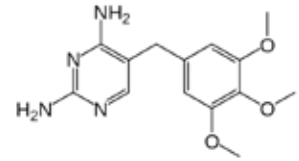


# APPLICATION

## Trimethoprim USP

### Overview

The related substances test of the USP monograph outlines the separation of all relevant impurities from Trimethoprim. This method was studied and improvements were made to provide higher resolution (Rs) and a faster separation time within allowable adjustments.



**Trimethoprim**

### USP Monograph: Trimethoprim Details

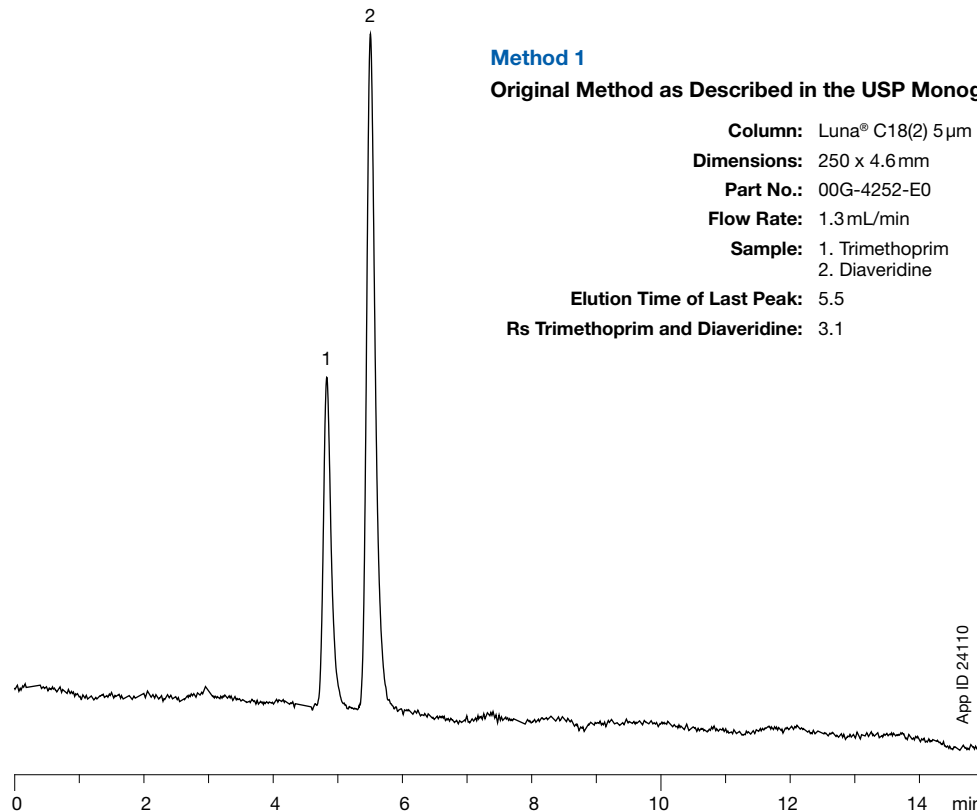
<b>Buffer Solution</b>	Prepare a 10mM sodium perchlorate solution in water, adjust with phosphoric acid to pH 3.6, and mix
<b>Resolution Solution</b>	Dissolve accurately weighed quantities of USP Trimethoprim RS and Diaveridine; and dilute quantitatively with mobile phase to obtain a solution having known concentrations of about 10 µg per mL and 5 µg per mL, respectively
<b>Test Solution</b>	Transfer about 25.0 mg of Trimethoprim to a 25 mL volumetric flask, dissolve in and dilute with mobile phase to volume, and mix

### Column

<b>Size</b>	250 x 4.6 mm
<b>Stationary Phase</b>	L1: Octadecyl silane chemically bonded to porous or non-porous silica or ceramic microparticles, 1.5 to 10 µm in diameter, or a monolithic rod
<b>Mobile Phase</b>	Prepare a filtered and degassed mixture of Buffer Solution and methanol (7:3)
<b>Flow Rate</b>	1.3 mL/min
<b>Detection</b>	Spectrophotometer @ 280 nm
<b>Injection</b>	20 µL

