

Scaling and Migration of a Method for Naphazoline Hydrochloride, Pheniramine Maleate and Associated Related Substances to an ACQUITY™ Arc™ System

Margaret Maziarz

Waters Corporation

Abstract

When analytical methods are scaled across different particle size columns and migrated between systems, demonstrating equivalent results is critical to ensure product consistency and regulatory compliance. This work describes scaling and migration of a method for the analysis of naphazoline hydrochloride (HCl) and pheniramine maleate active pharmaceutical ingredients (APIs) and their associated related substances. The method developed on a 1.7 μm (2.1 x 100 mm) column is geometrically scaled to a 2.5 μm (4.6 x 150 mm) column with equivalent chemistry and migrated from an ACQUITY™ UPLC™ H-Class Plus System to an ACQUITY Arc™ System. Equivalent performance is demonstrated by evaluating chromatographic separation, system suitability data, and assay results for APIs and related substances content.

Benefits

- The Waters™ Columns Calculator facilitates method scaling by calculating operating parameters that produce equivalent chromatographic performance across the different particle sized columns
 - Accounting for differences in systems dwell volume and extra-column dispersion increases the success of method scaling and migration between chromatographic systems
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Introduction

Analytical methods developed on the sub-2 μm particle size columns using low-dispersion liquid chromatography (LC) systems offer fast and high throughput analysis and the benefit of reduced method development times. When quality control laboratories supporting manufacturing processes are not equipped with low dispersion systems, there is need to scale these methods to larger particles suitable for analysis on higher-dispersion HPLCs.¹

When scaling and migrating a method across different instruments, the goal is to obtain identical chromatographic separation and method performance. Numerous factors must be considered to ensure the quality of the chromatographic separation. Maintaining ratio of column length (L) to particle size (dp) preserves the resolving power of the method. Additionally, HPLC systems designed for use with larger particle size generally have larger dwell volume and extra-column dispersion compared to systems used with sub-2 μm particles. These differences will impact chromatographic separation and must be considered when scaling methods.²

In this work, a method for naphazoline hydrochloride, pheniramine maleate and associated related substances, developed on a 1.7 μm particle size column, is scaled to a 2.5 μm column and migrated from an ACQUITY UPLC H-Class Plus System to an ACQUITY Arc System. Chromatographic separation, system suitability results and assay results are examined to measure the success of method scaling and migration.

Experimental

Standard solutions

Compounds used in the study (Table 1) were purchased from Sigma-Aldrich and Toronto Research Chemicals (TRC).

Individual stock solutions were prepared in methanol at 4.0 mg/mL. Stock solutions were diluted with 80:20 water/methanol diluent to make a mixture standard solution for method migration experiments containing naphazoline HCl and pheniramine maleate APIs at 0.1 mg/mL and related substances at 10 $\mu\text{g}/\text{mL}$.

Compound	Name	Formula
Pheniramine maleate	PHEN API	$C_{20}H_{24}N_2O_4$
2-benzylpyridine	PHEN Imp. A	$C_{12}H_{11}N$
4-benzylpyridine	PHEN Imp. B	$C_{12}H_{11}N$
Naphazoline HCl	NAPH API	$C_{14}H_{15}ClN_2$
N-(2-Aminoethyl)-2-(naphthalen-1-yl) acetamide	NAPH Imp. A	$C_{14}H_{16}N_2O$
1-naphthylacetic acid	NAPH Imp. B	$C_{12}H_{10}O_2$
1-Naphthylacetonitrile	NAPH Imp. C	$C_{10}H_7CH_2CN$
β -naphazoline	NAPH Imp. D	$C_{14}H_{14}N_2$

Table 1. Active pharmaceutical ingredients (APIs) and their associated related substances.

Ophthalmic Sample Solutions

Ophthalmic sample solutions were prepared by dilution in 80:20 water/methanol diluent to the working concentrations of 500 μ g/mL pheniramine maleate and 40 μ g/mL naphazoline HCl.

