

# Separation of Rivaroxaban and its Chiral Impurities per USP Monograph

Alyssa Nodland and Bryan Tackett, PhD  
Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA

## Overview

Rivaroxaban is a factor Xa inhibitor, an anticoagulant, that decreases the clotting ability of the blood and helps prevent clots from forming in the blood vessels. In this application note, we report the separation of Rivaroxaban and its related chiral impurities using a Lux 5 µm i-Cellulose-5 column compared with a CHIRALPAK 5 µm IC column according to the USP monograph for Rivaroxaban, which specifies a column containing L119 (Cellulose tris-(3,5-dichlorophenylcarbamate)) packing with dimensions of 250 x 4.6 mm.

System suitability per USP Monograph for Rivaroxaban Chiral Impurities is resolution no less than (NLT) 1.5 between the Rivaroxaban R-enantiomer and Rivaroxaban, a percent relative standard deviation (%RSD) of no more than (NMT) 5 %, and a signal-to-noise (S/N) ratio of NLT 10. All system suitability requirements for Rivaroxaban Chiral Impurities were met by both the Lux i-Cellulose-5 and CHIRALPAK IC.

The Lux i-Cellulose-5 column is a suitable substitute for the CHIRALPAK IC column for the USP Rivaroxaban chiral enantiomeric impurities method. The S/N ratio was higher using the Lux i-Cellulose-5 column with a slightly lower resolution, compared to the CHIRALPAK IC column. Problems were encountered throughout the method due to Rivaroxaban not being completely soluble in Acetonitrile. After sitting in the cooled autosampler for a day, precipitate formed in the vials. This clogged the column and resulted in unusual noise. New samples were prepped daily and flushing the column before each run was done to try and avoid this problem.

All solutions were prepared as indicated in the USP Monograph for Rivaroxaban. USP Rivaroxaban RS (Catalog No. 1604530) and USP Rivaroxaban R-Enantiomer RS (Catalog No. 1604541) were purchased from USP.

## LC-UV Conditions

**Columns:** Lux™ 5 µm i-Cellulose-5 ([00G-4756-E0](#))  
CHIRALPAK® 5 µm IC

**Dimensions:** 250 x 4.6 mm

**Mobile Phase:** Acetonitrile

**Flow Rate:** 0.7 mL/min (Isocratic)

**Injection Volume:** 20 µL

**Temperature:** 30 °C

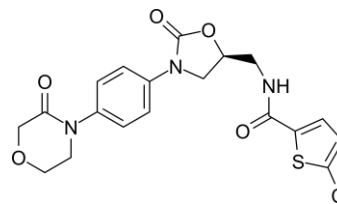
**Detector:** UV @ 254 nm

**System:** Agilent® 1260

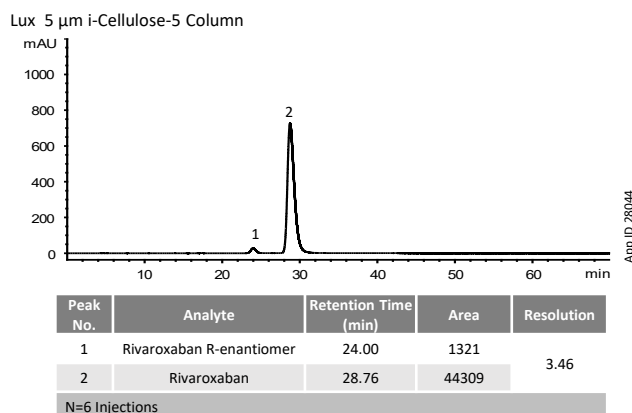
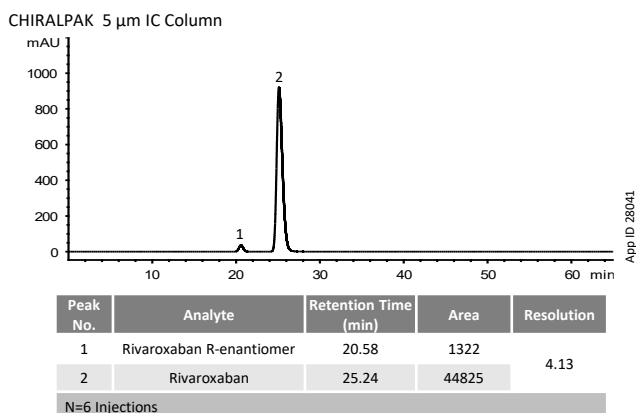
**Table 1.** Preparation of Test and Reference Solutions

Solution	Composition
Standard Solution	0.5 µg/mL of USP Rivaroxaban RS in Acetonitrile.
System Suitability Solution	0.5 mg/mL of USP Rivaroxaban RS and 0.015 mg/mL of USP Rivaroxaban R-Enantiomer RS in Acetonitrile.
Sensitivity Solution	0.25 µg/mL of USP Rivaroxaban R-Enantiomer RS in Acetonitrile.

**Figure 1.** Rivaroxaban Structure.

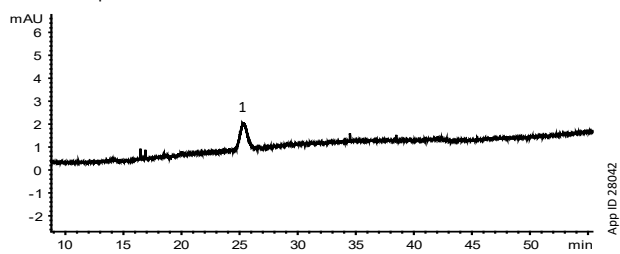


**Figure 2.** System Suitability Solution.



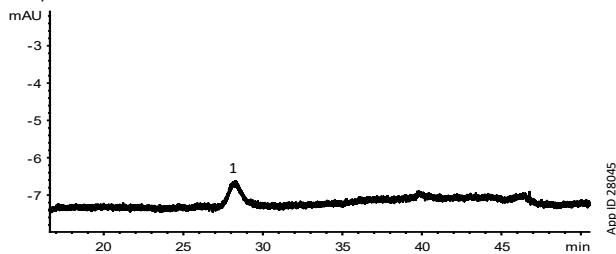
**Figure 3.** Standard Solution.

