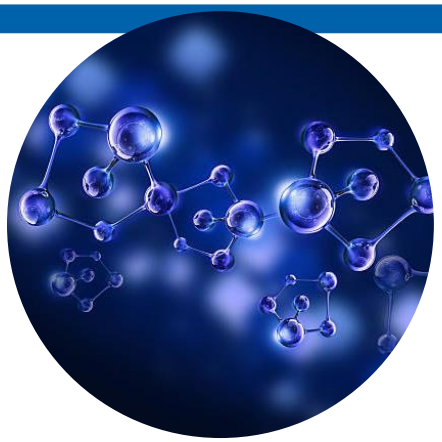


TN-1328

Modernization of USP and Ph. Eur. Method – Irbesartan Organic Impurities/Related Substances

Krishna Chaitanya Routhu[1], Swetha Kotikalapudi[1], Heiko Behr, PhD[2], and Bryan Tackett, PhD[3]

[1]India Phenologix Lab, Phenomenex India, Hitech Defence and Aerospace Park Industrial Area, Mahadeva Kodigehalli, Holbi, Jala Taluka, Bengaluru 562149, India

[2]Phenomenex Ltd., Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany

[3]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Irbesartan is a potent selective angiotensin II type 1 (AT1) receptor antagonist and is used in the treatment of hypertensive patients and diabetic nephropathy. This study for Irbesartan and its related substances is based on USP and Ph. Eur monographs where an L1 (USP) or end-capped octadecylsilyl silica gel (Ph. Eur.) stationary phase is used under isocratic conditions. In this technical note, we demonstrate the potential method improvements that can be achieved within the defined allowable adjustments of chromatographic conditions per the USP and Ph. Eur. monographs using Luna™ C18(2) and Kinetex™ C18 columns.

USP General Chapter <621> and the Ph. Eur. 2.2.46 chromatography chapter have been harmonized and updated from Dec 2022 and July 2022, respectively. The adjustments of chromatographic conditions have suggested the extent to which the various parameters of a chromatographic analysis method may be adjusted without fundamentally modifying the pharmacopoeia analytical procedures. Based on these allowable adjustments several "Methods" were developed and listed in **Table 1**.

System suitability per USP Monograph for Irbesartan Organic Impurities is relative standard deviation (%RSD) no more than (NMT) 2.0 %, and system suitability per Ph. Eur. Monograph 2465 for Irbesartan Related Substances is a minimum resolution between Related Impurity A and Irbesartan of 3.0. NOTE: The USP Irbesartan Assay method uses the same column and isocratic mobile phase conditions; however, the system suitability requirement is for resolution no less than (NLT) 2.0 between Irbesartan and Irbesartan related compound A.

All the reference solutions were prepared as indicated in Ph. Eur. monograph 1649 for Azithromycin. The following certified reference standards (CRS) were 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):

- Y0001166, Irbesartan CRS
- Y0001156, Irbesartan Related Impurity A CRS

Table 1. Different Methods Within Allowable Adjustments of Chromatographic Conditions USP <621> and Ph. Eur. 2.2.46.

