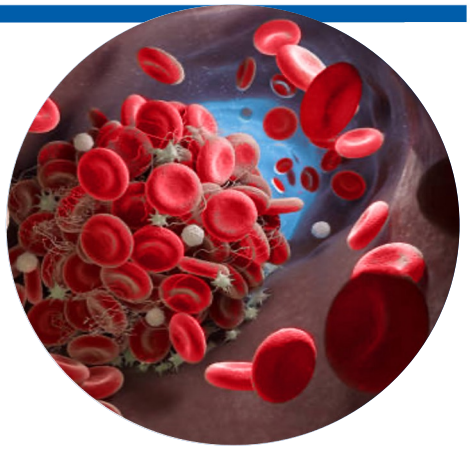

TN-1413

Apixaban Assay, Organic Impurities and Limit of Apixaban Related Compounds -H, -G, and -F per USP monograph

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Introduction

Apixaban is an anticoagulant medication used to treat and prevent blood clots and stroke in people with nonvalvular atrial fibrillation through directly inhibiting factor Xa.

The USP monograph is available for Apixaban Assay, Organic Impurities, and Related Compounds. The column mentioned in the monograph for the determination of Assay and Limit of Apixaban Related compound-H, Apixaban Related compound-G and Apixaban Related compound-F is L1, 250 x 4.6 mm, 5 µm packing. The column mentioned in the monograph for determination of Organic Impurities is L7 column, 250 x 4.6 mm, 5 µm packing.

In this technical note, we demonstrate the usefulness of the fully porous Luna C18(2), the thermally modified Luna Omega C18, and the superficially porous Kinetex C18 columns as alternate columns in determination of Assay and Limit of Apixaban Related compound-H, Apixaban Related compound-G and Apixaban Related compound-F per Apixaban USP monograph. For the separation of Organic Impurities in Apixaban, the fully porous Luna C8(2) and the superficially porous Kinetex C8 were evaluated.

System suitability for Apixaban assay is a tailing factor no more than (NMT) 2.0 and a %RSD of NMT 0.73 % from the Standard Solution. For Organic Impurities, system suitability is a resolution no less than (NLT) 2.0 between Apixaban Related Compound-D and Related Compound-E from the System Suitability Solution, a tailing factor NMT 2.0 and a %RSD NMT 5.0 % from the Standard Solution, and a Signal-to-Noise ratio (S/N) NLT 10 from the Sensitivity Solution. The system suitability for the Limits of the Related Compounds is a resolution NLT 1.5 between Related Compound-H and Related Compound-G, and between Related Compound-G and Related Compound-F, and a %RSD NMT 5.0 % for each impurity from the Standard Solution.

All the solutions were prepared as indicated in the monograph of Apixaban. The following Reference Standards were purchased locally from Clearsynth Labs Limited.

- Apixaban (CAS Number: 503612-47-3)
- Apixaban Related Compound-D (CAS Number: 1351611-14-7)
- Apixaban Related Compound-E (CAS Number: 1074365-84-6)
- Apixaban Related Compound-F (CAS Number: 536759-91-8)
- Apixaban Related Compound-G (CAS Number: 881386-12-5)
- Apixaban Related Compound-H (CAS Number: 1421823-20-2)

LC Conditions: Assay

Columns: Luna 5 µm C18(2) (00G-4252-E0)
Luna Omega 5 µm C18 (00G-4785-E0)
Kinetex 5 µm C18 (00G-4601-E0)
Dimensions: 250 mm x 4.6 mm
Mobile Phase: Buffer / Acetonitrile 48:52 v/v
(Buffer: 0.77g Ammonium Acetate in Water)
Diluent Water / Acetonitrile 50:50 v/v
Gradient: Isocratic
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL
Temperature: 45 °C
LC System: Waters Arc HPLC with PDA
Detection: UV @ 280 nm

LC Conditions: Organic Impurities

Columns: Luna 5 µm C8(2) (00G-4249-E0)
Kinetex 5 µm C8 (00G-4608-E0)
Dimensions: 250 mm x 4.6 mm
Mobile Phase: A: 0.1 % Trifluoroacetic Acid in Water
B: Acetonitrile
Diluent Water / Acetonitrile (60:40, v/v)
Gradient:

Time(min)	%B
0	30
10	30
30	70
35	70
40	30
50	30

Flow Rate: 1.0 mL/ min
Injection Volume: 10 µL
Temperature: 25 °C
Autosampler Temperature: 5 °C
LC System: Waters Arc HPLC with PDA
Detection: UV @ 280 nm

