

Ph. Eur. Monograph 1368: Ramipril Related Substances on NUCLEOSIL® 3 µm C18, Luna™ 3 µm C18(2), Luna Omega 3 µm C18, and Gemini™ 3 µm NX-C18 Column

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Overview

Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications and it is used to treat high blood pressure, heart failure, and diabetic kidney disease.

In this application note we show the separation of Ramipril from its related substances following Ph. Eur. Monograph 1368. We use a Luna 3 µm C18(2), Luna Omega 3 µm C18, and Gemini 3 µm NX-C18 column and compare them to the NUCLEOSIL 3 µm C18 column originally used in the monograph. All the Phenomenex columns and the NUCLEOSIL column used for this study met the system suitability criteria of a resolution (R_s) minimum of 3.0 between the peaks due to Impurity A and Ramipril in the chromatogram obtained with Reference Solution (a), the symmetry factor should be 0.8 to 2.0 for the peak due to Ramipril in the chromatogram obtained with the Test Solution, and a signal-to-noise ratio (S/N) minimum of 3 for the principal peak in the chromatogram obtained with Reference Solution (c).

All reference solutions were prepared as indicated in Ph. Eur. monograph 1368 for Ramipril. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- R0145000, Ramipril CRS
- R0145005, Ramipril Impurity A CRS
- R0145010, Ramipril Impurity B CRS
- R0145015, Ramipril Impurity C CRS
- R0145020, Ramipril Impurity D CRS

Experimental Preparation

The following procedure has been used to precondition the system and column before each test series. The instrument needs to be equilibrated with the mobile phase at the initial composition for at least 35 minutes; if a suitable baseline cannot be obtained, use another grade of Triethylamine.

LC-UV Conditions

Columns: NUCLEOSIL 3 µm C18 ([CH0-9321](#))
 Luna 3 µm C18(2) ([00G-4251-E0](#))
 Luna Omega 3 µm C18 ([00G-4784-E0](#))
 Gemini 3 µm NX-C18 ([00G-4453-E0](#))

Dimensions: 250 x 4.6 mm

Mobile Phase: [Mobile Phase](#) (Table 1)

