Robust Separation of Hydrochlorothiazide and Chlorothiazide in Hydrochlorothiazide Tablets Using the Kinetex® 5 µm C18 column

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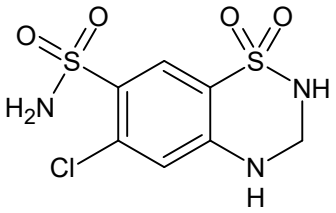


Overview

This application highlights the robust and reproducible separation of Hydrochlorothiazide and its related impurity Chlorothiazide under the USP monograph conditions for assay of Hydrochlorothiazide Tablets. The application demonstrates the potential method improvements that can be achieved per the allowable adjustments outlined in USP General Chapter <621> relative to the original column and conditions referenced in the monograph.

Monograph: 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 ReferenceStandard (RS)

Column	
Size	Method 1: 250 x 4.6 mm, Method 2: 250 x 4.6 mm
Stationary Phase	Method 1: Symmetry® 5 µm C18, Method 2: Kinetex 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:

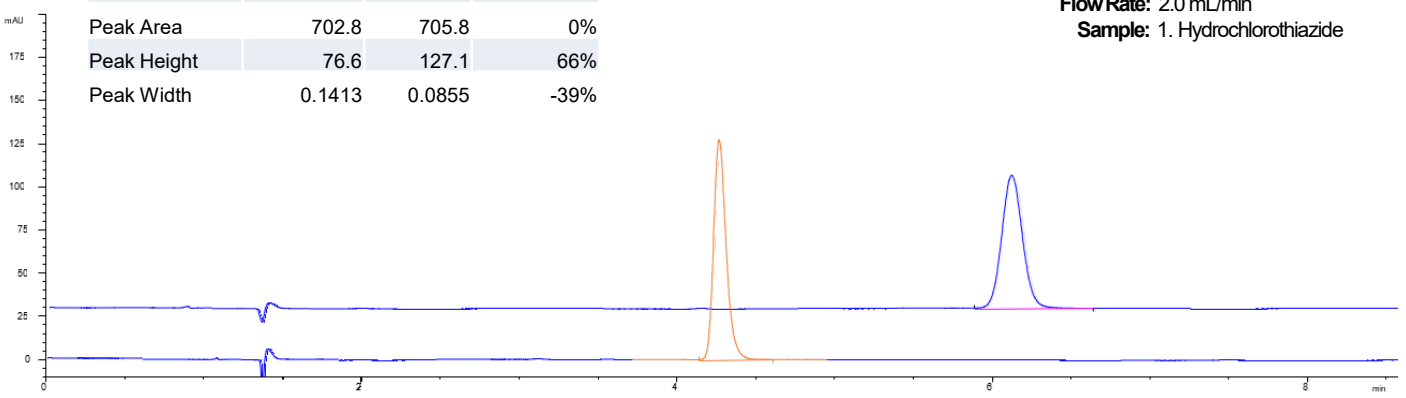
- Resolution (Rs): NLT 2.0 between Chlorothiazide and Hydrochlorothiazide for System Suitability solution
- Relative Standard Deviation: NMT 1.5 % for Standard solution (5 replicate injections)

Method Overlay

Kinetex 5 µm C18 and Symmetry 5 µm C18 Overlay Standard solution

Standard Solution	Symmetry C18	Kinetex C18	Percent Difference
Retention Time	6.31	4.27	-32%
Peak Area	702.8	705.8	0%
Peak Height	76.6	127.1	66%
Peak Width	0.1413	0.0855	-39%