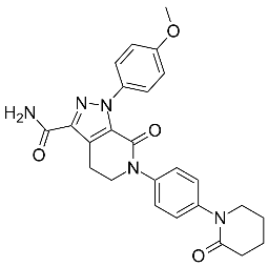
LC Conditions: Limit of Related Compounds

Columns: Luna 5 µm C18(2) (00G-4252-E0)
Luna Omega 5 µm C18 (00G-4785-E0)
Kinetex 5 µm C18 (00G-4601-E0)
Dimensions: 250 mm x 4.6 mm
Mobile Phase: MP-A: Buffer: Acetonitrile 80:20
MP-B: Acetonitrile
(Buffer: 0.77g Ammonium Acetate in Water, adjust pH 6.6 with Ammonia Water or Acetic Acid)
Diluent: Acetonitrile
Gradient:

Time(min)	%B
0	20
5	20
25	80
27	20
40	20

Flow Rate: 1.0 mL/ min
Injection Volume: 10 µL
Temperature: 30 °C
LC System: Waters Arc HPLC with PDA
Detection: UV @ 280 nm

Figure 1. Structure of Apixaban



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Results and Discussion

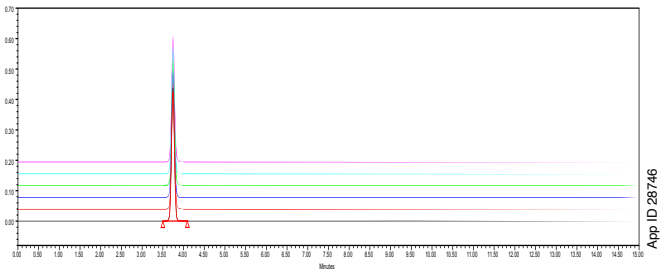
The USP method for assay of Apixaban was successfully verified on the Luna C18(2), the Luna Omega C18, and Kinetex C18 columns (**Figure 2**). All the system suitability parameters for Assay were well within the acceptance criteria in all the columns in terms of % RSD and Tailing. Apixaban peak was observed at similar RT ~3.5 minuteson both the Luna C18(2) column and the Luna Omega C18 column. Whereas on Kinetex C18 column, the peak was eluted at RT ~2.8 minutes. %RSD for Apixaban peak was <0.05 % and provided excellent reproducibility on all columns. In addition, the tailing factor for the Apixaban peak was observed slightly higher on the Kinetex C18 column than the Luna C18(2) and Luna Omega C18 columns.

The USP method for Organic Impurities in Apixaban was successfully verified on the Luna C8(2) and the Kinetex C8 columns. **Figure 3** shows the effective separation of Apixaban Related Compound-D and Apixaban Related Compound-E from Apixaban on the Luna C8(2) and Kinetex C8 columns. **Figure 4** corresponds to chromatograms of the Sensitivity Solution showing the excellent S/N ratio on both the columns. **Figure 5** shows the Apixaban Standard Solution overlay of 6 injections on the Luna C8(2) column and Kinetex C8 column. All the system suitability parameters for Organic Impurities were well within the acceptance criteria on both the columns in terms of %RSD, tailing factor, and resolution. The S/N ratio for Apixaban peak from the Sensitivity Solution was observed 5-8 times of the acceptance criteria in both columns. The reproducibility and % RSD for Apixaban is well within the acceptance criteria in both the columns. The resolution between the Apixaban RC-D and Apixaban RC-E was observed more in Kinetex C8 column than Luna C8(2).

The USP method for Limit of Apixaban Related Compound-H, Related Compound-G, and Related Compound-F was verified on the Luna C18(2), Luna Omega C18, and Kinetex C18 columns. All the system suitability parameters for Limit of Apixaban Related Cpompound-H, Related Compound-G, and Related Compound-F were well within the acceptance criteria on all the columns in terms of %RSD and resolution. The reproducibility and %RSD for all the impurities was well within the acceptance criteria on all the columns. The retention times and the resolution observed between the Apixaban Related Compound-G and Apixaban Related Compound-F were relatively similar on the Luna C18(2) column and the Luna Omega C18 as shown in **Figure 6**. Additionally, the reproducibility of the method was confirmed through six replicate injections with the % RSD <0.5 % for the Luna C18(2) column and Luna Omega C18 column. The Kinetex C18 column showed lower resolution compared to the Luna C18(2) and Luna Omega C18 columns.

