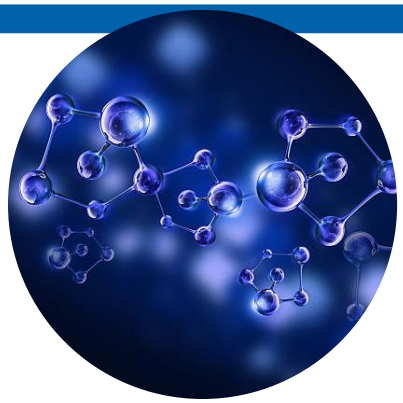

TN-1340

Separation of Teriflunomide and its Organic Impurities per the Proposed USP Monograph in PF 48(5)

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Introduction

Teriflunomide is a pyrimidine synthesis inhibitor that inhibits the function of specific immune cells that have been implicated in Multiple Sclerosis (MS). It is related to Leflunomide, a drug used to treat rheumatoid arthritis. This study for Teriflunomide and its organic impurities is based on the newly proposed USP monograph where an end-capped octadecylsilyl silica gel stationary phase is used under gradient conditions. Because there is no existing USP monograph for this drug substance, a new monograph based on validated methods of analysis was proposed in PF 48(5). In this technical note, we report the separation of Teriflunomide and its related organic impurities using a Kinetex™ 2.6 µm C18 column compared to a Ascentis® Express 2.7 µm C18 column originally used in the proposed monograph.

System suitability per USP Monograph for the Teriflunomide Assay requires a tailing factor no more than (NMT) 2.0 and a percent relative standard deviation (%RSD) of NMT 0.85 % for six replicate injections. System suitability per USP Monograph for the Teriflunomide Related Organic Impurities requires resolution no less than (NLT) 1.5 between Teriflunomide related Compound B and Leflunomide Related Compound A, %RSD NMT 5.0 % each for Terflunomide, Teriflunomide Related Compound B, and Leflunomide Related Compound A, and a Signal-to-Noise (S/N) ratio NLT 10.

According to USP General Chapter <621> , the configuration of the equipment employed may significantly alter the resolution, retention time, and relative retentions described. Differences in system dwell volume can have an impact on the results obtained for gradient methods. Monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for analytical procedure development and that actually used for implementation. The system used in this study had a lower dwell volume (0.45 mL) than described in the monograph (1.1 mL)*, so the time points stated in the gradient table must be replaced by the adapted time points calculated using the following equation:

$$t_c = t - \frac{(D - D_0)}{F}$$

t_c = adapted time point (min)
t = time point indicated in the monograph (min)
D = Dwell volume (mL)
D_0 = Dwell volume used for development of the method (mL)
F = flow rate (mL/min)

The adapted time points for the Ascentis Express 2.7 µm C18 column would be:

$$t_c = 2 - \frac{(0.45 - 1.1)}{1}$$

$$t_c = 2.65$$

Each time point in the gradient would be adjusted by +0.65 minutes and is shown in the gradient table.

Adjustments to column dimensions for gradient methods will be allowed provided that the L/dp ratio remains constant or within the range between -25 % to +50 % of the prescribed L/dp ratio indicated in the monograph. In this monograph, the indicated column length was 150 mm, and the particle size was 2.7 µm; therefore, the Kinetex 2.6 µm column used here would be an allowed adjustment. When the particle size is changed, the flow rate requires adjustment because smaller-particle columns will require higher linear velocities for the same performance. The flow rate is adjusted for particle size using the following equation:

$$F_2 = F_1 \, x \, \frac{dc_2^2 \, x \, dp_1}{dc_1^2 \, x \, dp_2}$$

F_1 = flow rate indicated in the monograph (mL/min)
F_2 = adjusted flow rate (mL/min)
dc_1 = internal diameter of the column indicated in the monograph (mm)
dc_2 = internal diameter of the column used (mm)
dp_1 = particle size indicated in the monograph (µm)
dp_2 = particle size of the column used (µm)

The adjusted flow rate for the Kinetex 2.6 µm column would be:

$$F_2 = 1 \, x \, \frac{4.6^2 \, x \, 2.7}{4.6^2 \, x \, 2.6}$$

$$F_2 = 1.04$$

A change in column dimensions, and thus in column volume, impacts the gradient volume which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume. The new gradient time for each gradient segment can be calculated using the following equation:

$$t_{G2} = t_{G1} \, x \, \left(\frac{F_1}{F_2}\right) \, x \, \left(\frac{L_2 \, x \, dc_2^2}{L_1 \, x \, dc_1^2}\right)$$

t_{G1} = gradient time indicated in the monograph, or adjusted for dwell volume (min)
t_{G2} = adjusted gradient time (min)
F_1 = flow rate indicated in the monograph (mL/min)
F_2 = adjusted flow rate (mL/min)
L_1 = column length indicated in the monograph (mm)
L_2 = new column length (mm)
dc_1 = internal diameter of the column indicated in the monograph (mm)
dc_2 = internal diameter of the column used (mm)