Gradient: Time (min)	%B
0	10
6	10
7	25
20	35
30	75
50	75
50.1	10
60	10

Flow Rate: 1 mL/min

Injection: 10 µL

Temperature: 65 °C

Detector: UV @ 210 nm

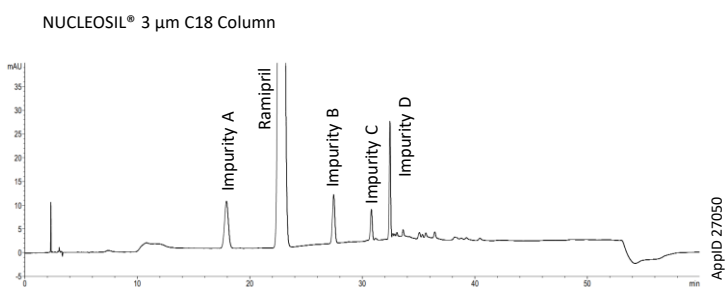
System: Agilent® 1260

Table 1. Preparation of Test and Reference Solutions

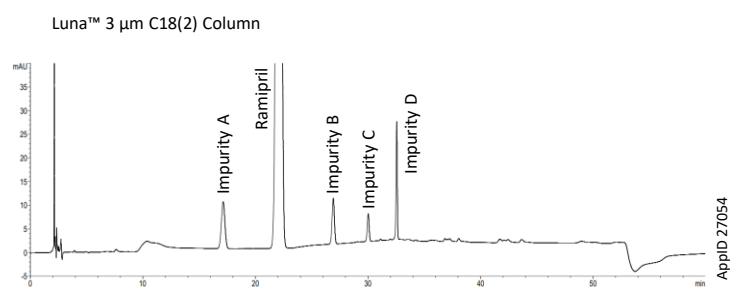
Solution	Composition
Mobile Phase	A: Dissolve 2.0 g of Sodium Perchlorate in a mixture of 0.5 mL of Triethylamine and 800 mL of HPLC water; adjust to pH 3.6 with Phosphoric Acid and add 200 mL of Acetonitrile. B: Dissolve 2.0 g of Sodium Perchlorate in a mixture of 0.5 mL of Triethylamine and 300 mL of HPLC water; adjust to pH 2.6 with Phosphoric Acid and add 700 mL of Acetonitrile.
Test Solution	Dissolve 20 mg of Ramipril CRS in Mobile Phase A , and dilute to 20.0 mL with Mobile Phase A .
Reference Solution (a)	Dissolve 2 mg of Ramipril Impurity A CRS, 2 mg of Ramipril Impurity B CRS, 2 mg of Ramipril Impurity C CRS and 2 mg of Ramipril Impurity D CRS in Mobile Phase A and dilute to 25 mL with Mobile Phase A . To 1 mL of this solution, add 5 mL of the Test Solution and dilute to 10 mL with Mobile Phase B .
Reference Solution (b)	Dilute 5.0 mL of the Test Solution to 100.0 mL with Mobile Phase B . Dilute 5.0 mL of this solution to 50.0 mL with Mobile Phase B .
Reference Solution (c)	Dilute 1.0 mL of Reference Solution (b) to 10.0 mL with Mobile Phase B .



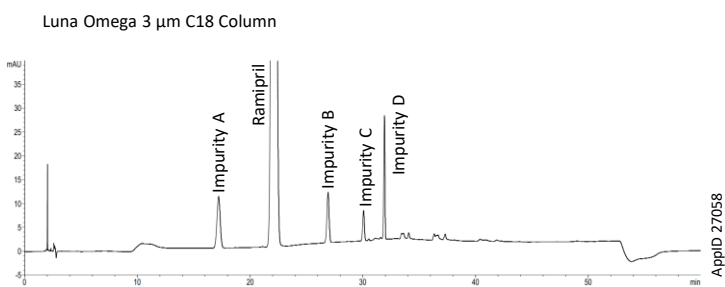
Figure 1. System Suitability Test Using Reference Solution (a)



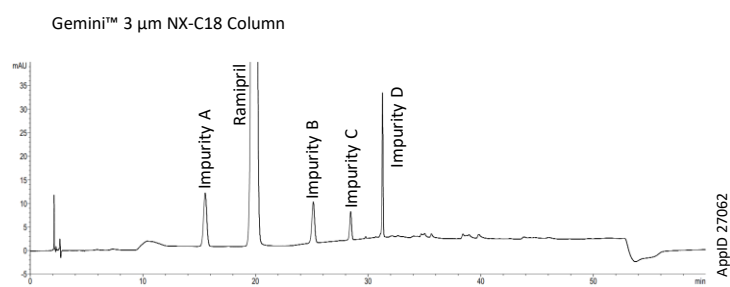
Inj. No.	Impurity A		Ramipril		R _s Ramipril/ Impurity A
	t _R	Area	t _R	Area	
1	17.924	235.2	22.752	10532.2	7.23
2	17.907	238.7	22.728	10672.2	7.30
3	17.915	239.0	22.739	10628.9	7.21
4	17.897	238.6	22.717	10632.1	7.21
5	17.908	239.6	22.733	10649.4	7.22
6	17.854	239.7	22.645	10638.2	7.26
Average	17.901	238.5	22.719	10625.5	7.24
% RSD	0.138	0.698	0.168	0.455	0.490



