Comparison of Two High-Performance Particle Morphologies in the Separation of Hydrochlorothiazide and Chlorothiazide

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Overview

In this application, we build upon previous work and explore the high-performance separation of hydrochlorothiazide and chlorothiazide with two different LC column particle morphologies. Under the same system and method conditions we compared a traditional Fully Porous 5 µm C18 to both a thermally modified Fully Porous Luna Omega 5 µm C18 and a Core-Shell Kinetex 5 µm C18 of identical dimensions. The Luna Omega and Kinetex columns satisfied the analysis requirement for a Hydrochlorothiazide Sulfide tablet under USP guidelines and were compared to the traditional Fully Porous column originally referenced in the same guidelines.

Compound: Hydrochlorothiazide
pK_{a1}: 7.9, pK_{a2}: 9.2
LogP: -0.07

USP Monograph: Hydrochlorothiazide Tablet Assay	
Standard Solution	0.15 mg/mL of USP Hydrochlorothiazide RS in *Mobile Phase*
System Suitability Solution	0.015 mg/mL of Chlorothiazide RS and 0.015 mg/mL Hydrochlorothiazide RS in *Mobile Phase**

* Note: A volume of Acetonitrile not exceeding 10 % of the total volume of solution may be used to dissolve the USP Reference Standard (RS)

Column	
Size	Method 1: 250 x 4.6 mm, Method 2: 250 x 4.6 mm, Method 3: 250 x 4.6
Stationary Phase	Method 1: Symmetry® 5 µm C18, Method 2: Kinetex 5 µm C18, Method 3: Luna Omega 5 µm C18
Temperature	30 °C
Mobile Phase	Acetonitrile and 0.1 M Monobasic Sodium Phosphate (1:9). Adjust with Phosphoric Acid to a pH of 3.0 ± 0.1
Isocratic	Isocratic: (1:9, A:B) Total Run Time: 30 min
Flow Rate	2.0 mL/min
Detector	UV @ 254 nm
Injection Volume	20 µL of System Suitability solution and Standard solution

System Suitability – System Suitability solution and Standard solution

Sample: Standard solution and System Suitability solution:

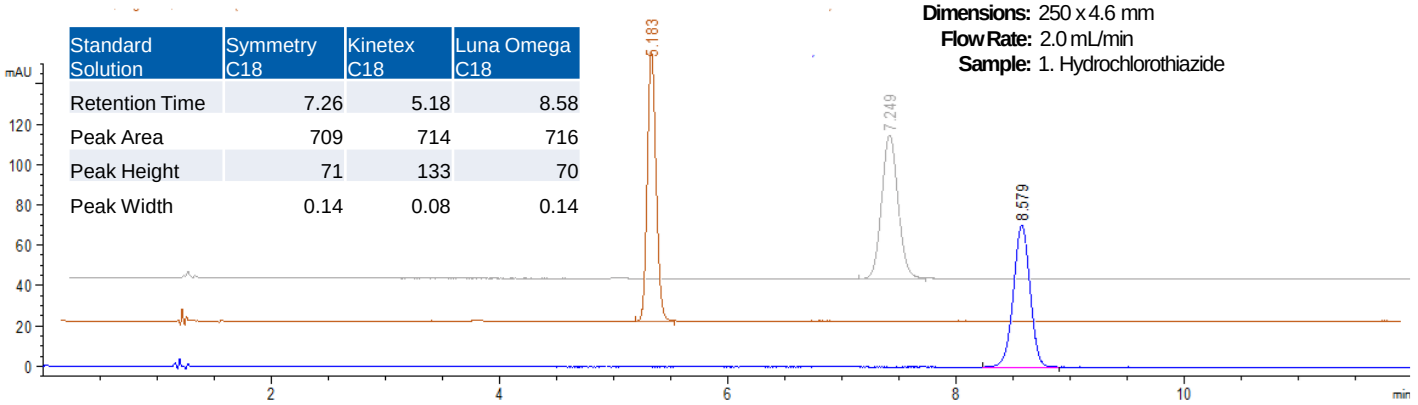
- Resolution (Rs): NLT 2.0 between Chlorothiazide and Hydrochlorothiazide for System Suitability solution
- Relative Standard Deviation: NMT 1.5 % for Standard solution (5 replicate injections)

Method: Standard solution Overlay

Kinetex 5 µm C18, Luna Omega 5 µm C18, and Symmetry 5 µm C18

Column: Symmetry 5 µm C18
Kinetex 5 µm C18
Luna Omega 5 µm C18
Dimensions: 250 x 4.6 mm
Flow Rate: 2.0 mL/min
Sample: 1. Hydrochlorothiazide

Standard Solution	Symmetry C18	Kinetex C18	Luna Omega C18
Retention Time	7.26	5.18	8.58
Peak Area	709	714	716
Peak Height	71	133	70
Peak Width	0.14	0.08	0.14