### System Suitability

Minimum resolution of 2.5 between Trimethoprim and Diaveridine  
Relative standard deviation for replicate injections is not more than 2.0 %



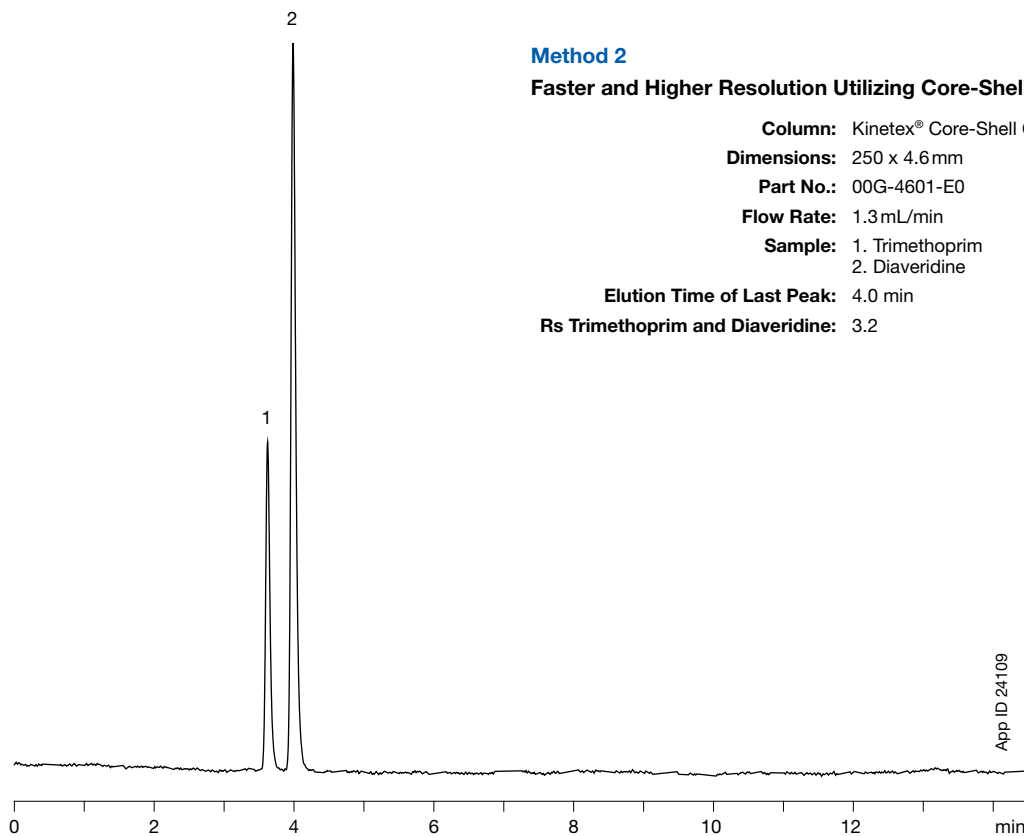
### Method 1

#### Original Method as Described in the USP Monograph

<b>Column:</b>	Luna <sup>®</sup> C18(2) 5µm Fully Porous
<b>Dimensions:</b>	250 x 4.6 mm
<b>Part No.:</b>	00G-4252-E0
<b>Flow Rate:</b>	1.3 mL/min
<b>Sample:</b>	1. Trimethoprim 2. Diaveridine
<b>Elution Time of Last Peak:</b>	5.5
<b>Rs Trimethoprim and Diaveridine:</b>	3.1

