TN-1391

Separation of Felodipine and its Organic Impurities per USP Monograph

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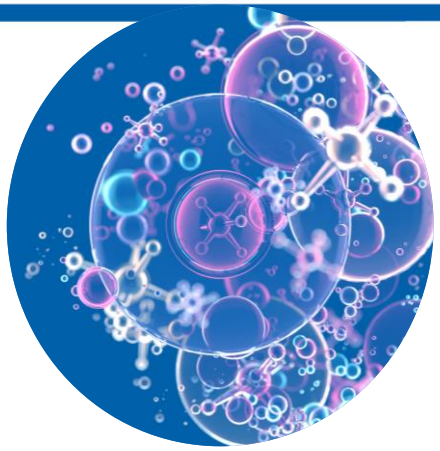


Introduction

Felodipine is a calcium channel blocker used to treat high blood pressure and angina. It works by relaxing blood vessels, improving blood flow, and reducing cardiac workload. It is also used to alleviate angina, a condition characterized by chest pain due to reduced blood supply.

In this technical note, we illustrate the appropriateness of Luna™ Omega C18 (5 µm 150 x 4.6 mm) and Kinetex™ C18 (5 µm 150 x 4.6 mm) columns for the assay, ethyl-3-aminocrotonate limits and the organic impurities of Felodipine, in accordance with the USP monograph. The USP monograph mandates the utilization of an L1 column (Waters® Symmetry™ C18, 5 µm, 150 x 4.6 mm). Both columns fulfill the acceptance criteria.

All reference solutions were prepared as per procedures indicated in USP monograph. USP reference standards used for the analysis.

- Felodipine RS (Lot: R079J0)
- Felodipine Related Compound A (Lot: R142H0)
- Ethyl 3-Aminocrotonate (Lot: F10540)

System Suitability, Standard and Sample Solution Preparation for Assay and Organic impurities methods

System suitability stock solution: 0.05 mg/mL of USP Felodipine RS and 0.1 mg/mL of USP Felodipine Related Compound A RS in mobile **phase System suitability solution:** 0.5 µg/mL of USP Felodipine RS and 1 µg/mL of USP Felodipine Related Compound A RS in mobile phase from the System suitability stock solution

Standard solution: 0.3 mg/mL of USP Felodipine RS in mobile phase

Sample solution: 0.3 mg/mL of Felodipine in mobile phase.

Sensitivity solution: 0.15 µg/mL of USP Felodipine RS in Mobile phase from the Standard solution.

Note: All the solution filtered through a suitable filter of 0.2 µm pore size and were prepared freshly before analysis.

Suitability requirements for Assay

Resolution: NLT 2.5 between Felodipine Related Compound A and Felodipine, System Suitability solution

Tailing factor: NMT 1.5, Standard solution

Relative standard deviation: NMT 0.37 %, Standard solution

Suitability requirements for organic impurities

Resolution: NLT 2.5 between the Felodipine Related Compound A and Felodipine, System Suitability solution

Relative standard deviation: NMT 10.0 %, Sensitivity solution

System suitability solution, Standard and sample Preparation for Ethyl 3-Aminocrotonate

Standard solution: 30 µg/mL of USP Ethyl 3-Aminocrotonate RS in Mobile phase.

Sample solution: 20 mg/mL of Felodipine in Mobile phase. Sonicate the solution for 10 min.

Suitability requirements

Requirements Relative standard deviation: NMT 3.0 %, Standard solution

LC Conditions for Assay and Organic impurities

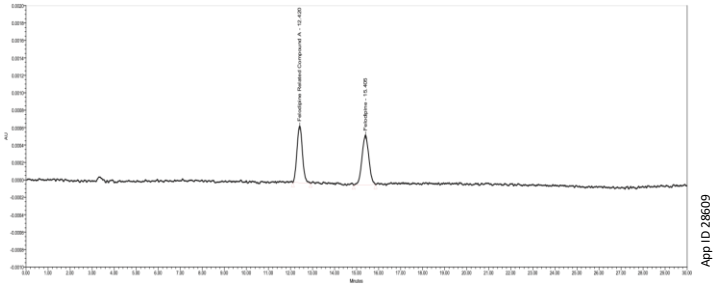
Column:	Luna™ Omega C18, 5 µm (Part No: 00F-4785-E0) Kinetex™ C18, 5 µm (Part No. 00F-4601-E0) Waters® Symmetry™ C18, 5 µm (WAT045905)
Dimensions:	150 x 4.6 mm
Buffer:	Dissolve 6.9 g of Sodium Phosphate Monobasic Dihydrate in 400 mL of water, add 8.0 mL of 1 M Phosphoric Acid, and dilute with Water to 1 L. [Note: The pH of this solution is around 3.0]
Mobile Phase:	Acetonitrile, Methanol, and Buffer (40:20:40 V/V/V)
Diluent	Mobile phase
Mode:	Isocratic
Flow Rate:	1 mL/min
Injection Volume:	20 µL for the System Suitability solution 40 µL for the Sensitivity solution, Standard solution and the Sample solution
Column Temperature:	25 °C
LC System:	Waters® Arc HPLC with PDA
Detection:	UV @ 254nm