Method Conditions

Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Column temperature:	42 °C
Detection:	λ range: 210–400 nm, Derived at 260 nm Sampling rate: 10 pts/sec
Vials:	LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV

Sample temperature: 10 °C

Wash solvents: Purge/sample: 80:20 water/methanol

Seal wash: 90:10 water/acetonitrile

Method conditions used for 1.7 and 2.5 µm particle size columns are summarized in Table 2. The flow rate, injection volume, and gradient elution were geometrically scaled from 1.7 to 2.5 µm using the Waters Columns Calculator.³

Parameter	Conditions for 1.7 µm column	Scaled conditions to 2.5 µm column																																																																						
Systems	ACQUITY™ UPLC™ H-Class Plus System, ACQUITY™ PDA, active pre-heating	ACQUITY™ Arc™ System, 2998 PDA, passive pre-heater																																																																						
Column	ACQUITY™ UPLC™ CSH C ₁₈ , 2.1 x 100 mm, 1.7 µm (P/N: 186005297)	XSELECT™ CSH C ₁₈ , 4.6 x 150 mm, 2.5 µm (P/N: 186006729)																																																																						
L/dp	58,824	60,000																																																																						
Flow rate	0.3 mL/min	1.2 mL/min																																																																						
Gradient	<table border="1"><thead><tr><th>Step</th><th>Time</th><th>%A</th><th>%B</th><th>Curve</th></tr></thead><tbody><tr><td>1</td><td>Initial</td><td>90.0</td><td>10.0</td><td>6</td></tr><tr><td>2</td><td>0.10</td><td>90.0</td><td>10.0</td><td>6</td></tr><tr><td>3</td><td>8.00</td><td>10.0</td><td>90.0</td><td>6</td></tr><tr><td>4</td><td>8.50</td><td>10.0</td><td>90.0</td><td>6</td></tr><tr><td>5</td><td>8.60</td><td>90.0</td><td>10.0</td><td>6</td></tr><tr><td>6</td><td>13.00</td><td>90.0</td><td>10.0</td><td>6</td></tr></tbody></table>	Step	Time	%A	%B	Curve	1	Initial	90.0	10.0	6	2	0.10	90.0	10.0	6	3	8.00	10.0	90.0	6	4	8.50	10.0	90.0	6	5	8.60	90.0	10.0	6	6	13.00	90.0	10.0	6	<table border="1"><thead><tr><th>Step</th><th>Time</th><th>%A</th><th>%B</th><th>Curve</th></tr></thead><tbody><tr><td>1</td><td>Initial</td><td>90.0</td><td>10.0</td><td>6</td></tr><tr><td>2</td><td>0.18</td><td>90.0</td><td>10.0</td><td>6</td></tr><tr><td>3</td><td>14.40</td><td>10.0</td><td>90.0</td><td>6</td></tr><tr><td>4</td><td>15.30</td><td>10.0</td><td>90.0</td><td>6</td></tr><tr><td>5</td><td>15.50</td><td>90.0</td><td>10.0</td><td>6</td></tr><tr><td>6</td><td>21.00</td><td>90.0</td><td>10.0</td><td>6</td></tr></tbody></table> <p>Gradient start 1.2 min after injection</p>	Step	Time	%A	%B	Curve	1	Initial	90.0	10.0	6	2	0.18	90.0	10.0	6	3	14.40	10.0	90.0	6	4	15.30	10.0	90.0	6	5	15.50	90.0	10.0	6	6	21.00	90.0	10.0	6
Step	Time	%A	%B	Curve																																																																				
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5	15.50	90.0	10.0	6																																																																				
6	21.00	90.0	10.0	6																																																																				
Inj. volume	1.0 µL	7.2 µL																																																																						

Table 2. Operating conditions for methods run using 1.7 and 2.5 µm particle size columns.

Data Management

Chromatography software: Empower™ 3 Feature Release 5 Service Release 5 (FR5 SR5)

Data acquisition and processing performed using Empower Software. Summary reports generated using the report templates are available in the Empower project.

Results and Discussion

Dwell Volumes of LC Systems

Systems designed for sub-2 μm particle size columns generally have smaller volumes compared to systems designed for use with 2.5 to 5 μm particles. The differences in system volumes may cause poor chromatographic separation and peak distortion in gradient methods. Therefore, system dwell volumes must be accounted for when migrating methods across different LC systems to achieve the same chromatographic separation.² The recommended procedure for measuring dwell volume of an LC system is described in the white paper.⁴

Method Scaling

To preserve the resolving power of the method, the ratio of column length (L) to particle size (dp) must be the same as (or very similar to) the original method. Other parameters including flow rate, injection volume and gradient times are scaled geometrically to maintain the same chromatographic separation quality.

In this work, a method for related substances of pheniramine maleate and naphazoline HCl APIs was scaled from 1.7 μm to 2.5 μm particle size using Columns Calculator³. The original method utilized a 100 mm long, 1.7 μm particle size column, resulting in $L/dp = 58,824$. For the target method, a 150 mm long, 2.5 μm column was selected, with an L/dp of 60,000. Due to the larger dwell volume of the ACQUITY Arc System (1.1 mL) compared to ACQUITY UPLC H-Class Plus System (0.4 mL), the gradient start time for the target method was delayed to maintain the integrity of the chromatographic separation. Using the Gradient StartSmart in the instrument method for the ACQUITY Arc System, the gradient was adjusted to start 1.2 minutes after the injection (Figure 1). The Gradient SmartStart feature compensated for the differences in systems dwell volume, eliminating the need for manual adjustments to the gradient table.