Method Parameter	Allowable Adjustments	Method 1 (Monograph Method)	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7
Stationary Phase	No change of the identity of the substituent (e.g., no replacement of C18 by C8); the other Physico-chemical characteristics of the stationary phase (i.e. chromatographic support, surface modification and extent of chemical modification) must be similar; a change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns is allowed provided the above-mentioned requirements are met.	L1 per USP and end-capped octadecylsilyl silica gel per Ph. Eur. (As Specified)	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Column Dimension (particle size and length)	The particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range −25 % to +50 % of the prescribed L/dp ratio.	Length = 250 mm Particle Size = 5 µm L/dp = 50	Length = 250 mm Particle Size = 5 µm L/dp = 50	Length = 250 mm Particle Size = 5 µm L/dp = 50	Length = 150 mm Particle Size = 3µm L/dp = 50	Length = 250 mm Particle Size = 5 µm L/dp = 50	Length = 250 mm Particle Size = 5 µm L/dp = 50	Length = 100 mm Particle Size = 1.7 µm L/dp = 58.8 (Deviation = +17.65 %) (Allowed)
Column Internal Diameter	In the absence of a change in particle size and/or length of the column, the internal diameter of the column may be adjusted.	ID = 4.0 mm (As specified)	ID = 4.6 mm (Allowed)	ID = 4.6 mm (Allowed)	ID = 4.6 mm (Allowed)	ID = 4.6 mm (Allowed)	ID = 4.6 mm (Allowed)	ID = 2.1 mm (Allowed)
Flow Rate	When the particle size is changed, the flow rate requires adjustment. After an adjustment due to a change in column dimensions, an additional change in flow rate of ± 50 % is permitted.	1.0 mL/min (As Specified)	1.0 mL/min (Deviation from linear flow rate after adjustment = -32.3 %) (Allowed)	1.323 mL/min (Linear flow rate adjusted)	0.5 mL/min (Deviation from linear flow rate after adjustment = -45.65 %) (Allowed)	1.0 mL/min (Deviation from linear flow rate after adjustment = -32.3%) (Allowed)	1.323 mL/min (Linear flow rate adjusted)	0.5 mL/min (Deviation from Linear flow rate after adjustment = -38.35 %) (Allowed)
Column Temperature	± 10 °C	25 °C (As Specified)	25 °C (As Specified)	25 °C (As Specified)	25 °C (As Specified)	25 °C (As Specified)	25 °C (As Specified)	25 °C (As Specified)
Minor Solvent Composition	± 30 % relative	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
pH of the Aqueous Content of the Mobile Phase	± 0.2 units	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Concentration of Salts in the Buffer Component of a Mobile Phase	± 10 %	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Detector Wavelength	No adjustment permitted	220 nm (As Specified)	As Specified	As Specified	As Specified	As Specified	As Specified	As Specified
Injection Volume	when the column dimensions are changed, the injection volume adjustment equation may be used for adjusting the injection volume.	10 µL (As specified)	13.2 µL (Calculated injection volume as per the new column dimensions)	13.2 µL (Calculated injection volume as per the new column dimensions)	8.0 µL (Calculated injection volume as per the new column dimensions)	13.2 µL (Calculated injection volume as per the new column dimensions)	13.2 µL (Calculated injection volume as per the new column dimensions)	1.1 µL (Calculated injection volume as per the new column dimensions)

LC Conditions

Column: Luna™ 5 µm C18(2), 250 x 4.0 mm (00G-4252-D0) – Method 1
Luna 5 µm C18(2), 250 x 4.6 mm (00G-4252-E0) – Method 2, 3
Luna 3 µm C18(2), 150 x 4.6 mm (00F-4251-E0) – Method 4
Kinetex™ 5 µm C18, 250 x 4.6 mm (00G-4601-E0) – Method 5, 6
Kinetex 1.7 µm C18, 100 x 2.1 mm (00D-4475-AN) – Method 7

Mobile Phase: Acetonitrile / Buffer pH 3.2 (33:67, v/v)

Buffer: Mix 5.5 mL of Phosphoric Acid and 950 mL of Water, adjust pH to 3.2 with Triethylamine.

Flow Rate: 1.0 mL/min – Method 1, 2, 5
1.323 mL/min – Method 3, 6
1.2 mL/min – Method 4
0.5 mL/min – Method 7

Injection Volume: 10 µL – Method 1
13.2 µL – Method 2, 3, 5, 6
8.0 µL – Method 4
1.1 µL – Method 7

Temperature: 25 °C

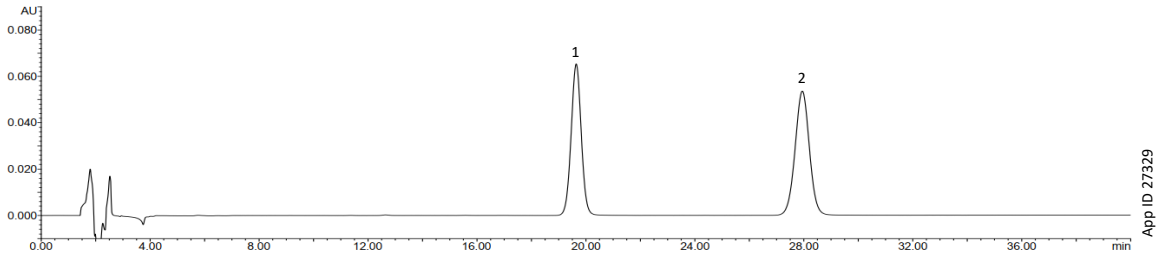
Detector: UV @ 220 nm

System: Waters® ACQUITY Arc® HPLC – Method 1, 2, 3, 4, 5, 6
Waters ACQUITY® H-Class UHPLC – Method 7