LC Conditions for Ethyl-3-Aminocrotonate

Buffer:	2.84 g/L of Sodium Phosphate, Dibasic, anhydrous in water prepared as follows. Dissolve 2.84 g of Sodium Phosphate, Dibasic, anhydrous in 1000 mL of water. Adjust with Phosphoric Acid to a pH of 7.0.
Mobile phase:	Acetonitrile and Buffer (57:43 V/V)
Diluent	Mobile phase
Mode:	Isocratic
Flow Rate:	1 mL/min
Injection Volume:	20 µL
Column Temperature:	35 °C
LC System:	Waters® Arc HPLC
Detection:	Waters® RI 2414

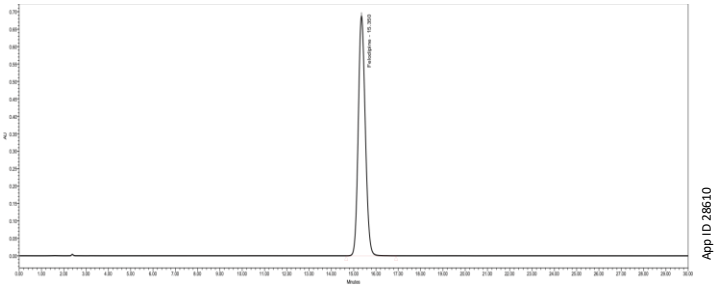
Results and Discussion on Waters Symmetry C18, for Assay

Figure 1. System suitability solution on Waters Symmetry C18, for Assay and organic impurities



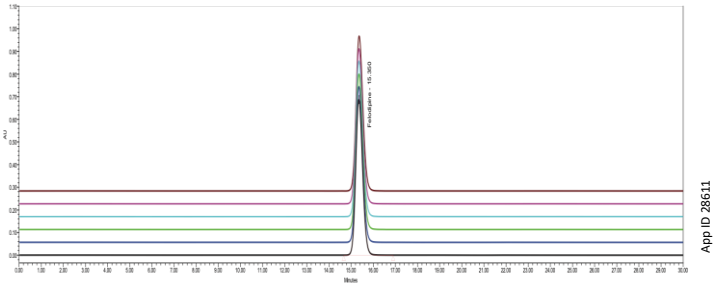
Peak No.	Data Point	Retention Time	Area	% Area	Height	RT Ratio	Resolution
1	Felodipine Related Compound A	12.420	11232	48.02	649	0.81	
2	Felodipine	15.405	12156	51.98	570	1.00	5.8

Figure 2. Standard solution on Waters Symmetry C18, for Assay



Peak No.	Analyte	Retention Time	Area	% Area	Height	USP Tailing
1	Felodipine	15.405	15042379	100.00	687393	1.1

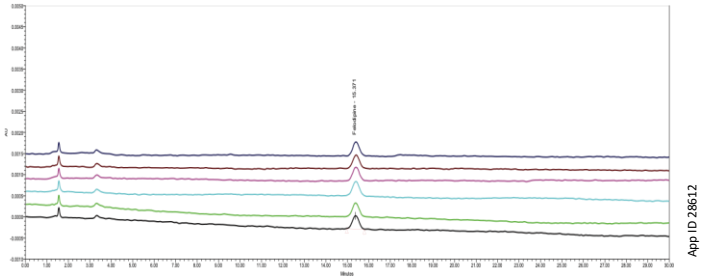
Figure 3. Overlayed chromatograms of replicate injections (N=6) of standard solution on Waters Symmetry for Assay



Injection	Retention Time	Area
Mean	15.355	15019263
SD	0.004	26667
%RSD	0.0	0.2
N=6 Injection		