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



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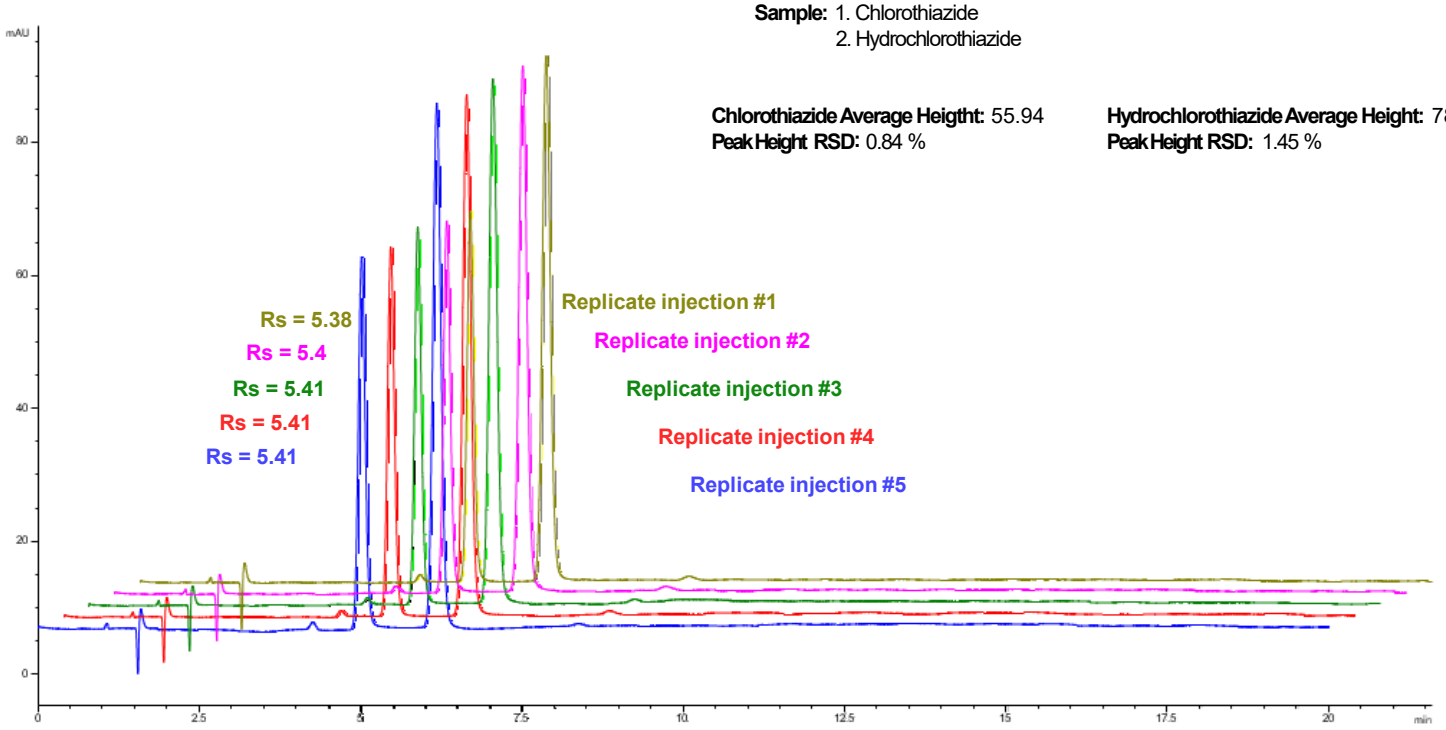
Method 1