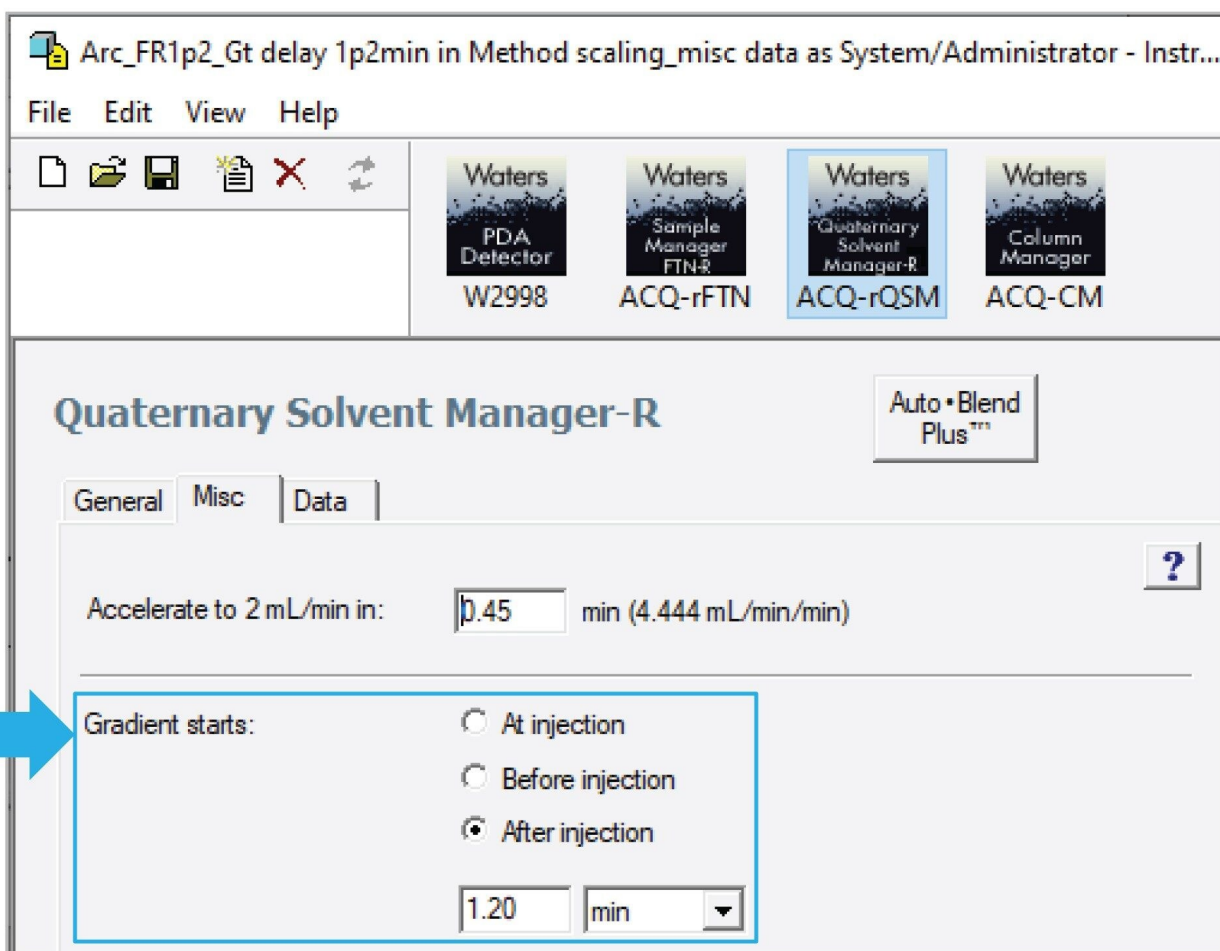


Figure 1. Instrument method in the ACQUITY Arc System with the Gradient StartSmart to compensate for differences in systems dwell volume in method migration. Gradient start time adjusted by 1.2 minutes after the injection.