Inj. No.	Impurity A		Ramipril		R _s Ramipril/ Impurity A
	t _R	Area	t _R	Area	
1	17.145	209.8	22.089	9285.7	8.00
2	17.141	220.6	22.085	9762.5	7.99
3	17.134	221.1	22.070	9810.5	7.95
4	17.130	218.5	22.070	9708.2	7.99
5	17.151	217.2	22.089	9672.5	8.04
6	17.115	217.5	22.053	9622.1	8.06
Average	17.136	217.5	22.076	9643.6	8.01
% RSD	0.074	1.873	0.065	1.943	0.492



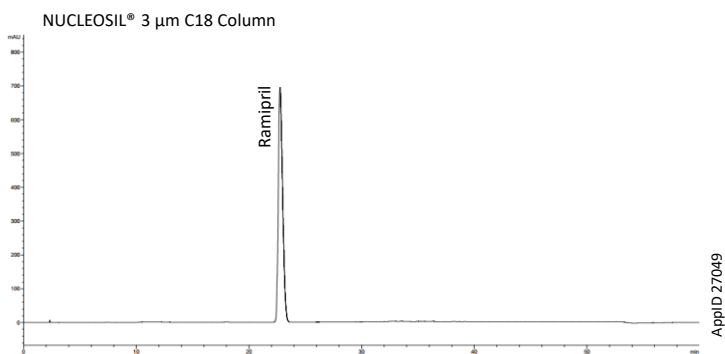
Inj. No.	Impurity A		Ramipril		R _s Ramipril/ Impurity A
	t _R	Area	t _R	Area	
1	17.240	216.8	22.150	9666.1	8.58
2	17.155	220.7	22.087	9740.1	8.62
3	17.235	221.3	22.146	9775.5	8.59
4	17.206	218.3	22.101	9676.7	8.58
5	17.220	217.9	22.136	9633.8	8.61
6	17.237	216.1	22.153	9585.1	8.61
Average	17.216	218.5	22.129	9679.6	8.60
% RSD	0.188	0.954	0.126	0.717	0.200



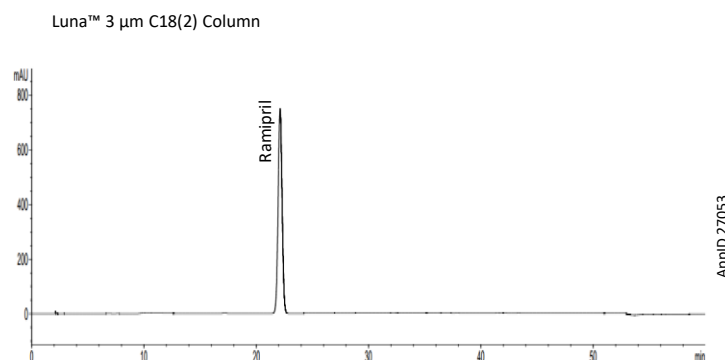
Inj. No.	Impurity A		Ramipril		R _s Ramipril/ Impurity A
	t _R	Area	t _R	Area	
1	15.466	226.9	19.856	10081.7	7.74
2	15.563	229.6	19.922	10172.8	7.69
3	15.473	230.1	19.792	10145.2	7.60
4	15.533	229.2	19.874	10155.2	7.64
5	15.543	229.5	19.895	10163.5	7.62
6	15.505	228.2	19.840	10162.3	7.59
Average	15.514	228.9	19.863	10146.8	7.65
% RSD	0.252	0.512	0.228	0.327	0.757



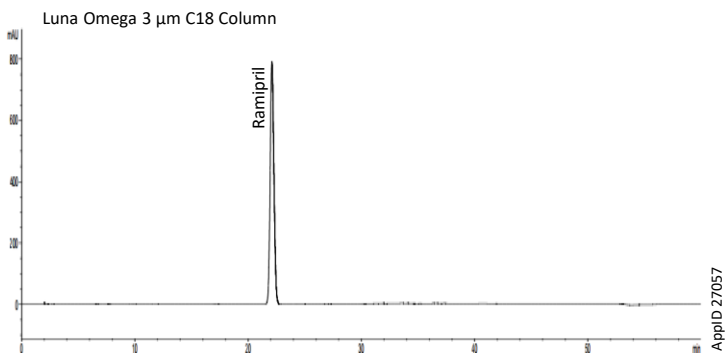
Figure 2. System Suitability Test Using Test Solution