App ID 24110





**Method 2**

**Faster and Higher Resolution Utilizing Core-Shell Technology**

**Column:** Kinetex<sup>®</sup> Core-Shell C18 5 $\mu$ m

**Dimensions:** 250 x 4.6 mm

**Part No.:** 00G-4601-E0

**Flow Rate:** 1.3 mL/min

**Sample:** 1. Trimethoprim  
2. Diaveridine

**Elution Time of Last Peak:** 4.0 min

**Rs Trimethoprim and Diaveridine:** 3.2

**Adjustments for Meeting System Suitability**

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1	Method 2
<b>Mobile Phase pH</b>	$\pm 0.2$ units	As specified	As specified
<b>Concentration of Salts in Buffer</b>	$\pm 10\%$	As specified in Monograph Details Table	As specified
<b>Composition of the Mobile Phase</b>	$\pm 30\%$ Relative; cannot exceed $\pm 10\%$ Absolute change; cannot be reduced to zero	As specified in Monograph Details Table	As specified
<b>Wavelength of Detector</b>	No deviations permitted	280 nm (as specified)	As specified
<b>Injection Volume</b>	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements	20 $\mu$ L (as specified)	As specified
<b>Column Temperature</b>	$\pm 10^\circ\text{C}$	Ambient (as specified)	As specified
<b>Stationary Phase</b>	No change of the identity of the substituent permitted (e.g. no replacement of C18 by C8)	L1 (as specified)	As specified
<b>Column Length</b>	Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%*	250 mm (as specified)	As specified
<b>Column Internal Diameter</b>	Can be adjusted so long as linear velocity is maintained	4.6 mm (as specified)	As specified
<b>Particle Size</b>	Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%*	5 $\mu$ m (as specified)	As specified
<b>Flow Rate</b>	$\pm 50\%$ (at given ID)	1.3 mL/min (as specified)	As specified

\*Alternatively (as for the application of particle size adjustment to superficially porous particles), other L/dp combinations can be used provided that the number of theoretical plates (N) is within -25% to +50%

## Kinetex® Ordering Information

5 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges <sup>†</sup>
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4601-AN	00B-4601-AN	00D-4601-AN	00F-4601-AN	AJO-8782 for 2.1 mm ID

5 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges <sup>†</sup>
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00B-4601-YO	00D-4601-YO	00F-4601-YO	AJO-8775 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges <sup>†</sup>
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJO-8768 for 4.6 mm ID

5 µm Semi-Preparative Columns (mm)			SecurityGuard SemiPrep Cartridges <sup>***</sup>
Phases	150 x 10	250 x 10	3/pk
C18	00F-4601-N0	00G-4601-N0	AJO-9278 for 9-16 mm ID

<sup>†</sup>SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

<sup>\*\*\*</sup>SemiPrep SecurityGuard Cartridges require holder, Part No.: AJO-9281

## Luna® Ordering Information

5 µm Microbore and Minibore Columns (mm)								SecurityGuard™ Cartridges (mm)
Phases	50 x 1.0	150 x 1.0	250 x 1.0	30 x 2.0	50 x 2.0	150 x 2.0	250 x 2.0	4 x 2.0*
C18(2)	00B-4252-A0	00F-4252-A0	00G-4252-A0	00A-4252-B0	00B-4252-B0	00F-4252-B0	00G-4252-B0	AJO-4286 for ID: 2.0-3.0 mm

5 µm MidBore and Analytical Columns (mm)								SecurityGuard™ Cartridges (mm)	
Phases	30 x 3.0	50 x 3.0	150 x 3.0	250 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	4 x 2.0*	4 x 3.0*
C18(2)	00A-4252-Y0	00B-4252-Y0	00F-4252-Y0	00G-4252-Y0	00A-4252-E0	00B-4252-E0	00C-4252-E0	AJO-4286 for ID: 2.0-3.0 mm	AJO-4287 3.2-8.0 mm

5 µm Analytical and Semi-Prep Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	100 x 4.6	150 x 4.6	250 x 4.6	250 x 10	4 x 3.0*	10 x 10 <sup>‡</sup>
C18(2)	00D-4252-E0	00F-4252-E0	00G-4252-E0	00G-4252-N0	AJO-4287 for ID: 3.2-8.0 mm	AJO-7221 9-16 mm

\*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJO-4282

<sup>‡</sup>SemiPrep SecurityGuard Cartridges require holder, Part No.: AJO-9281



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to a competing product of the same particle size, similar phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.



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