The chromatographic data of a sample containing 100 µg/mL of pheniramine maleate and naphazoline HCl APIs with 10 µg/mL of related compounds, acquired on both systems, is shown in Figure 2. The chromatographic separation produced on an ACQUITY Arc System was comparable with results obtained on the ACQUITY UPLC H-Class Plus System. Additionally, the relative retention times (RRT) for related substances and USP Resolution (Rs) between peaks produced on the ACQUITY Arc System were in agreement with the results obtained on an ACQUITY H-Class Plus System (Table 3). The RRT values were calculated using Empower Software by comparing retention of each related substance to the associated API. The RRT values aid peak identification in chromatographic separation, therefore it is imperative that they are the same when moving a method across different particle sized columns and different LC systems.

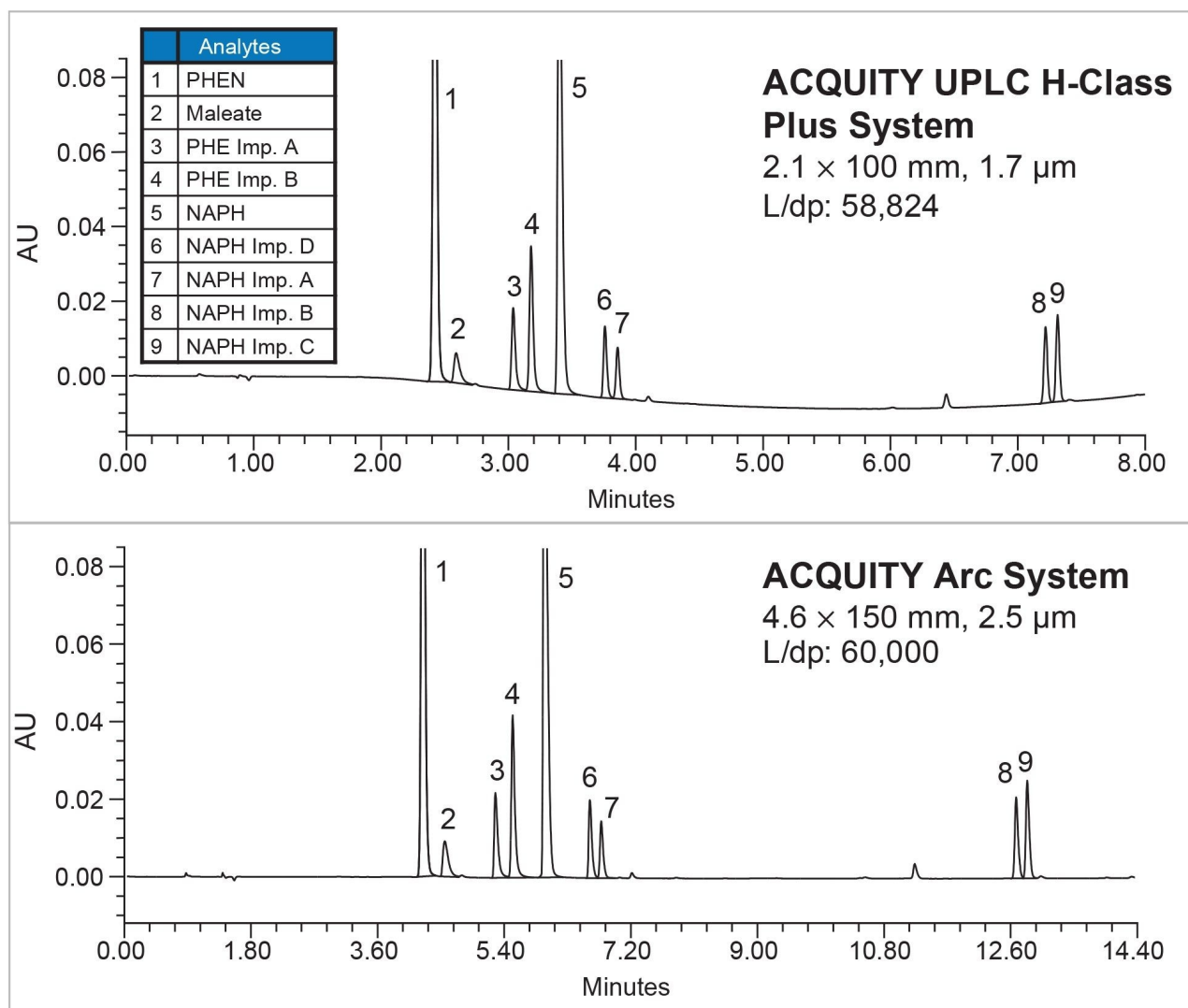


Figure 2. Chromatographic data for method scaling from 1.7 to 2.5 μm particle size column and migration between systems. UV at 260 nm.