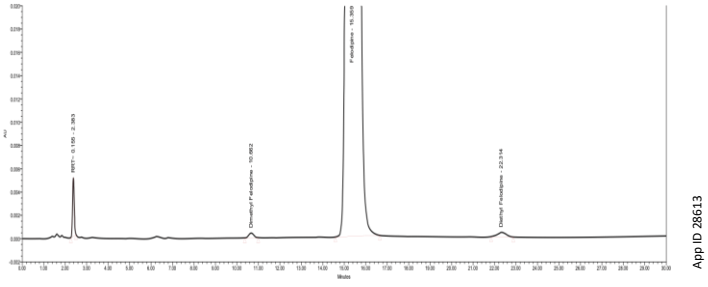
Results and Discussion on Waters Symmetry C18, for Organic impurities

Figure 4. Overlayed chromatogram of six replicate injection of sensitivity solution on Waters Symmetry C18, for organic impurities



Injection	Retention Time	Area
Mean	15.393	6679
SD	0.021	174
%RSD	0.1	2.6
N=6 Injection		

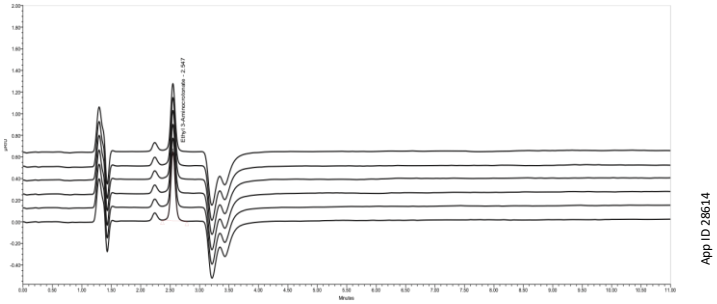
Figure 5. Sample solution on Waters Symmetry C18, for organic impurities



Peak No.	Data Point	Retention Time	Area	% Area	Height
1	Unknown impurity 1	2.383	27995	0.18	5077
2	Dimethyl Felodipine	10.662	7034	0.05	437
3	Felodipine Related Compound A	12.420			
4	Felodipine	15.359	15130765	99.69	691111
5	Diethyl Felodipine	22.314	11727	0.08	414

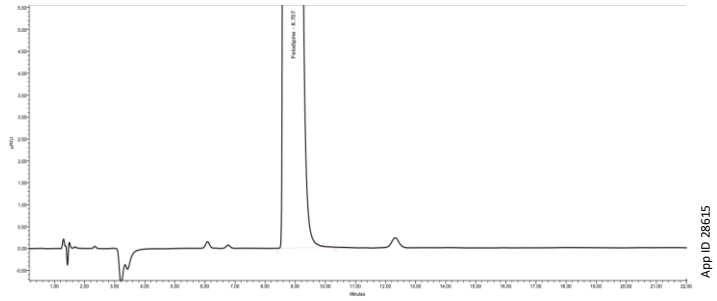
Results and Discussion on Waters Symmetry C18, for Ethyl-3-Aminocrotonate

Figure 6. Overlayed chromatogram of six replicate injection of sensitivity solution on Waters Symmetry C18, for Ethyl-3-Aminocrotonate



Injection	Retention Time	Area
Mean	2.547	3366
SD	0.000	32
%RSD	0.0	0.9
N=6 Injection		

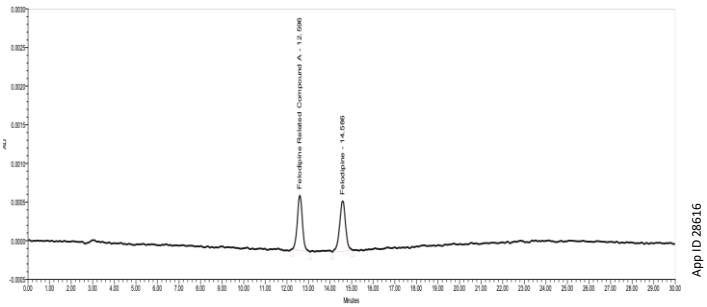
Figure 7. Sample solution on Waters Symmetry C18, for Ethyl-3-Aminocrotonate



Peak No.	Analyte	Retention Time	Area	% Area	Height
1	Ethyl 3-Aminocrotonate	2.547			
2	Felodipine	8.707	4371422	100.00	220676