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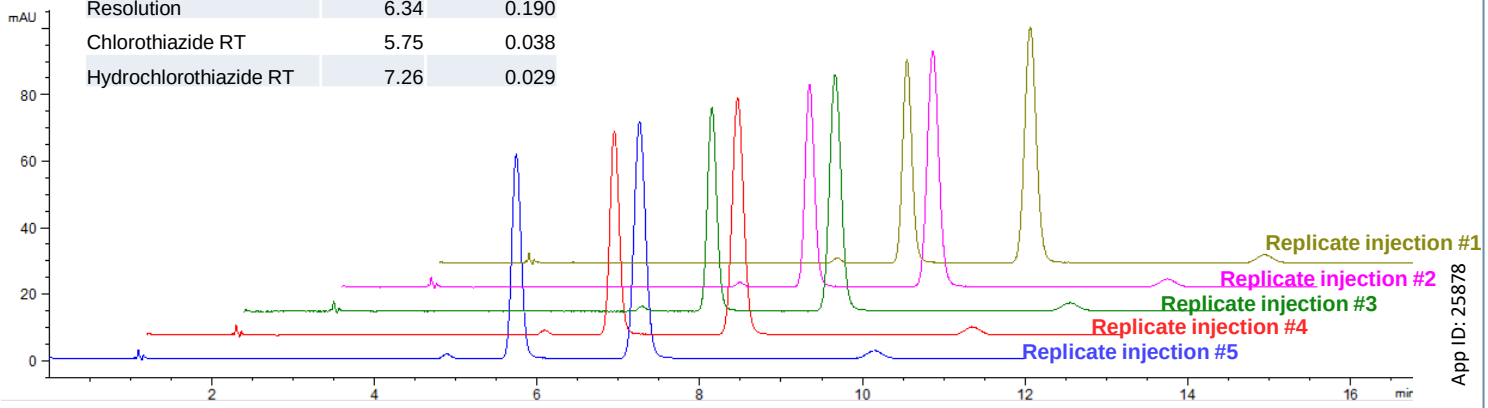
Method 1

System Suitability solution: Hydrochlorothiazide Symmetry® 5 µm C18

Column: Symmetry 5 µm C18 Traditional Fully Porous
Dimensions: 250 x 4.6 mm
Flow Rate: 2.0 mL/min
Sample: 1. Chlorothiazide
2. Hydrochlorothiazide

Standard Solution	Symmetry C18	Percent RSD
Resolution	6.34	0.190
Chlorothiazide RT	5.75	0.038
Hydrochlorothiazide RT	7.26	0.029

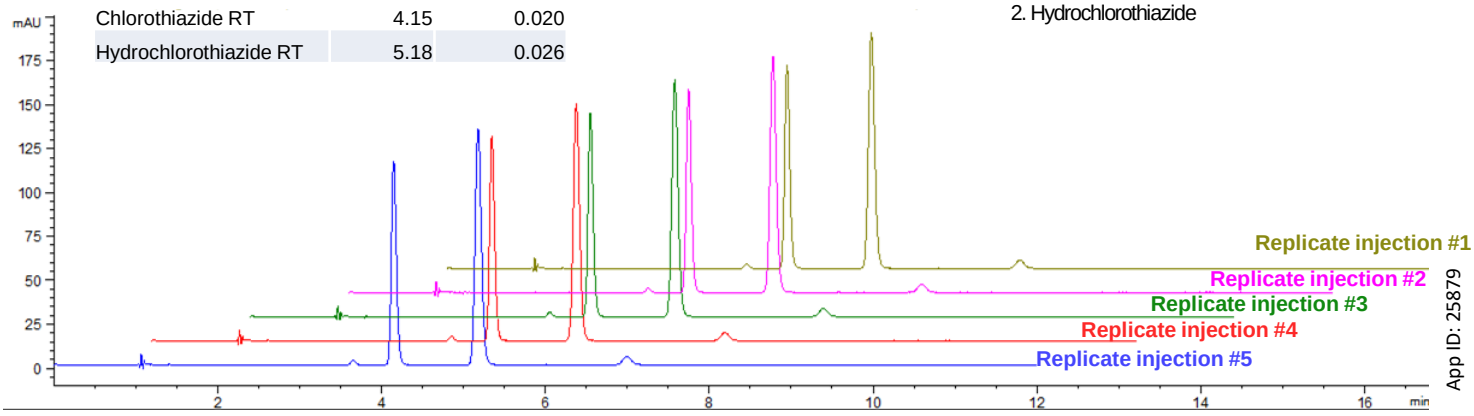


Method 2

System Suitability solution: Hydrochlorothiazide Kinetex® 5 µm C18

Column: Kinetex 5 µm C18 Core-Shell
Dimensions: 250 x 4.6 mm
Part No.: 00G-4601-E0
Flow Rate: 2.0 mL/min
Sample: 1. Chlorothiazide
2. Hydrochlorothiazide

Standard Solution	Kinetex C18	Percent RSD
Resolution	8.11	0.055
Chlorothiazide RT	4.15	0.020
Hydrochlorothiazide RT	5.18	0.026