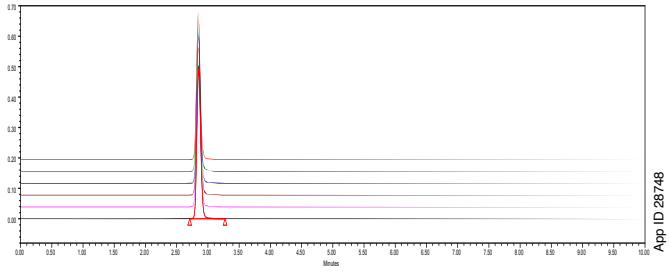
Figure 2. Standard Solution – Assay

Luna 5 µm C18(2) Column



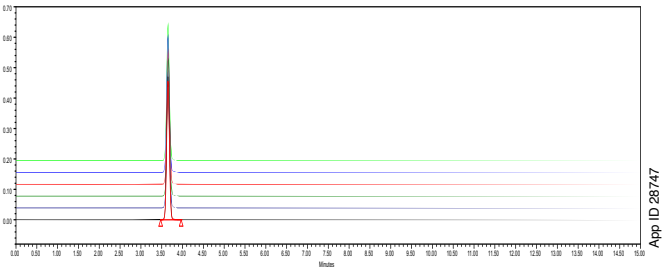
Peak No.	Analyte	Retention Time	Area	USP Tailing	USP Plate Count	Area % RSD (N=6)
1	Apixaban	3.743	2125863	1.02	12712	0.05

Kinetex 5 µm Polar C18 Column



Peak No.	Analyte	Retention Time	Area	USP Tailing	USP Plate Count	Area % RSD (N=6)
1	Apixaban	2.853	2125052	1.12	9966	0.04

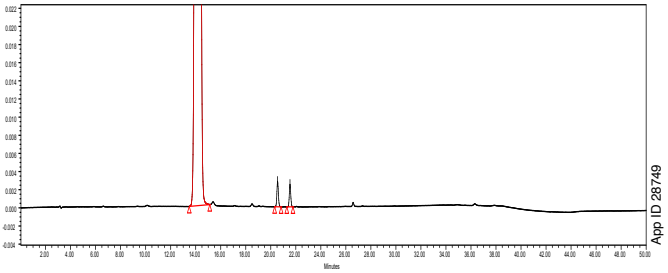
Luna Omega 5 µm C18 Column



Peak No.	Analyte	Retention Time	Area	USP Tailing	USP Plate Count	Area % RSD (N=6)
1	Apixaban	3.651	2127201	1.03	13884	0.04

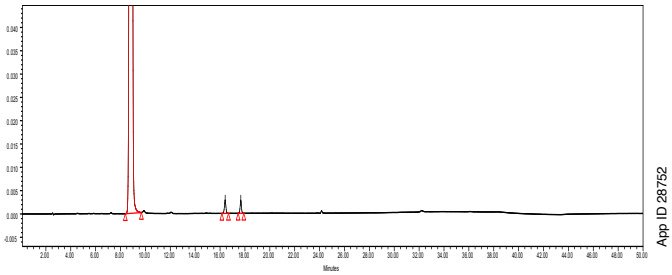
Figure 3. System Suitability Solution – Organic Impurities

Luna 5 µm C8(2) Column



Peak No.	Analyte	Retention Time	Area	% Area	RT Ratio	USP Resolution
1	Apixaban	14.150	15292548	99.71		
2	Apixaban Related Compound-D	20.545	23597	0.15	1.452	19.01
3	Apixaban Related Compound-E	21.537	20322	0.13	1.522	4.45

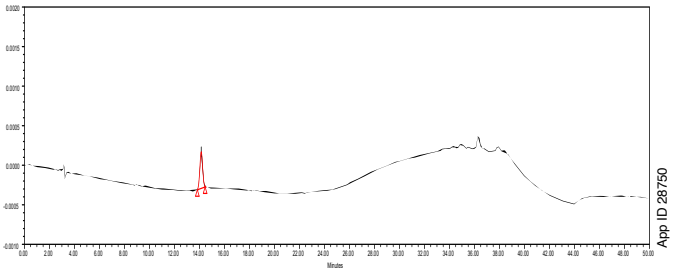
Kinetex 5 µm C8 Column



Peak No.	Analyte	Retention Time (min.)	Area	% Area	RT Ratio	USP Resolution
1	Apixaban	8.834	15393949	99.72		
2	Apixaban Related Compound-D	16.409	23332	0.15	1.858	32.69
3	Apixaban Related Compound-E	17.668	20478	0.13	2.000	6.06