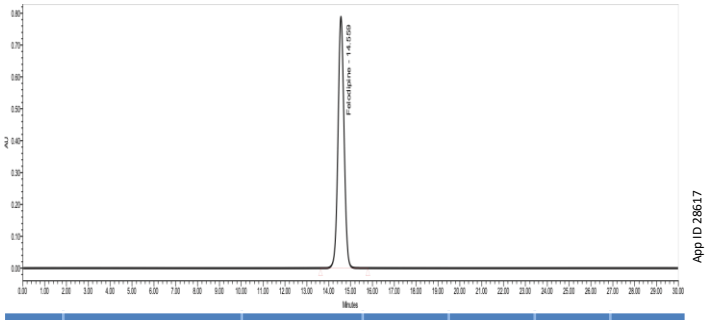
Results and Discussion on Luna Omega C18, for Assay

Figure 8. System suitability solution on Luna Omega C18, for Assay and organic impurities



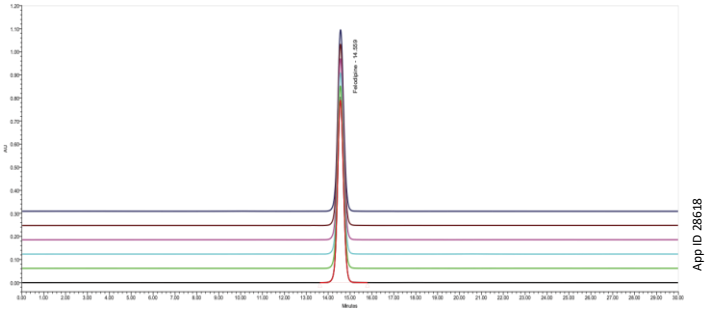
Peak No.	Analyte	Retention Time	Area	% Area	Height	Resolution
1	Felodipine Related Compound A	12.596	11640	48.46	704	
2	Felodipine	14.586	12382	51.54	640	4.4

Figure 9. Standard solution on Luna Omega C18, for Assay



Peak No.	Name	Retention Time	Area	% Area	Height	USP Tailing
1	Felodipine	14.559	15136934	100.00	789102	1.0

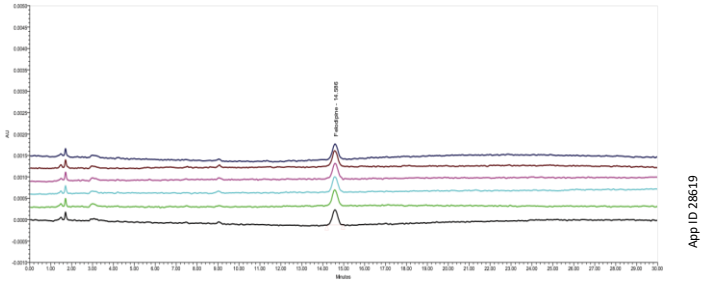
Figure 10. Overlayed chromatogram of six replicate injections of standard solution on Luna Omega C18, for Assay



Injection	Retention Time	Area
Mean	14.561	15092306
SD	0.003	43078
%RSD	0.0	0.3
N=6 Injection		

Results and Discussion on Luna Omega C18, for Organic impurities

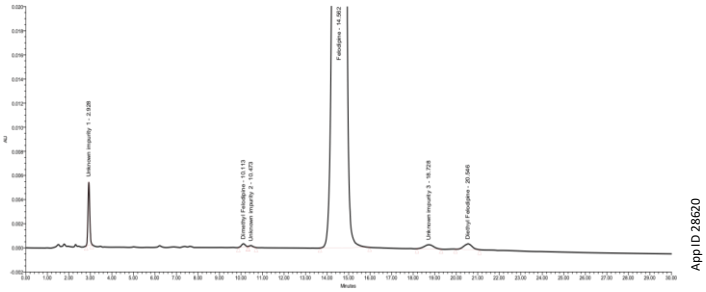
Figure 11. Overlayed chromatogram of six replicate injections of sensitivity solution on Luna Omega C18, for organic impurities



App ID 28619

Injection	Retention Time	Area
Mean	14.581	6645
SD	0.010	188
%RSD	0.1	2.8
N=6 Injection		