CHIRALPAK 5 µm IC Column



Peak No.	Analyte	Retention Time (min)	Area	%RSD
1	Rivaroxaban	25.35	54.26	3.48
N=5 Injections				

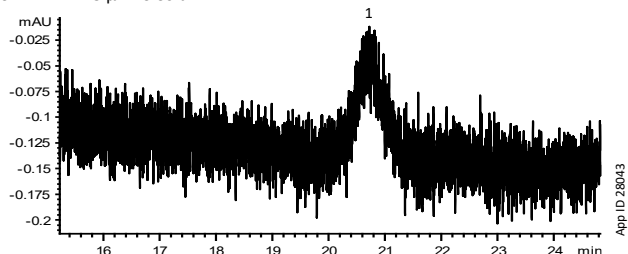
Lux 5 µm i-Cellulose-5 Column



Peak No.	Analyte	Retention Time (min)	Area	%RSD
1	Rivaroxaban	28.55	51.93	3.80
N=5 Injections				

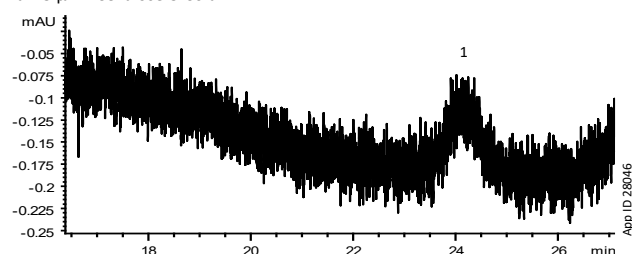
**Figure 4.** Sensitivity Solution.

CHIRALPAK 5 µm IC Column



Peak No.	Analyte	Retention Time (min)	Area	S/N Ratio
1	Rivaroxaban R-enantiomer	20.79	6.32	11.16
N=5 Injections				

Lux 5 µm i-Cellulose-5 Column



Peak No.	Analyte	Retention Time (min)	Area	S/N Ratio
1	Rivaroxaban R-enantiomer	24.33	7.22	12.60
N=5 Injections				



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**Australia**

t: +61 (0)2-9428-6444  
auinfo@phenomenex.com

**Austria**

t: +43 (0)1-319-1301  
anfrage@phenomenex.com

**Belgium**

t: +32 (0)2 503 4015 (French)  
t: +32 (0)2 511 8666 (Dutch)  
beinfo@phenomenex.com

**Canada**

t: +1 (800) 543-3681  
info@phenomenex.com

**China**

t: +86 400-606-8099  
cninfo@phenomenex.com

**Czech Republic**

t: +420 272 017 077  
cz-info@phenomenex.com

**Denmark**

t: +45 4824 8048  
nordicinfo@phenomenex.com

**Finland**

t: +358 (0)9 4789 0063  
nordicinfo@phenomenex.com

**France**

t: +33 (0)1 30 09 21 10  
franceinfo@phenomenex.com

**Germany**

t: +49 (0)6021-58830-0  
anfrage@phenomenex.com

**Hong Kong**

t: +852 6012 8162  
hkinfo@phenomenex.com

**India**

t: +91 (0)40-3012 2400  
indiainfo@phenomenex.com

**Indonesia**

t: +62 21 3952 5747  
indoinfo@phenomenex.com

**Ireland**

t: +353 (0)1 247 5405  
eireinfo@phenomenex.com

**Italy**

t: +39 051 6327511  
italiainfo@phenomenex.com

**Japan**

t: +81 (0) 120-149-262  
jpinfo@phenomenex.com

**Luxembourg**

t: +31 (0)30-2418700  
nlinfo@phenomenex.com

**Mexico**

t: 01-800-844-5226  
tecnicomx@phenomenex.com

**The Netherlands**

t: +31 (0)30-2418700  
nlinfo@phenomenex.com

**New Zealand**

t: +64 (0)9-4780951  
nzinfo@phenomenex.com

**Norway**

t: +47 810 02 005  
nordicinfo@phenomenex.com

**Poland**

t: +48 22 51 02 180  
pl-info@phenomenex.com

**Portugal**

t: +351 221 450 488  
ptinfo@phenomenex.com

**Singapore**

t: 800-852-3944  
sginfo@phenomenex.com

**Slovakia**

t: +420 272 017 077  
sk-info@phenomenex.com

**Spain**

t: +34 91-413-8613  
espinfo@phenomenex.com

**Sweden**

t: +46 (0)8 611 6950  
nordicinfo@phenomenex.com

**Switzerland**

t: +41 (0)61 692 20 20  
swissinfo@phenomenex.com

**Taiwan**

t: +886 (0) 0801-49-1246  
twinfo@phenomenex.com

**Thailand**

t: +66 (0) 2 566 0287  
thaiinfo@phenomenex.com

**United Kingdom**

t: +44 (0)1625-501367  
ukinfo@phenomenex.com

**USA**

t: +1 (310) 212-0555  
info@phenomenex.com

🌐 **All other countries/regions**  
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