, demands a systematic approach for method development. AQbD (Analytical Quality by Design) ensures robust method performance by considering critical method parameters, risk assessment, and process variability. Given the absence of a monograph method for related substances for Valsartan and Sacubitril tablets, an AQbD approach provides a scientifically sound and adaptable methodology, leading to reliable and consistent analytical results.

Furthermore, the impurities arising due to acid degradation of Valsartan and Sacubitril in the tablets may introduce unique challenges, necessitating a method that is specifically tailored to this formulation. Acid degradation of the sample was chosen because it has been shown that acid degradation with Hydrochloric Acid induces maximum degradation over other degradation methods. An AQbD approach empowers method developers to comprehensively understand the impact of various factors on method performance, select the appropriate column, mobile phase, and detection conditions, and establish a method that meets the stringent regulatory requirements for related substances determination.

The aim of the study in this technical note was to develop a stability indicating method that accurately measures Valsartan and Sacubitril without interferences from degradants in stability samples. The method should separate Valsartan and Sacubitril from degradants resulting from forced degradation.

Sample Preparation

Two tablets containing the combination of Valsartan and Sacubitril were crushed and precisely weighed, yielding 100 mg of the formulated powder and transferred into a 100 mL volumetric flask. 70 mL of Diluent Solution (Water / Acetonitrile (50:50, v/v)) was added to the flask. 1000 µL of 2 N Hydrochloric Acid was added followed by vigorous vortexing and incubated in a light protected environment for 1 hour. Subsequently, 1000 µL of 2 N Sodium Hydroxide was added to neutralize the solution, then made up to 100 mL volume with Diluent. The solution was centrifuged, and the resulting supernatant was filtered using 4 mm, 0.2 µm Phenex™ nylon syringe filter (Part No.: AF3-3207-12). This forced degradation sample preparation exhibited no significant secondary degradation effects throughout the study.

Initial Study

A preliminary chromatographic run was performed on a Kinetex™ 5 µm F5 column with the LC conditions listed below. Both compounds were readily soluble in the Diluent Solution. **Figure 1** suggested that a gradient elution mechanism was preferred, as this resulted in a reasonably fast separation with good peak shapes and resolution of the Valsartan and Sacubitril. The UV max was adjusted to 250 nm. A 18 % degradation of the main peaks were observed after acid degradation with multiple degradation peaks being observed (**Figure 2**).

Initial LC Conditions

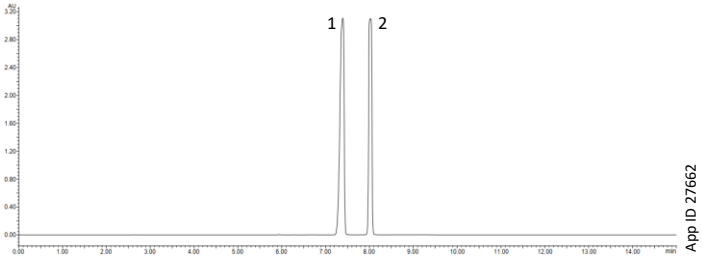
Column: Kinetex 5 µm F5
Dimensions: 250 x 4.6 mm
Part No.: 00G-4724-E0
Mobile Phase: A: 0.1 % Formic Acid in Water
B: Acetonitrile

Gradient:

Time (min)	%B
0	35
15	65

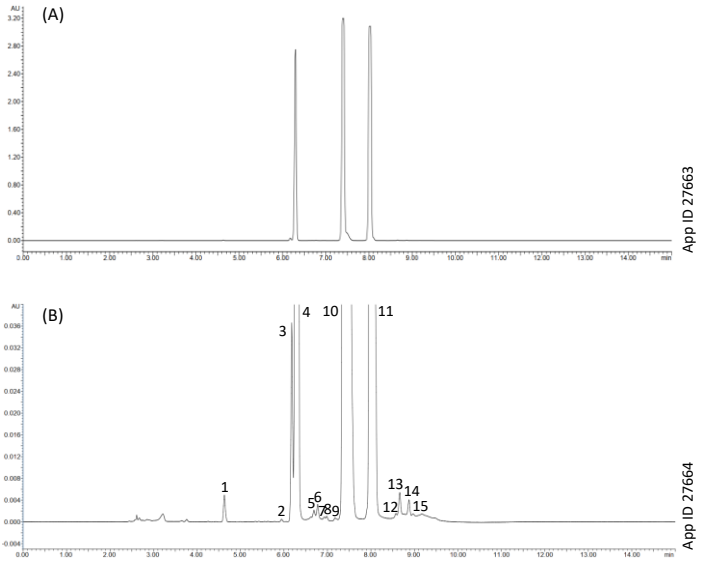
Flow Rate: 0.8 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
LC System: Waters® ACQUITY Arc® HPLC
Detection: UV @ 250 nm

Figure 1. A Preliminary Chromatographic Run of Valsartan and Sacubitril Before Degradation.