Table 1. Preparation of Test and Reference Solutions

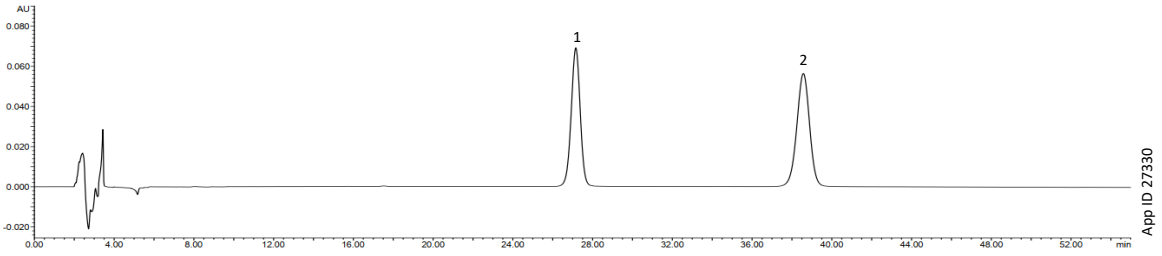
Solution	Composition
System Suitability Solution – Assay (USP)	Dissolve 5 mg of Irbesartan and 5 mg Irbesartan Impurity A CRS in Methanol and dilute to 10 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with Methanol.
Standard Solution – Organic Impurities (USP)	
Reference Solution (b) (Ph. Eur.)	

Figure 1. System Suitability Solution (Reference Solution (b)) for Method 1 on a Luna 5 µm C18(2), 250 x 4.0 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	19.64	1690347	0.49	65352	10.07	1.02
2	Irbesartan	27.95	1952362	0.53	53571		1.00
N = 6 Injections							

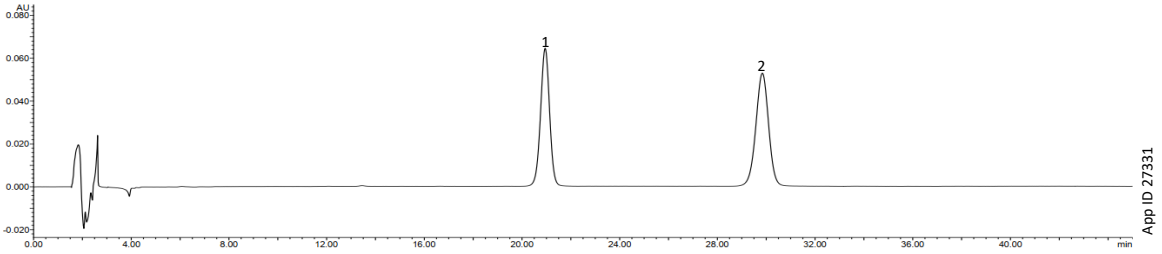
Figure 2. System Suitability Solution (Reference Solution (b)) for Method 2 on a Luna 5 µm C18(2), 250 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	27.16	2183162	0.27	69105	11.36	0.98
2	Irbesartan	38.58	2517525	0.34	56412		0.97
N = 6 Injections							

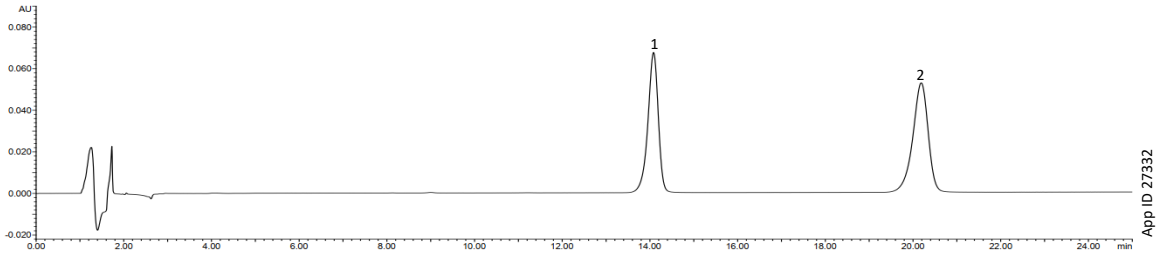
*NLT 2.0 per USP

Figure 3. System Suitability Solution (Reference Solution (b)) for Method 3 on a Luna™ 5 µm C18(2), 250 x 4.6 mm Column.