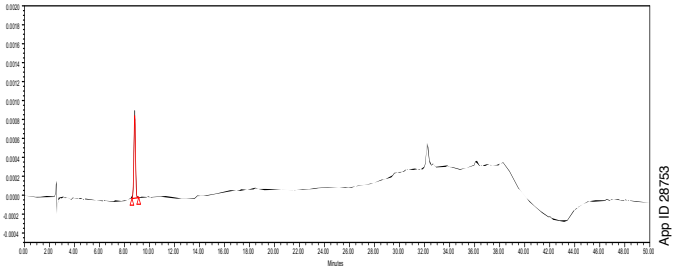
Figure 4. Sensitivity Solution – Organic Impurities

Luna Omega 5 µm C8(2) Column



Peak No.	Analyte	Retention Time	Area	Height	USP S/N
1	Apixaban	14.147	7233	464	67.18

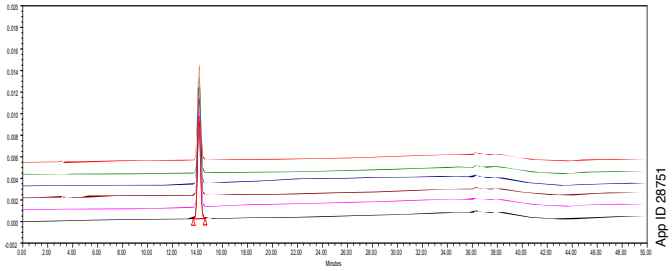
Kinetex 5 µm C8 Column



Peak No.	Analyte	Retention Time	Area	Height	USP S/N
1	Apixaban	8.822	7951	874	88.28

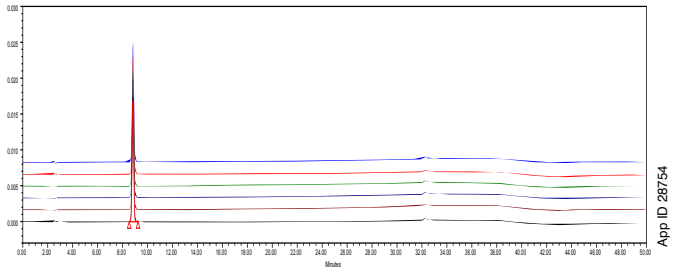
Figure 5. Standard Solution – Organic Impurities

Luna Omega 5 µm C8(2) Column



Peak No.	Analyte	Retention Time	Area	USP Tailing	USP Plate Count	Area % RSD (N=6)
1	Apixaban	14.119	150939	1.04	16430	0.15

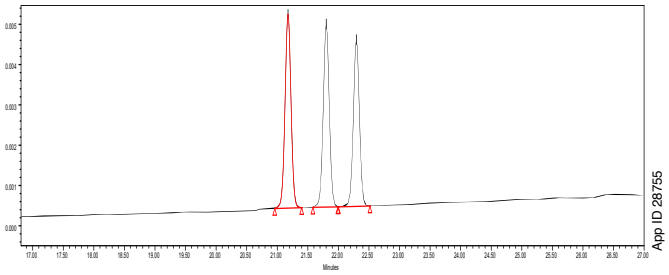
Kinetex 5 µm C8 Column



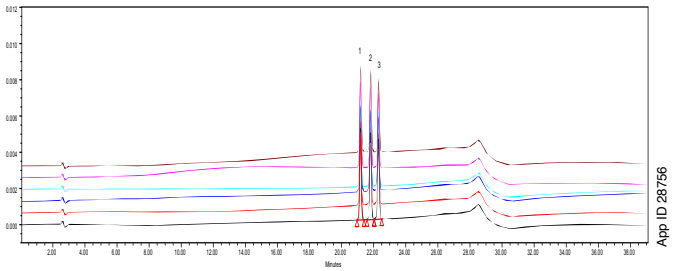
Peak No.	Analyte	Retention Time	Area	USP Tailing	USP Plate Count	Area % RSD (N=6)
1	Apixaban	8.831	153121	0.99	21685	0.07