Peak No.	Name	Retention Time (min)	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Valsartan	7.392	20337489	57.04	3108800	4.88	0.92	35818
2	Sacubutril	8.026	15314254	42.96	3099100		0.90	95925

Figure 2. A Preliminary Chromatographic Run of Valsartan and Sacubitril After Degradation (A) and a Zoomed Scale View (B).



Peak No.	Name	Retention Time (min)	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1		4.630	14809	0.04	4953		1.13	56678
2		5.946	1309	0.00	474	17.27	1.04	101561
3		6.179	72964	0.20	31639	3.38	0.92	152823
4		6.299	7897974	21.36	2744327	1.75	0.96	117969
5		6.688	3084	0.01	1141	5.59	0.67	165283
6		6.777	5919	0.02	2316	1.31	1.10	152938
7		6.926	565	0.00	254	2.30	0.84	207955
8		6.988	1019	0.00	485	1.03	1.23	226165
9		7.173	1382	0.00	436	2.49	1.21	101977
10	Valsartan	7.405	14559258	39.38	3202945	2.53	0.96	100319
11	Sacubutril	8.022	14395236	38.93	3085514	6.44	0.97	107006
12		8.572	983	0.00	460	7.03	0.92	346395
13		8.664	12121	0.03	4096	1.36	1.02	199696
14		8.875	7736	0.02	2788	2.76	0.94	224113
15		8.972	891	0.00	321	1.30	1.18	226595

Screening Study

Alternative stationary phase selectivities, mobile phase pH, and gradient run times were included as variables in the first set of experiments. The screening phase of method development is based on early risk assessment of variables like mobile phase pH, column chemistry, and run time. DryLab 4 AQbD software was used to generate the 3D model to identify the robust area. The method was further optimized by studying the column temperature variation from 25 °C to 55 °C and the effect of a strong solvent between Methanol and Acetonitrile. The columns and methods used for the screening phase are listed below.

Screening Phase LC Conditions

Column: Kinetex™ 5 µm F5 (00G-4724-E0)
Luna™ Omega 5 µm PS C18 (00G-4753-E0)
Gemini™ 5 µm NX-C18 (00G-4454-E0)
Synergi™ 4 µm Polar-RP (00G-4336-E0)

Dimensions: 250 x 4.6 mm

Mobile Phase: A1: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 2.0
A2: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 2.5
A3: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 3.0
A4: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 6.0
A5: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 6.5
A6: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 7.0
B: Methanol
C: Acetonitrile

Gradient:

	Condition 1		Condition 2	
	Time (min)	%B	Time (min)	%B
	0	5	0	5
	30	90	90	90
	35	90	95	90
	38	5	98	5
	45	5	100	5

Flow Rate: 0.8 mL/min

Injection Volume: 10 µL

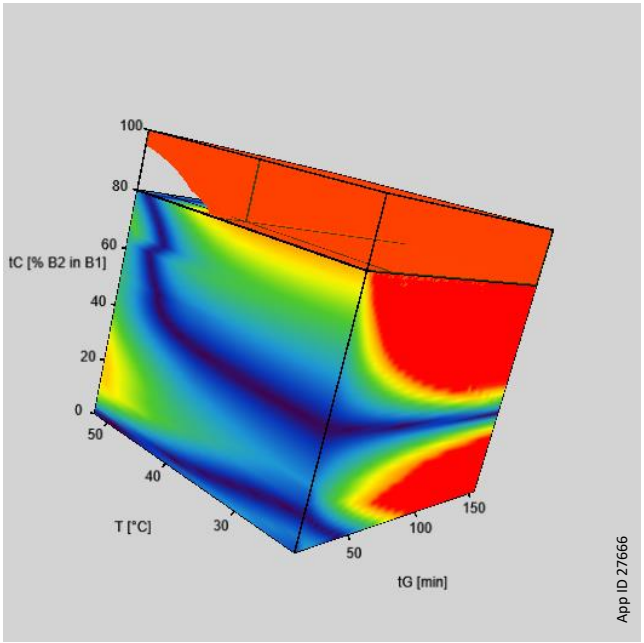
Temperature: 25 °C to 55 °C

LC System: Waters® ACQUITY Arc® HPLC

Detection: UV @ 250 nm

Modeling Software: DryLab® 4 (Molnár-Institute)

Figure 3. 3D Model on a Kinetex F5 Column.