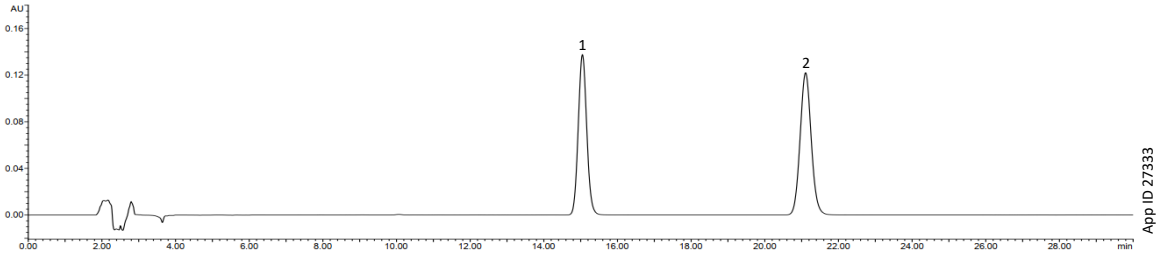
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	20.95	1660038	0.10	64446	10.87	0.98
2	Irbesartan	29.84	1913290	0.08	52756		0.98
N = 6 Injections							

Figure 4. System Suitability Solution (Reference Solution (b)) for Method 4 on a Luna 3 µm C18(2), 150 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	14.08	1111806	0.21	67439	11.53	0.87
2	Irbesartan	20.19	1281927	0.21	52645		0.87
N = 6 Injections							

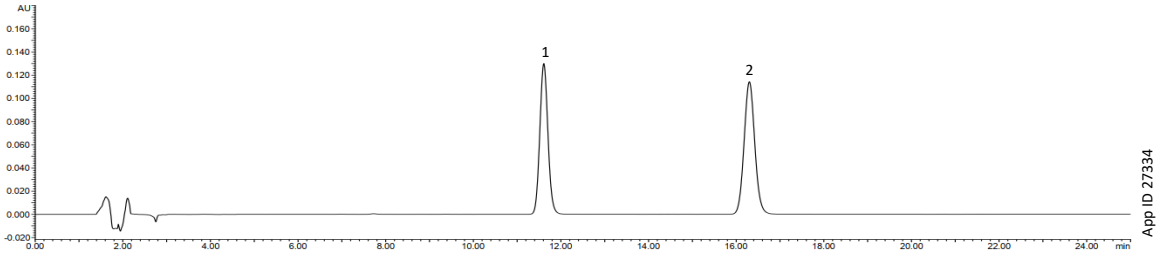
Figure 5. System Suitability Solution (Reference Solution (b)) for Method 5 on a Kinetex™ 5 µm C18, 250 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	15.05	2213344	0.20	137712	12.37	1.08
2	Irbesartan	21.11	2555501	0.24	122101		1.07
N = 6 Injections							

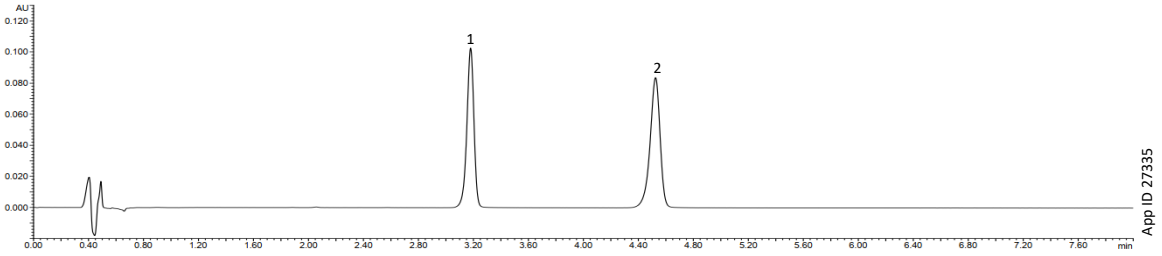
*NLT 2.0 per USP

Figure 6. System Suitability Solution (Reference Solution (b)) for Method 6 on a Kinetex™ 5 µm C18, 250 x 4.6 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	11.61	1677128	0.14	129969	11.88	1.08
2	Irbesartan	16.30	1938559	0.12	114343		1.07
N = 6 Injections							

Figure 7. System Suitability Solution (Reference Solution (b)) for Method 7 on a Kinetex 1.7 µm C18, 100 x 2.1 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Height	Resolution (minimum of 3.0)*	Symmetry Factor
1	Irbesartan Impurity A	3.18	368368	1.09	102700	12.00	0.90
2	Irbesartan	4.53	424730	1.03	83744		0.87
N = 6 Injections							

*NLT 2.0 per USP

Conclusions

The results in the above seven methods show that the system suitability criteria (Resolution, % RSD of area) met and surpassed the requirement. The use of a Kinetex 5 µm C18, 250 x 4.6 mm column, a Luna™ 3 µm C18(2), 150 x 4.6 mm column, and a Kinetex 1.7 µm C18, 100 x 2.1 mm column is an allowed adjustment to the original column dimensions per the newly revised European Pharmacopoeia Chapter 2.2.46 and USP General Chapter <621> for isocratic methods, with the flow rates scaled accordingly. The use of a Kinetex core-shell column over the original fully porous column has reduced the total analysis time by 25 % (from 40 minutes to 30 minutes with a 1.0 mL/min flow rate) and 37.5 % (from 40 minutes to 25 minutes with a relative flow rate of 1.3 mL/min). With the Kinetex 1.7 µm C18, 100 x 2.1 mm column, we demonstrated a reduction in total analysis time by 80 % (from 40 minutes to 8 minutes with a 0.5 mL/min flow rate).