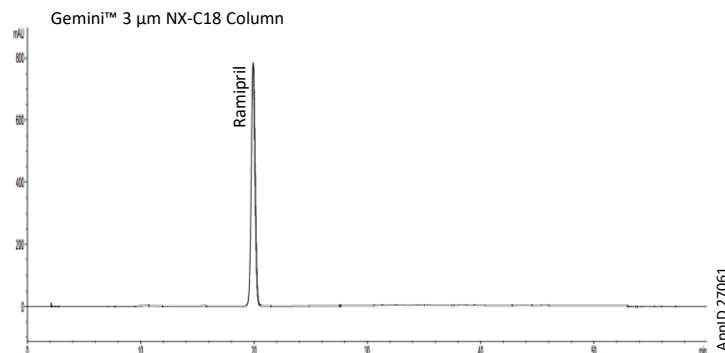
Inj. No.	Ramipril		
	t_R	Area	Symmetry Factor
1	22.756	18576.3	1.34
2	22.754	18598.5	1.34
3	22.769	18575.8	1.34
4	22.744	18579.1	1.34
5	22.753	18602.6	1.34
6	22.819	18577.3	1.30
Average	22.770	18584.9	1.33
% RSD	0.120	0.070	1.22



Inj. No.	Ramipril		
	t_R	Area	Symmetry Factor
1	22.116	18472.9	1.030
2	22.092	18410.3	1.030
3	22.092	18384.2	1.025
4	22.089	18394.3	1.023
5	22.065	18382.4	1.030
6	22.031	18396.9	1.030
Average	22.081	18406.8	1.028
% RSD	0.133	0.184	0.308