Optimized Study

The best overall outcome from the screening experiments suggested the Kinetex™ F5 column using 10 mM Potassium Dihydrogen Phosphate Buffer, pH 2.5 for mobile phase A and Acetonitrile for mobile phase B. The initial gradient composition was increased to 35 % B as no peaks were observed to elute between 5 % and 35 % mobile phase B under the screening conditions. Column temperature was not a critical factor over the range of 25 to 55 °C, therefore the midpoint of 40 °C was selected. The final optimized conditions are listed below.

Optimized LC Conditions

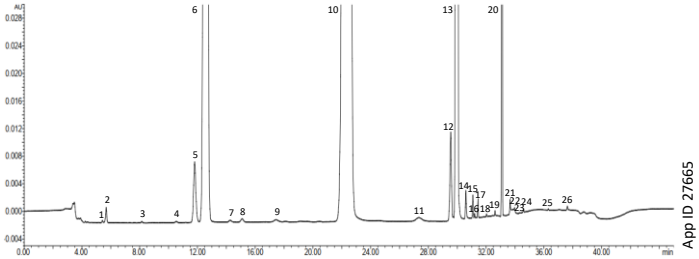
Column: Kinetex 5 µm F5
Dimensions: 250 x 4.6 mm
Part No.: 00G-4724-E0
Mobile Phase: A: 10 mM Potassium Dihydrogen Phosphate Buffer, pH 2.5 with Orthophosphoric Acid
B: Acetonitrile

Gradient:

Time (min)	%B
0	35
25	35
32	90
35	90
38	35
45	35

Flow Rate: 0.8 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
LC System: Waters® ACQUITY Arc® HPLC
Detection: UV @ 250 nm

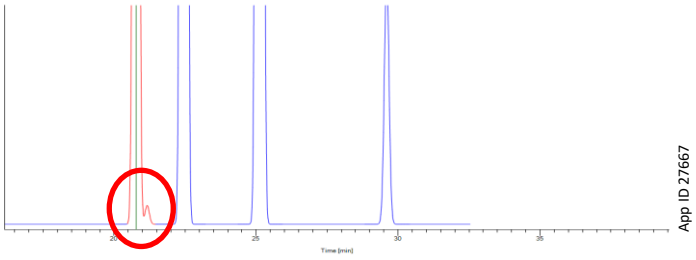
Figure 4. Chromatogram of the Optimized Method on a Kinetex F5 Column.



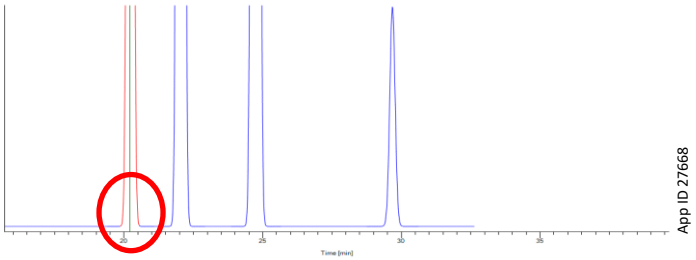
Peak No.	Name	Retention Time (min)	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1		5.419	1548	0.00	336		0.88	29220
2		5.682	12617	0.02	2206	2.24	0.90	23334
3		8.133	1237	0.00	202	14.67	1.05	26172
4		10.532	1721	0.00	191	9.96	0.95	25977
5		11.807	96618	0.17	8737	4.33	1.09	25958
6		12.560	8504897	15.12	719083	2.49	1.00	25858
7		14.290	3007	0.01	261	5.87	0.83	42445
8		15.106	6801	0.01	492	2.58	0.84	28775
9		17.435	6056	0.01	332	5.40	1.16	19008
10	Valsartan	22.358	25167181	44.74	1211226	9.30	0.93	26133
11		27.367	15012	0.03	531	7.50	1.16	19559
12		29.551	91663	0.16	12539	4.46	0.86	360971
13	Sacubutril	29.959	21459633	38.15	3087033	2.30	0.97	572535
14		30.595	18390	0.03	4084	4.70	1.17	1190692
15		31.082	10713	0.02	3304	4.89	0.92	2045397
16		31.205	1791	0.00	628	2.21	0.95	2588932
17		31.433	8388	0.01	2753	2.87	0.96	2408064
18		32.023	1426	0.00	446	7.51	0.69	2821159
19		32.616	2130	0.00	742	7.92	1.33	3159030
20		33.093	830809	1.48	322783	6.72	0.97	3723791
21		33.658	6757	0.01	1951	7.49	0.98	2675847
22		33.973	1554	0.00	484	3.74	1.12	2498162
23		34.300	373	0.00	104	6.81	0.97	174492023
24		34.601	1629	0.00	412	5.66	0.73	2093527
25		36.303	662	0.00	188	20.43	1.08	4176799
26		37.625	2972	0.01	580	14.19	1.50	1706673