Luna™ Ordering Information

3 µm MidBore™ and Analytical Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	**30 x 3.0**	**50 x 3.0**	**150 x 3.0**	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**4 x 2.0***	**4 x 3.0***
									/10pk	/10pk
Silica(2)	—	00B-4162-Y0	00F-4162-Y0	00A-4162-E0	00B-4162-E0	—	00D-4162-E0	00F-4162-E0	AJ0-4347	AJ0-4348
C8(2)	00A-4248-Y0	00B-4248-Y0	00F-4248-Y0	00A-4248-E0	00B-4248-E0	00C-4248-E0	00D-4248-E0	00F-4248-E0	AJ0-4289	AJ0-4290
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJ0-4286	AJ0-4287
CN	—	00B-4254-Y0	00F-4254-Y0	00A-4254-E0	00B-4254-E0	00C-4254-E0	00D-4254-E0	00F-4254-E0	AJ0-4304	AJ0-4305
Phenyl-Hexyl	—	00B-4256-Y0	00F-4256-Y0	—	00B-4256-E0	00C-4256-E0	00D-4256-E0	00F-4256-E0	AJ0-4350	AJ0-4351
NH_2	—	00B-4377-Y0	00F-4377-Y0	—	00B-4377-E0	—	00D-4377-E0	00F-4377-E0	AJ0-4301	AJ0-4302
HILIC	—	00B-4449-Y0	00F-4449-Y0	—	—	—	00D-4449-E0	00F-4449-E0	AJ0-8328	AJ0-8329
PFP(2)	—	00B-4447-Y0	00F-4447-Y0	—	00B-4447-E0	—	00D-4447-E0	00F-4447-E0	AJ0-8326	AJ0-8327
									for ID: 2.0-3.0 mm	3.2-18.0 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‡**
					/10pk	/3pk
Silica(2)	00D-4274-E0	00F-4274-E0	00G-4274-E0	00G-4274-N0	AJ0-4348	AJ0-7223
C5	00D-4043-E0	00F-4043-E0	00G-4043-E0	00G-4043-N0	AJ0-4293	AJ0-7372
C8(2)	00D-4249-E0	00F-4249-E0	00G-4249-E0	00G-4249-N0	AJ0-4290	AJ0-7222
C18(2)	00D-4252-E0	00F-4252-E0	00G-4252-E0	00G-4252-N0	AJ0-4287	AJ0-7221
CN	00D-4255-E0	00F-4255-E0	00G-4255-E0	00G-4255-N0	AJ0-4305	AJ0-7313
Phenyl-Hexyl	00D-4257-E0	00F-4257-E0	00G-4257-E0	00G-4257-N0	AJ0-4351	AJ0-7314
NH_2	00D-4378-E0	00F-4378-E0	00G-4378-E0	00G-4378-N0	AJ0-4302	AJ0-7364
SCX	00D-4398-E0	00F-4398-E0	00G-4398-E0	00G-4398-N0	AJ0-4308	AJ0-7369
HILIC	00D-4450-E0	00F-4450-E0	00G-4450-E0	00G-4450-N0	AJ0-8329	AJ0-8902
PFP(2)	00D-4448-E0	00F-4448-E0	00G-4448-E0	00G-4448-N0	AJ0-8327	AJ0-8376
					for ID: 3.2-8.0 mm	9-16 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282
‡SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-9281

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

1.7 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges**
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
EVO C18	—	00B-4726-AN	00D-4726-AN	00F-4726-AN	AJ0-9298
Biphenyl	00A-4628-AN	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	AJ0-8782
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782
C8	00A-4499-AN	00B-4499-AN	00D-4499-AN	00F-4499-AN	AJ0-8784
HILIC	00A-4474-AN	00B-4474-AN	00D-4474-AN	—	AJ0-8786
Phenyl-Hexyl	—	00B-4500-AN	00D-4500-AN	00F-4500-AN	AJ0-8788
F5	—	00B-4722-AN	00D-4722-AN	00F-4722-AN	AJ0-9322
					for 2.1 mm ID

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

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Austria
t: +43 (0)1-319-1301
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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 5019 9707
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 22 104 21 72
pl-info@phenomenex.com

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

Singapore
t: +65 6559 4364
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
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