Figure 12. Sample solution on Luna Omega C18, for organic impurities

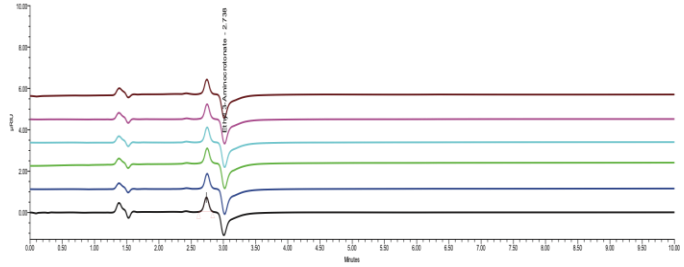


App ID 28620

Peak No.	Analyte	Retention Time	Area	% Area	Height
1	Unknown impurity 1	2.928	30369	0.20	5313
2	Dimethyl Felodipine	10.113	3352	0.02	286
3	Unknown impurity 2	10.473	1334	0.01	124
4	Felodipine Related Compound A	12.584			
5	Felodipine	14.562	15146086	99.62	790560
6	Unknown impurity 3	18.728	10224	0.07	359

Results and Discussion on Luna Omega C18, for Ethyl-3-Aminocrotonate

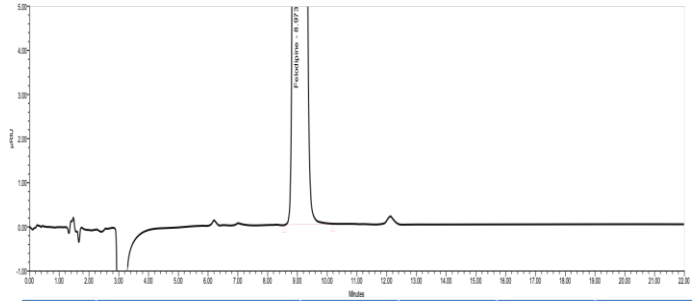
Figure 13. Overlayed chromatogram of replicate injections (N=6) of sensitivity solution on Luna Omega C18, for Ethyl-3-Aminocrotonate



App ID 28621

Injection	Retention Time	Area
Mean	2.746	3524
SD	0.004	32
%RSD	0.2	0.9
N=6 Injection		

Figure 14. Sample solution on Luna Omega C18, for Ethyl-3-Aminocrotonate

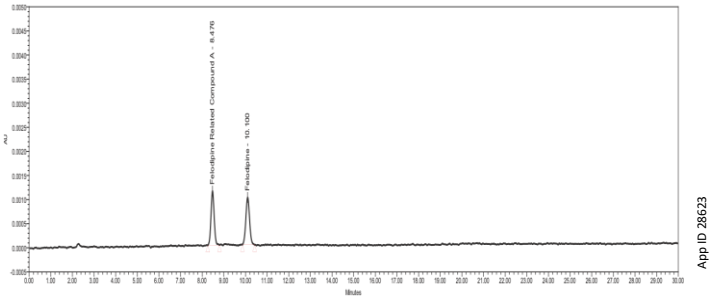


App ID 28622

Peak No.	Analyte	Retention Time	Area	% Area	Height
1	Ethyl 3-Aminocrotonate	2.758			
2	Felodipine	8.973	3273583	100.00	222369

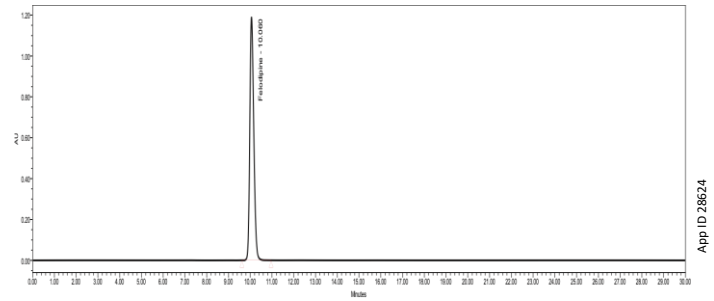
Results and Discussion on Kinetex C18, for Assay

Figure 15. System suitability solution on Kinetex C18, for Assay and organic impurities