For the second gradient segment, the adjusted gradient time would be:

$$t_{G2} = 2.65 \, x \, \left(\frac{1}{1.04}\right) \, x \, \left(\frac{150 \, x \, 4.6^2}{150 \, x 4.6^2}\right)$$

$$t_{G2} = 2.55$$

A gradient adjustment factor can be calculated and used to determine the new gradient segment times using:

$$Gradient\ adjustment\ factor = \left(\frac{t_{G2}}{t_{G1}}\right)$$

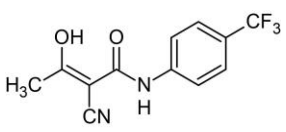
$$Gradient\ adjustment\ factor = 0.96$$

The new gradient timetable for the Kinetex 2.6 µm C18 column is shown in the gradient table.

All requirements for System Suitability for Teriflunomide Assay and Organic Impurities were met by all columns.

All solutions were prepared as indicated in the USP Monograph for Teriflunomide. USP Teriflunomide RS and USP Teriflunomide Related Compound B RS were unavailable, so EDQM Teriflunomide CRS (Catalog No. Y0002247) and EDQM Teriflunomide Impurity B CRS (Catalog No. Y0002254) were substituted and purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 Strasbourg (France). Leflunomide Related Compound A RS (Catalog No. 1357045) was purchased from USP.

Figure 1. Teriflunomide Structure



*Dwell volume noted in Ph. Eur. Teriflunomide monograph using the same column and chromatographic conditions.

LC Conditions

Column: Ascentis® Express 2.7 µm C18, 150 x 4.6 mm
Kinetex™ 2.6 µm C18, 150 x 4.6 mm (00F-4462-E0)

Mobile Phase: A: Acetonitrile / Buffer (10:90, v/v)
B: Buffer / Acetonitrile (10:90, v/v)

Buffer: 3.85 g/L of Ammonium Acetate in Water. Adjust with glacial Acetic Acid to a pH of 5.5.

		Ascentis Express	Kinetex	
Gradient:	**Time (min)**	**Adjusted Time (min)**	**Adjusted Time (min)**	**%B**
	0	0	0	24
	2	2.65	2.55	24
	12	12.65	12.18	77
	13	13.65	13.14	24
	17	17.65	17	24

Flow Rate: 1.0 mL/min (Ascentis Express)
1.04 mL/min (Kinetex)