Figure 5. Effect of Strong Solvent (Acetonitrile Concentration) on a Critical Pair (1st and 2nd Peak). Resolution by DryLab® 4 Prediction.

20 % Acetonitrile in Methanol
Resolution = 1.33



40 % Acetonitrile in Methanol
Resolution = 0.09



60 % Acetonitrile in Methanol
Resolution = 0.94

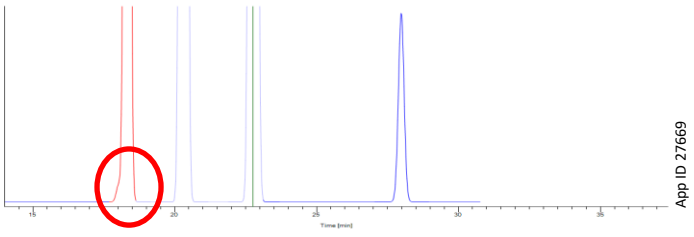
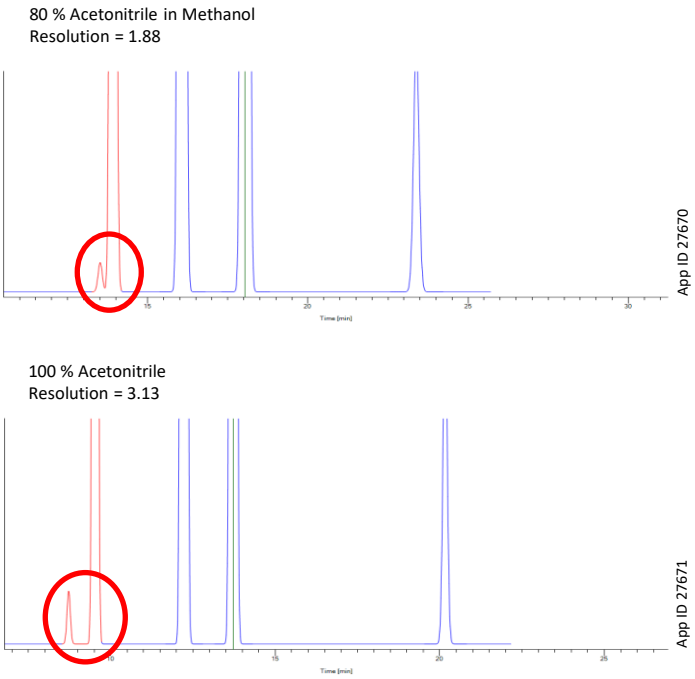


Figure 5 . Cont'd Effect of Strong Solvent (Acetonitrile Concentration) on a Critical Pair (1st and 2nd Peak). Resolution by DryLab® 4 Prediction.



Conclusion

The application of Analytical Quality by Design (AQbD) principles was integral to the development of a stability-indicating method for the Valsartan and Sacubitril tablet. This was executed through the utilization of DryLab AQbD software on the Waters® ACQUITY Arc® HPLC instrument. The exploration involved the assessment and the impacts of column chemistry, temperature, pH, organic modifier, and gradient slope on both selectivity and resolution.