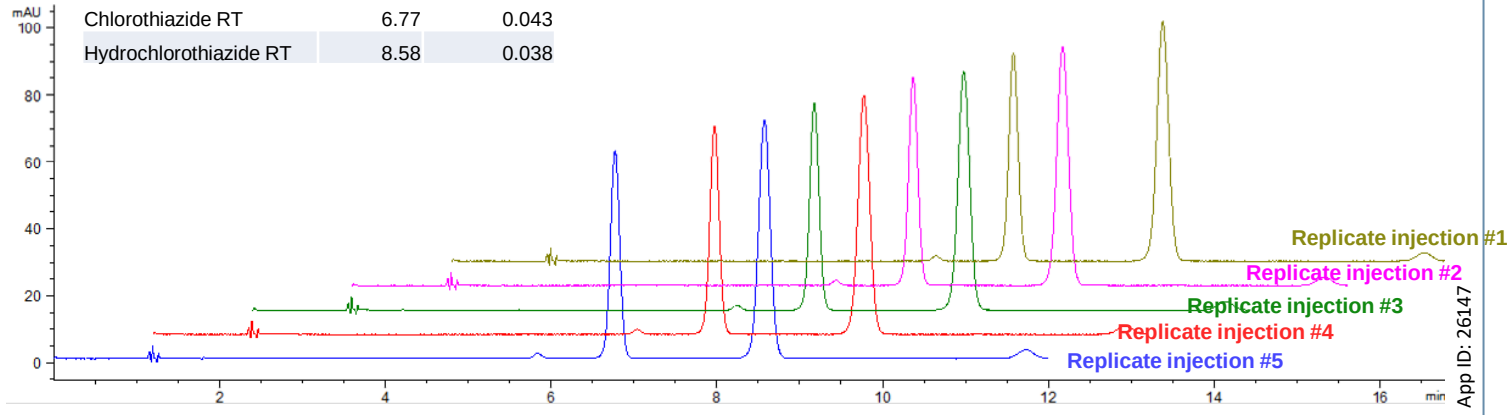


Method 3

System Suitability solution: Hydrochlorothiazide Luna® Omega 5 µm C18

Column: Luna Omega 5 µm C18 Thermally Modified Fully Porous
Dimensions: 250 x 4.6 mm
Part No.: 00G-4785-E0
Flow Rate: 2.0 mL/min
Sample: 1. Chlorothiazide
2. Hydrochlorothiazide

Standard Solution	Luna Omega C18	Percent RSD
Resolution	7.56	0.172
Chlorothiazide RT	6.77	0.043
Hydrochlorothiazide RT	8.58	0.038



Adjustments for Meeting System Suitability

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1	Method 2 & 3
Mobile Phase pH	± 0.2 units	As specified	As specified
Concentration of Salts in Buffer	± 10%	As specified	As specified
Composition of the Mobile Phase	± 30% Relative; cannot exceed ± 10% Absolute adjustment; cannot be reduced to zero	As specified	As specified
Wavelength of Detector	No deviations permitted	254 nm (as specified)	As specified
Injection Volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements	20 µL (as specified)	10 µL (Allowed)
Column Temperature	± 10°C	30 °C (Allowed)	30 °C (Allowed)
Stationary Phase	No change of the identity of the substituent permitted (e.g. no replacement of C18 by C8)	L1 (as specified)	As specified
Column Length	Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%*	250 mm (as specified)	250 mm As specified
Column Internal Diameter	Can be adjusted so long as linear velocity is maintained	4.6 mm (as specified)	4.6 mm As specified
Particle Size	Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%*	5 µm (as specified)	5 µm As specified
Flow Rate	± 50 % (at given ID)	2.0 mL/min (as specified)	2.0 mL/min 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%.

Allowable Column Adjustments: L/dp Ratio -25 % to 50 %

Column	Length (mm)	ID (mm)	dp (µm)	L/dp	Allowable Range 37,500-75,000
Original	250	4.6	5	50,000	37,500 – 75,000
Alternative	250	4.6	5	50,000	ALLOWED

Method Summary and Comparison

	Method 1	Method 2	Method 3
Column	Symmetry® 5 µm C18	Kinetex® 5 µm C18	Luna® Omega 5 µm C18
System Suitability Chlorothiazide Average Rt	5.75 min	4.15 min	6.77 min
System Suitability Hydrochlorothiazide Average Rt	7.26 min	5.18 min	8.58 min
System Suitability Hydrochlorothiazide Average Rs	6.34	8.112	7.562
System Suitability Hydrochlorothiazide Average Peak Height	71.1	134.53	71.32
System Suitability Hydrochlorothiazide Average Peak Area	715.80	722.15	722.98
System Suitability Hydrochlorothiazide Peak Height RSD (n=5)	0.183 %	0.081 %	0.154 %
System Suitability Hydrochlorothiazide Peak Area RSD (n=5)	0.316 %	0.102 %	0.142 %
Backpressure (Bar)	350	346	326

Conclusion

Both the Kinetex and Luna Omega 5 µm C18 met the system suitability requirement for this monograph method, and provided improved resolution and method performance in comparison to a traditional fully porous C18. This application demonstrated the use of allowable adjustments to improve method performance without requiring a re-validation. Also, this application illustrates selectivity difference based on particle morphology and the benefit of a reproducible and robust particle starting point in method development and lifecycle management.

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