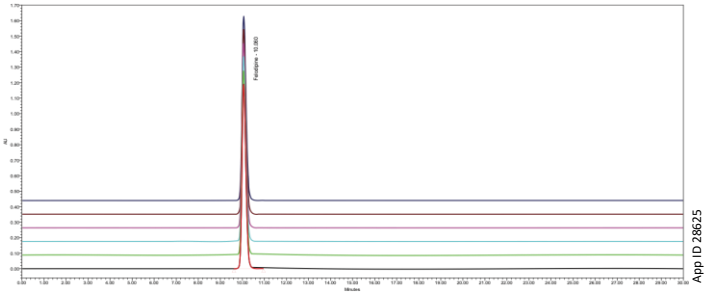
Peak No.	Analyte	Retention Time	Area	% Area	Height
1	Felodipine Related Compound A	8.476	11448	49.21	1127
2	Felodipine	10.100	11817	50.79	983

Figure 16. Standard solution on Kinetex C18, for Assay



Peak No.	Analyte	Retention Time	Area	% Area	Height	USP Tailing
1	Felodipine	10.060	14977680	100.00	1189361	1.2

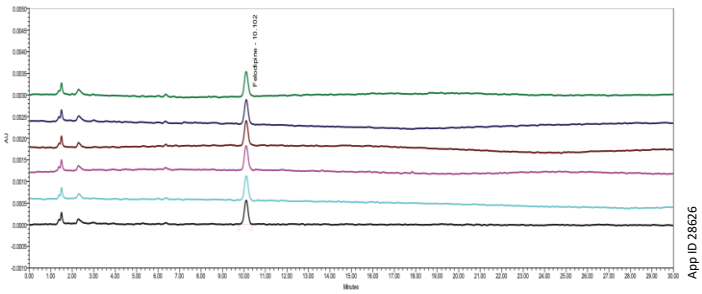
Figure 17. . Overlayed chromatogram of replicate injections (N=6) of standard solution on Kinetex C18, for Assay



Injection	Retention Time	Area
Mean	10.059	15003499
SD	0.004	20926
%RSD	0.0	0.1
N=6 Injection		

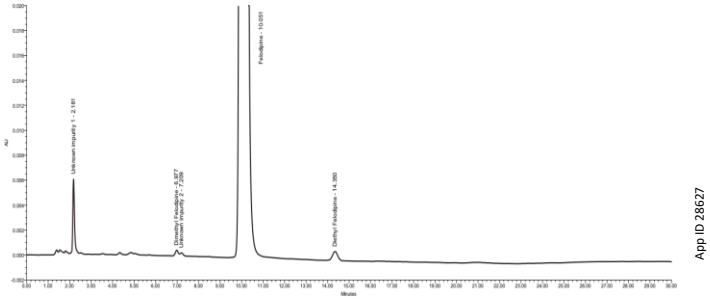
Results and Discussion on Kinetex C18, for Organic impurities

Figure 18. Overlayed chromatograms of replicate injections (N=6) of sensitivity solution on Kinetex C18, for organic impurities



Injection	Retention Time	Area
Mean	10.103	6920
SD	0.004	219
%RSD	0.0	3.2
N=6 Injection		

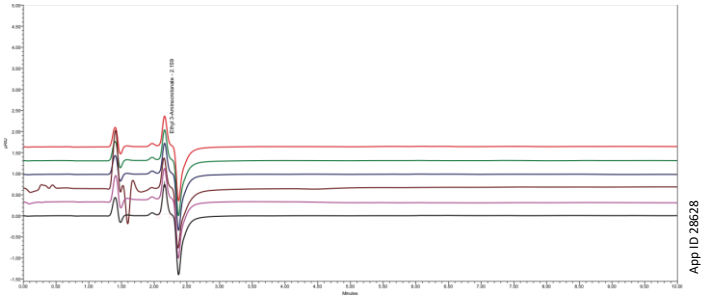
Figure 19. Sample solution on Kinetex C18, for organic impurities



Peak No.	Analyte	Retention Time	Area	% Area	Height	RT Ratio
1	Unknown impurity 1	2.181	30377	0.20	5910	0.22
2	Dimethyl Felodipine	6.977	3200	0.02	425	0.69
3	Unknown impurity 2	7.209	1288	0.01	179	0.72
4	Felodipine Related Compound A	8.482				
5	Felodipine	10.051	15056985	99.69	1197289	1.00
6	Diethyl Felodipine	14.350	12301	0.08	755	1.43