Injection Volume: 5 µL

Temperature: 40 °C

Detector: UV @ 249 nm

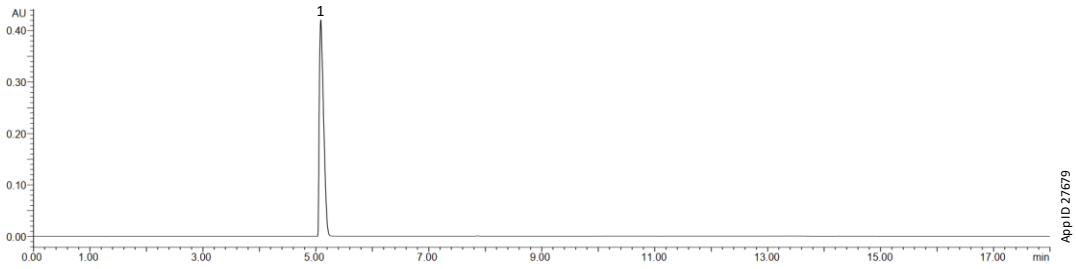
System: Waters® ACQUITY® H-Class UHPLC

Table 1. Preparation of Solutions

Solution	Composition
Diluent	Acetonitrile / Buffer (80:20, v/v) Buffer: 3.85 g/L of Ammonium Acetate in Water. Adjust with glacial Acetic Acid to a pH of 5.5.
Standard Solution (Assay)	0.2 mg /mL of *Teriflunomide CRS* in Diluent.
Sample Solution (Assay and Organic Impurities)	Same as Standard Solution.
Sensitivity Solution (Organic Impurities)	0.1 µg/mL of *Teriflunomide CRS* in Diluent.
Standard Stock Solution A (Organic Impurities)	0.04 mg/mL of *USP Leflunomide Related Compound A RS* in Acetonitrile. Sonicate to dissolve.
Standard Stock Solution B (Organic Impurities)	0.04 mg/mL of *Teriflunomide Related Compound B CRS* in Acetonitrile. Sonicate to dissolve.
Standard Solution (Organic Impurities)	0.2 µg/mL each of *Teriflunomide CRS*, *Teriflunomide Related Compound B CRS*, and 0.02 µg/mL of *USP Leflunomide Related Compound A RS*, prepared as follows: Transfer a suitable amount of *Teriflunomide CRS* to a suitable volumetric flask. Add Diluent equivalent to 20 % of the flask volume and sonicate to dissolve. Add a suitable volume of **Standard Stock Solution A** and Standard Stock Solution B and dilute with Diluent to volume.

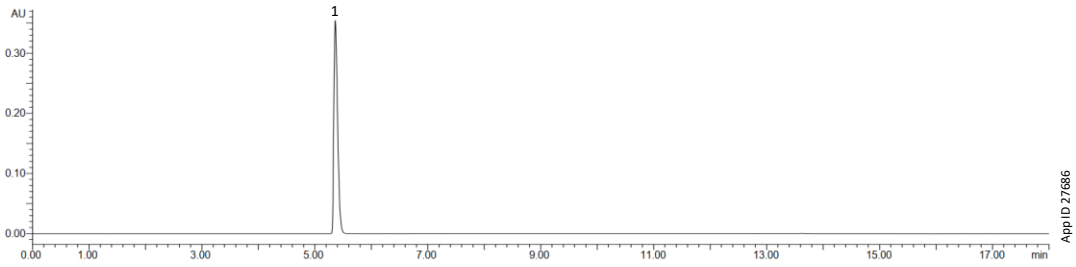
Figure 2. Standard Solution - Assay

Ascentis Express 2.7 µm C18, 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Symmetry Factor
1	Teriflunomide	5.09	1659767	0.41	339289.67	1.59
N = 6 Injections						

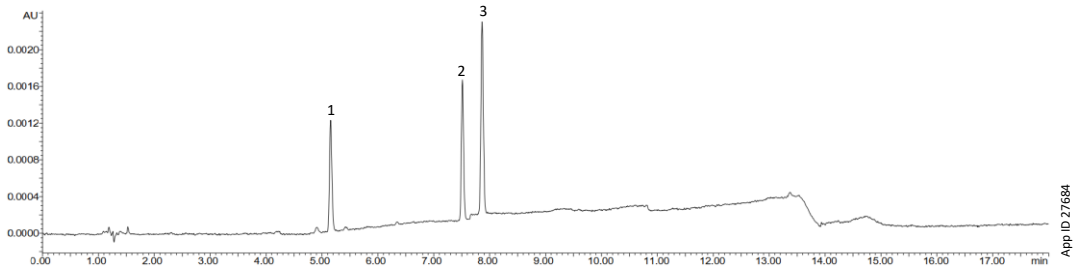
Kinetex 2.6 µm C18, 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Symmetry Factor
1	Teriflunomide	5.36	1638156.17	0.44	355644.67	1.51
N = 6 Injections						

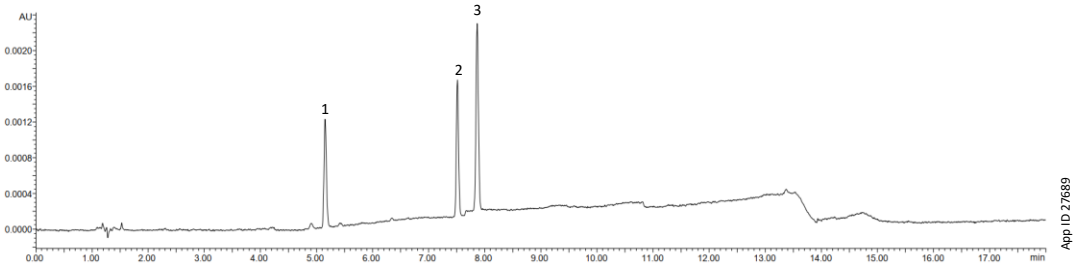
Figure 3 . Standard Solution – Organic Impurities

Ascentis® Express 2.7 µm C18, 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Resolution	Symmetry Factor
1	Teriflunomide	5.157	3723.5	0.352	-	1.14
2	Teriflunomide Related Compound B	7.513	4246.167	1.041	4.498	1.098
3	Leflunomide Related Compound A	7.866	6435.33	0.759		1.067
N = 3 Injections						