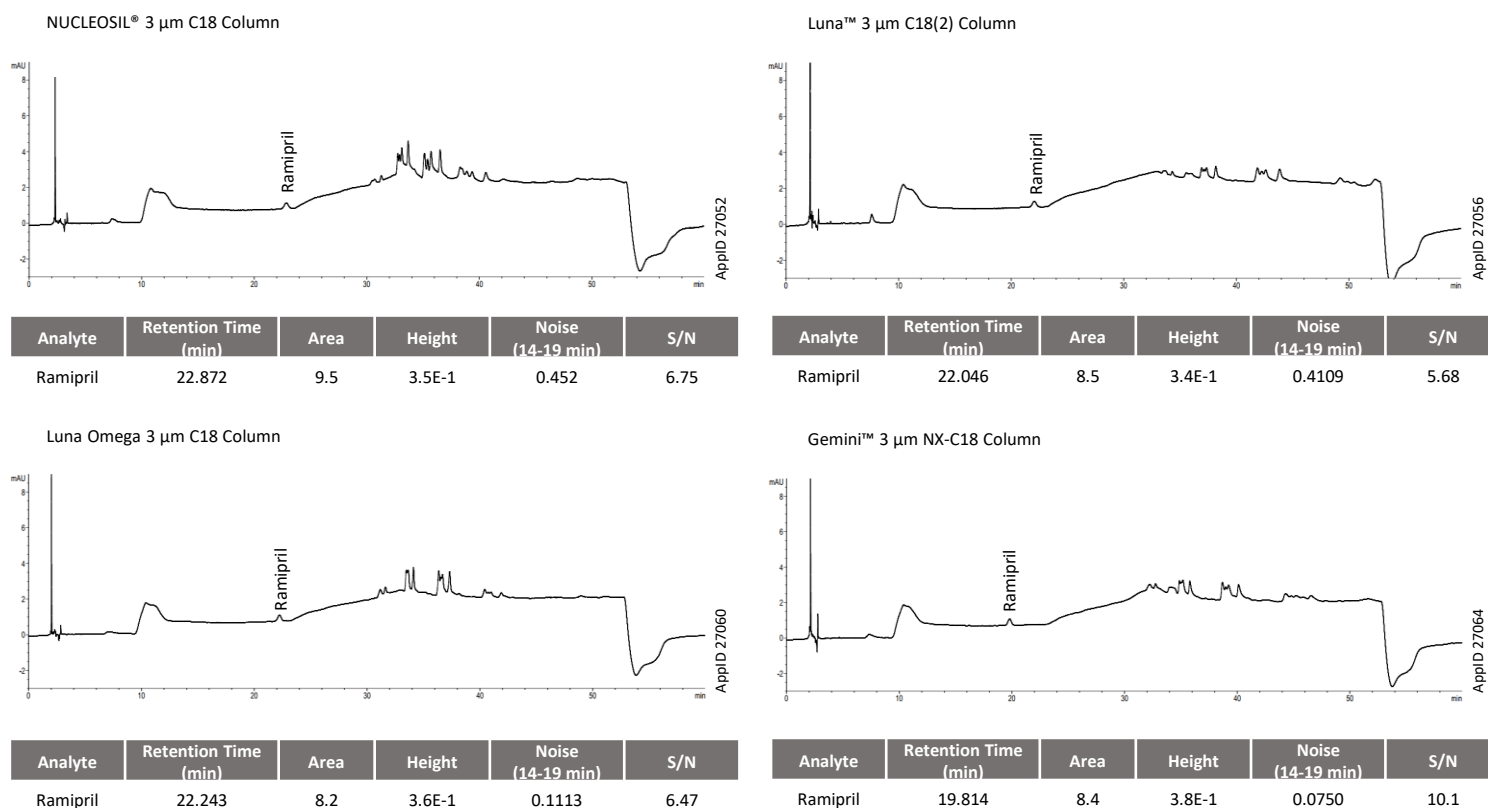


Inj. No.	Ramipril		
	t_R	Area	Symmetry Factor
1	22.097	18167.1	1.126
2	22.111	18165.5	1.122
3	22.121	18180.6	1.125
4	22.117	18185.6	1.127
5	21.726	18170.0	1.123
6	22.101	18187.2	1.124
Average	22.046	18176.0	1.125
% RSD	0.711	0.053	0.166



Inj. No.	Ramipril		
	t_R	Area	Symmetry Factor
1	19.919	18402.4	1.043
2	19.874	18423.8	1.043
3	19.911	18386.4	1.042
4	19.910	18433.2	1.039
5	19.873	18377.2	1.033
6	19.944	18399.4	1.031
Average	19.905	18403.7	1.039
% RSD	0.138	0.116	0.509



Figure 3. System Suitability Test Using Reference Solution (c)


Conclusion

By comparison with the NUCLEOSIL 3 µm C18 column, which was the column originally used for this Ph. Eur. Monograph 1368, the Gemini 3 µm NX-C18 column showed the best overall separation for all Ramipril related compounds. The system suitability criteria for the resolution between impurity A and Ramipril ($R_s \geq 3.0$) was achieved on all columns tested (Figure 1), with the Luna Omega 3 µm C18 providing the best resolution between impurity A and Ramipril, with an average $R_s = 8.60$ for six replicate injections. The system suitability requirement for symmetry factor for Ramipril in the test solution (Figure 2) was met by all columns with the Luna 3 µm C18(2) providing the best peak shape (average of 1.028 for six replicates). Lastly, the signal-to-noise ratio for the principal peak in the chromatogram was also achieved (Figure 3), and the study showed the Gemini 3 µm NX-C18 column had the highest S/N of 10.1. Therefore, the Luna 3 µm C18(2), Luna Omega 3 µm C18, and Gemini 3 µm NX-C18 columns are suitable for the analysis of Ramipril and related substances following the Ph. Eur. monograph 1368.



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