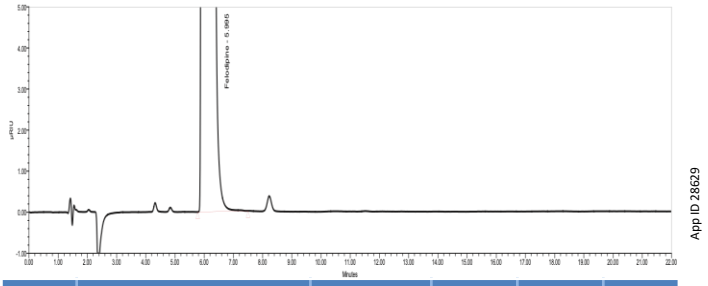
Results and Discussion on Kinetex C18, 5 µm for Ethyl-3-Aminocrotonate

Figure 20. Overlayed chromatograms of replicate injections (N=6) of sensitivity solution on Kinetex C18, for Ethyl-3-Aminocrotonate



Injection	Retention Time	Area
Mean	2.157	3242
SD	0.003	30
%RSD	0.2	0.9
N=6 Injection		

Figure 21. Sample solution on Kinetex C18, for Ethyl-3-Aminocrotonate



Peak No.	Analyte	Retention Time	Area	% Area	Height
1	Ethyl 3-Aminocrotonate	2.157			
2	Felodipine	5.995	4354148	100.00	292384

Summarization tables

Table 1. Summarization table for System suitability solution, Assay and Organic Impurities

Column Name	Waters Symmetry C18 5 µm 150 x 4.6 mm		Luna Omega C18 5 µm 150 x 4.6 mm		Kinetex C18 5 µm 150 x 4.6 mm		Limit as per USP Monograph
Analyte	RT	Resolution	RT	Resolution	RT	Resolution	
Felodipine related compound A	12.420	5.8	12.596	4.4	8.476	5.6	Resolution NLT 2.5 Felodipine related compound A and Felodipine
Felodipine	15.405		14.586		10.100		

Table 2. Summarization table for Standard solution, Assay

Column Name	Waters Symmetry C18 5 µm 150 x 4.6 mm			Luna Omega C18, 5 µm 150 x 4.6 mm			Kinetex C18 5 µm 150 x 4.6 mm			Limit as per USP Monograph
Analyte	RT	%RSD	Tailing factor	RT	%RSD	Tailing factor	RT	%RSD	Tailing factor	
Felodipine	15.355	0.2	1.1	14.561	0.3	1.0	10.059	0.1	1.2	%RSD NMT 0.37 and Tailing NMT 1.5

Table 3. Summarization table for Sensitivity solution, Organic Impurities and Limit of Ethyl 3-Aminocrotonate Standard solution

Column Name	Waters Symmetry C18 5 µm 150 x 4.6 mm		Luna Omega C18, 5 µm 150 x 4.6 mm		Kinetex C18, 5 µm 150 x 4.6 mm		Limit as per USP Monograph
Analyte	RT	%RSD	RT	%RSD	RT	%RSD	
Felodipine	15.393	2.6	14.581	2.8	10.103	3.2	%RSD NMT 10.0
Ethyl 3-Aminocrotonate	2.547	0.9	2.746	0.9	2.157	0.9	%RSD NMT 3.0

Conclusions

The monograph discusses the application of an L1 column (Waters Symmetry C18 (5 µm, 150 x 4.6 mm). We have assessed two L1 columns, namely Luna Omega C18 (5 µm, 150 x 4.6 mm) and Kinetex C18 (5 µm, 150 x 4.6 mm), to determine their appropriateness for the same monograph. All other method parameters remained constant. The system suitability test (SST) requirements with respect to resolution, tailing factor and relative standard deviation were successfully met using both the Luna Omega C18 and Kinetex columns for assay, organic impurities and the limit of ethyl 3-aminocrotonate methods. When analyzed with the Luna Omega C18, two additional unknown impurities were detected in the sample compared to the Waters Symmetry C18 column. In contrast, one additional unknown impurity was identified in the sample analyzed with the Kinetex C18 column relative to the Waters Symmetry C18 column.

This investigation validates that both the Luna Omega C18 and Kinetex columns satisfy the acceptance criteria for the Felodipine assay, limit of Ethyl 3-Aminocrotonate, and organic impurities methods as specified in the USP monograph and may serve as alternatives to the recommended L1 phase column. Utilizing a Kinetex C18 column the overall duration of the analysis was decreased by 33% while maintaining system suitability. In contrast, the Luna Omega C18 column offered comparable run times and resolutions when assessed against the Waters Symmetry column.

Ordering Information

Need a different column size or sample preparation format?

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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