System Suitability solution: Hydrochlorothiazide Symmetry® 5 µm C18

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

Chlorothiazide Average Heigtht: 55.94
Peak Height RSD: 0.84 %

Hydrochlorothiazide Average Height: 78.86
Peak Height RSD: 1.45 %



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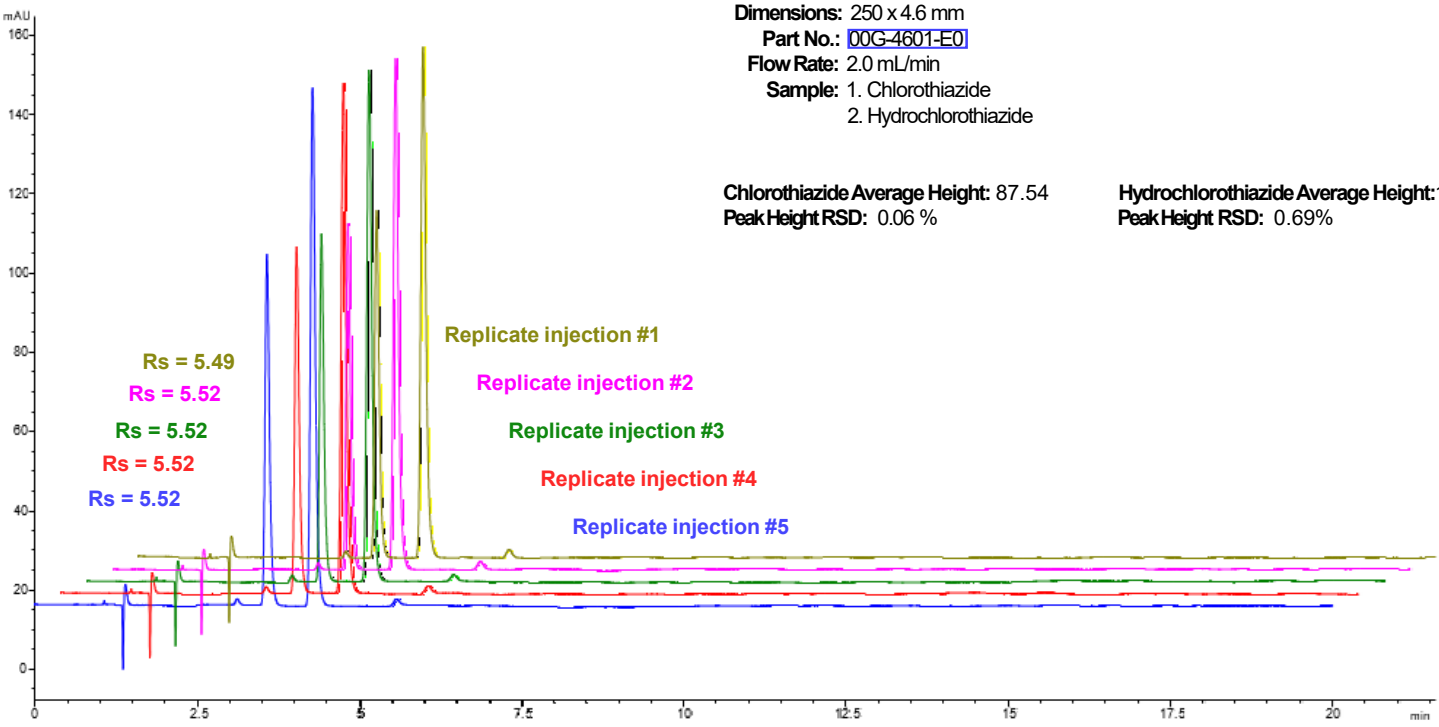
Method 2

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

Chlorothiazide Average Height: 87.54
Peak Height RSD: 0.06 %

Hydrochlorothiazide Average Height:128.84
Peak Height RSD: 0.69%



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Adjustments for Meeting System Suitability

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1	Method 2
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
Column	Symmetry® 5 µm C18	Kinetex 5 µm C18
System Suitability Solution Chlorothiazide Average Rt	5.1 min	3.64 min
System Suitability Solution Hydrochlorothiazide Average Rt	6.27 min	4.36 min
Relative Retention Times for Chlorothiazide and Hydrochlorothiazide are about 0.8 and 1.0, Respectively		
System Suitability Solution Hydrochlorothiazide Average Rs	5.4	5.51
System Suitability (Standard Solution) Hydrochlorothiazide Average Peak Height	74	135
System Suitability (Standard Solution) Hydrochlorothiazide Peak Height RSD (n=5)	0.9 %	1.48 %
Backpressure (Bar)	176	190

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