Figure 6. Standard Solution – Limit of Related Compounds

Luna 5 µm C18(2) Column

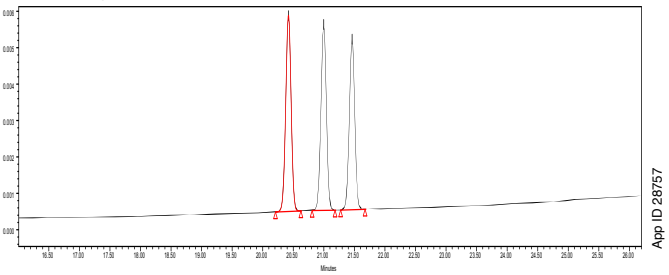


Luna 5 µm C18(2) Column – Overlay Chromatogram

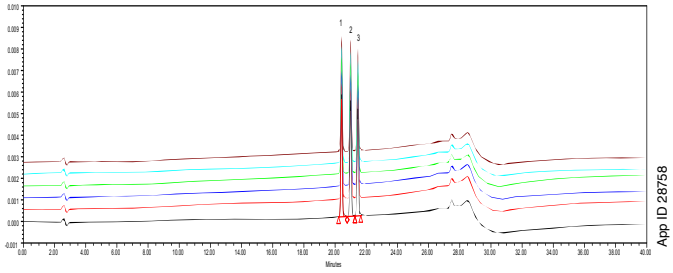


Peak No.	Analyte	Retention Time	Area	USP Resolution	Area % RSD (N=6)
1	Apixaban Related Compound-H	21.176	32948		0.25
2	Apixaban Related Compound-G	21.802	31286	3.41	0.33
3	Apixaban Related Compound-F	22.295	28612	2.68	0.22

Luna Omega 5 µm C18 Column

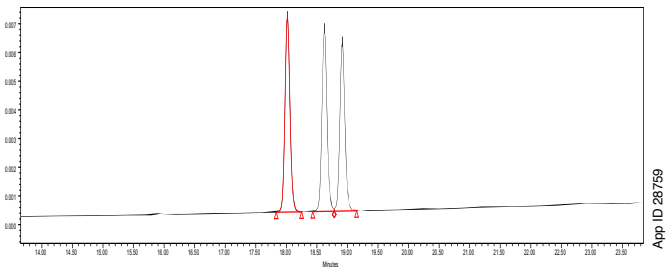


Luna Omega 5 µm C18 Column – Overlay Chromatogram

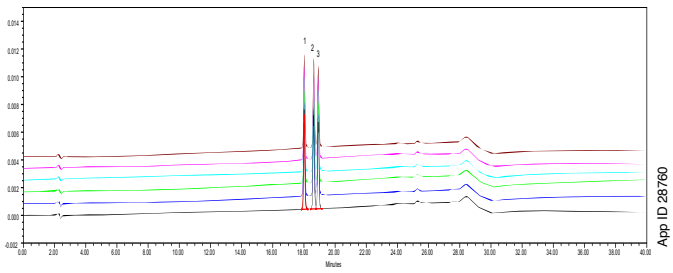


Peak No.	Analyte	Retention Time	Area	USP Resolution	Area % RSD (N=6)
1	Apixaban Related Compound-H	20.421	33452		0.53
2	Apixaban Related Compound-G	20.998	31837	3.45	0.37
3	Apixaban Related Compound-F	21.463	29210	2.77	0.30

Kinetex 5 µm C18 Column



Kinetex 5 µm C18 Column – Overlay Chromatogram



Peak No.	Analyte	Retention Time	Area	USP Resolution	Area % RSD (N=6)
1	Apixaban Related Compound-H	18.017	39277		0.37
2	Apixaban Related Compound-G	18.622	36870	3.94	0.48
3	Apixaban Related Compound-F	18.916	34425	1.90	0.48

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Conclusion

The determination of Assay and Limit of Apixaban Related compound-H, Apixaban Related compound-G and Apixaban Related compound-F were verified on a fully porous Luna C18(2), a thermally modified Luna Omega C18, and a superficially porous Kinetex C18 column as per Apixaban USP monograph. All three columns showed excellent reproducibility for both methods. All the system suitability parameters were well within the acceptance criteria for the methods on all three columns. Based on the experimental findings, all three columns can serve as an alternative for the analysis of Assay and Limit of Apixaban Related compound-H, Apixaban Related compound-G and Apixaban Related compound-F in accordance with the USP monograph.

Additionally, the effective separation of organic impurities in Apixaban as per USP monograph verified using a fully porous Luna C8(2) and a superficially porous Kinetex C8 column. S/N for Apixaban peak from the Sensitivity Solution was observed 5-8 times of the acceptance criteria in both columns and observed a 2 times greater peak height in the Kinetex C8 column. Based on the experimental findings, both the columns can serve as an alternative for the analysis of Apixaban organic impurities in accordance with the USP monograph.

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