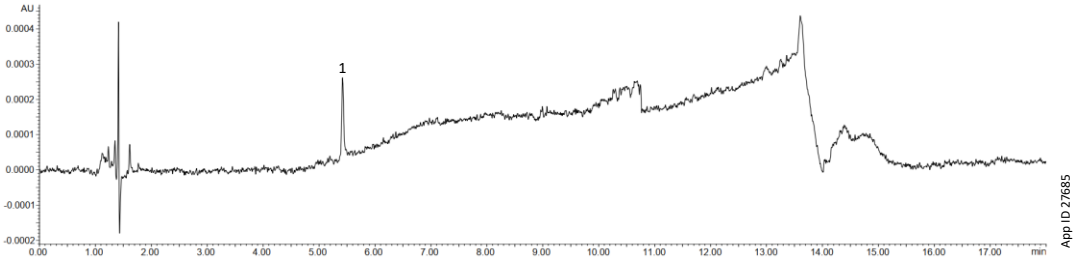
Kinetex™ 2.6 µm C18, 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Resolution	Symmetry Factor
1	Teriflunomide	5.157	3723.5	0.352	-	1.14
2	Teriflunomide Related Compound B	7.513	4241	1.067	4.502	1.097
3	Leflunomide Related Compound A	7.866	6410.67	0.682		1.068
N = 3 Injections						

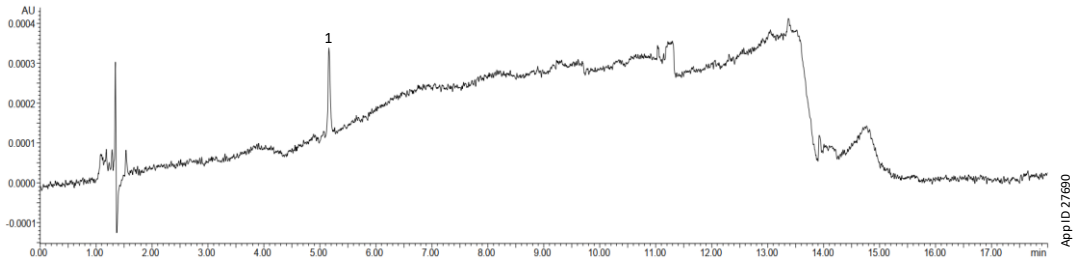
Figure 4 . Sensitivity Solution – Organic Impurities

Ascentis® Express 2.7 µm C18, 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Area	S/N Ratio	Symmetry Factor
1	Teriflunomide	5.421	608	16.28	1.24

Kinetex™ 2.6 µm C18, 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Area	S/N Ratio	Symmetry Factor
1	Teriflunomide	5.162	593	15.42	1.08

Conclusions

The USP General Chapter <621> now allows for adjustment of gradient methods. However, differences in system dwell volume and column dimensions (L and dp) must be properly accounted for to ensure performance of the method per the original monograph. Initial attempts to replicate the proposed USP monograph method for Teriflunomide using the original column (Ascentis Express 2.7 µm C18) resulted in failure to meet system suitability requirements. After accounting for the lower dwell volume of the system used in this study by increasing the isocratic hold time and subsequent gradient time points, system suitability requirements were met with the original column.

Further adjustments to the flow rate and gradient timetable were required when using the Kinetex 2.6 µm C18 column, with the L/dp ratio for the Kinetex column well within the allowable adjustment per USP <621>. Comparable results to the original column were obtained using the Kinetex 2.6 µm C18 column, demonstrating that this column is a suitable alternative for the proposed USP Teriflunomide monograph for Assay and Organic Impurities. In this technical note we have illustrated the various adjustments that must be made to successfully adjust for system differences and column dimensions.

Kinetex™ Ordering Information

2.6 µm Analytical Columns (mm)							SecurityGuard™ ULTRA Cartridges*
Phases	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
EVO C18	00A-4725-E0	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	00G-4725-E0	AJ0-9296
PS C18	00A-4780-E0	00B-4780-E0	—	00D-4780-E0	00F-4780-E0	00G-4780-E0	AJ0-8949
Polar C18	00A-4759-E0	00B-4759-E0	—	00D-4759-E0	00F-4759-E0	—	AJ0-9530
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	—	AJ0-9207
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	—	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	—	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	—	AJ0-8770
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	—	AJ0-8772
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	—	AJ0-8774
F5	00A-4723-E0	00B-4723-E0	—	00D-4723-E0	00F-4723-E0	—	AJ0-9320

for 4.6 mm ID

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

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No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

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