Initial screening was performed to select the column providing the best separation of the Valsartan and Sacubitril peaks. Amongst the screened columns, the Kinetex™ F5 column provided the best separation and peak shape because of its unique selectivity. This column was finalized for further AQbD analysis-based optimization in terms of column temperature, gradients, pH, and mobile phase composition. The initial AQbD experiments indicated that there was no significant impact of pH and temperature on the resolution, but mobile phase composition and gradient slope significantly impacted the overall separation. In this technical note we have highlighted the use of an AQbD approach to optimize the mobile phase composition for achieving the best resolution.

The result of such an approach was a method configuration on a Kinetex F5 column characterized by a column temperature of 40 °C, a strong solvent composition of 100 % acetonitrile and 10 mM Potassium Dihydrogen Phosphate Buffer, pH of 2.5. The systematic AQbD-based approach profoundly enriched the understanding of method variables, thereby substantially reducing the risk of setbacks during method validation and transfer. This approach yielded a markedly superior and robust method in significantly less time on the Kinetex F5 column.

Kinetex™ Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
F5	00B-4724-E0	00D-4724-E0	00F-4724-E0	00G-4724-E0	AJ0-9320
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
HILIC	—	—	00F-4606-E0	00G-4606-E0	AJ0-8772

for 4.6 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Phenex™ Syringe Filter Ordering Information

	4 mm Diameter for ≤ 2 mL sample volumes		**15 mm Diameter** for 2 – 10 mL sample volumes		**25 – 30 mm Diameter** for 11 – 100 mL sample volumes	
Membrane Type/Size	**Part No.**	**Unit**	**Part No.**	**Unit**	**Part No.**	**Unit**
0.20 µm						
Phenex-RC (Regenerated Cellulose)	AF0-3203-12 AF0-3203-52	100/pk 500/pk	AF0-2203-12 AF0-2203-52	100/pk 500/pk	AF0-8203-12 AF0-8203-52	100/pk 500/pk
Phenex-PES [2] (Polyethersulfone)	— —	— —	— —	— —	AF0-8208-12 AF0-8208-52	100/pk 500/pk
Phenex-PTFE (Polytetrafluoroethylene)	AF0-3202-12 AF0-3202-52	100/pk 500/pk	AF0-2202-12 AF0-2202-52	100/pk 500/pk	AF0-1202-12 AF0-1202-52	100/pk 500/pk
Phenex-NY (Nylon)	AF3-3207-12 AF3-3207-52	100/pk 500/pk	AF0-2207-12 AF0-2207-52	100/pk 500/pk	AF0-1207-12 AF0-1207-52	100/pk 500/pk
Phenex-GF/NY [1] (Glass Fiber/Nylon)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a Nylon (NY) membrane. Excellent for filtration of particle-laden samples, such as foods and beverages, environmental, biofuels, and dissolution samples. Use less hand pressure to filter even the most difficult samples. Outlet connection is luer lock.				AF0-1A47-12 AF0-1A47-52	100/pk 500/pk
Phenex-PVDF (Polyvinylidene Fluoride)	— —	— —	AF6-5206-12 AF6-5206-52	100/pk 500/pk	AF6-6206-12 AF6-6206-52	100/pk 500/pk
Phenex-GF/PVDF (Glass Fiber/Polyvinylidene Fluoride)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a PVDF membrane. The hydrophilic PVDF membrane provides high flow rates and through- put, low extractables and broad chemical compatibility. This membrane binds less protein than nylon or PTFE membranes.				AF6-6C06-12 AF6-6C06-52	100/pk 500/pk
Phenex-CA [3] (Cellulose Acetate)	— —	— —	— —	— —	AF0-8204-12 AF0-8204-52	100/pk 500/pk
Phenex-GF/CA [1,2,3] (Glass Fiber/Cellulose Acetate)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a CA membrane. Excellent for filtration of tissue culture media, general biological sample filtration and clarification. Outlet connection is luer lock.				AF0-8A09-12 AF0-8A09-52	100/pk 500/pk

1. Glass fiber filters are 28 mm diameter and made of borosilicate. They will remove 90% of all particles > 1.2 µm.
2. Housing material is methacrylate butadiene styrene (MBS) polymerisate. Also known as Cyrolite®.
3. Cellulose acetate is surfactant-free.

Above syringe filters are non-sterile. Housing is made of medical-grade polypropylene (PP). Luer lock inlet/slip outlet connections unless otherwise indicated. Additional dimensions and membrane types are available, including sterile filters. Please contact your local Phenomenex technical consultant or distributor for availability or assistance.

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