Compound	Retention time (min)		Relative retention time (RRT)		USP Resolution (Rs)	
	1.7 μm	2.5 μm	1.7 μm	2.5 μm	1.7 μm	2.5 μm
PHEN	2.423	4.244	n/a	n/a	n/a	n/a
Maleate	2.589	4.556	1.07	1.07	2.4	2.8
PHE Imp. A	3.038	5.277	1.25	1.24	6.6	6.4
PHE Imp. B	3.178	5.521	1.31	1.30	2.6	2.7
NAPH	3.401	5.978	n/a	n/a	3.7	4.5
NAPH Imp. D	3.758	6.619	1.11	1.11	6.2	6.4
NAPH Imp. A	3.858	6.780	1.13	1.13	2.0	1.9
NAPH Imp. B	7.218	12.678	2.12	2.12	65.1	68.9
NAPH Imp. C	7.313	12.836	2.15	2.15	1.8	1.8

Table 3. Data for method scaling and migration between systems. Method with 1.7 μm (2.1 x 100 mm) run on ACQUITY UPLC H-Class Plus System, while method with 2.5 μm particle size column run on ACQUITY Arc. UV at 260 nm.

Performance Verification: System Suitability

Performance of the method run on the ACQUITY Arc System with 2.5 μm column was assessed by measuring repeatability of six replicate injections using a 10 $\mu\text{g}/\text{mL}$ standard. The peak areas and retention times repeatability on the ACQUITY Arc System were excellent and comparable to the results acquired on the ACQUITY UPLC H-Class Plus System (Figure 3). The ACQUITY Arc System produced equivalent USP Resolution between critical pairs of peaks: 1.9 between Naph-Imp D and Naph-Imp A, as well as 1.8 between Naph-Imp B and Naph-Imp C.

A. ACQUITY UPLC H-Class Plus System

Empower 3		System Suitability	
Sample Set ID:	Sample Set Id 6792		
Result Set ID:	Result Set Id 8618		
Channel Name:	PD A Ch 1 260nm@4.8nm		
Sample:	API/RS-10ug-SS		

	Name	# of inj.	Ave RT (min)	USP Rs (Ave)	%RSD of PeakAreas	% RSD of RT
1	PHEN	6	2.398		0.30	0.14
2	Maleate	6	2.642	3.5	3.12	0.09
3	PHEN-Imp A	6	3.019	5.4	0.14	0.13
4	PHEN-Imp B	6	3.173	2.9	0.18	0.12
5	NAPH	6	3.384	4.0	0.25	0.13
6	NAPH-Imp D	6	3.715	6.5	0.31	0.14
7	NAPH-Imp A	6	3.813	1.9	0.41	0.13
8	NAPH-Imp B	6	7.211	64.8	0.18	0.05
9	NAPH-Imp C	6	7.304	1.8	0.06	0.05

B. ACQUITY Arc System

Empower 3		System Suitability	
Sample Set ID:	Sample Set Id 6588		
Result Set ID:	Result Set Id 8064		
Channel Name:	2998 Ch 1 260nm@4.8nm		
Sample:	APIs/RS-10ug-SS		

	Name	# of inj.	Ave RT (min)	USP Rs (Ave)	%RSD of PeakAreas	% RSD of RT
1	PHEN	6	4.208		0.40	0.04
2	Maleate	6	4.584	3.6	1.02	0.05
3	PHEN-Imp A	6	5.262	6.3	0.11	0.03
4	PHEN-Imp B	6	5.480	2.5	0.28	0.03
5	NAPH	6	6.036	6.6	0.44	0.03
6	NAPH-Imp D	6	6.634	7.3	0.15	0.02
7	NAPH-Imp A	6	6.796	1.9	0.45	0.02
8	NAPH-Imp B	6	12.676	68.9	0.20	0.01
9	NAPH-Imp C	6	12.834	1.8	0.08	0.01

Figure 3. System suitability results for method scaling and migration from an ACQUITY UPLC H-Class Plus System with 1.7 μm column (A) to an ACQUITY Arc System with 2.5 μm column (B).

Analysis of Ophthalmic Formulations

Representative chromatograms of the ophthalmic sample solution containing 500 $\mu\text{g}/\text{mL}$ of pheniramine maleate and 40 $\mu\text{g}/\text{mL}$ of naphazoline HCl acquired on an ACQUITY UPLC H-Class Plus System with 1.7 μm column and an ACQUITY Arc System with 2.5 μm column are shown in Figure 4.

The assay of APIs and related substances content in the ophthalmic sample solutions were calculated and compared across systems. The APIs assay or percent (%) recovery was determined by quantifying samples against a calibration curve generated in the range of 80 to 120% with respect to the working concentration of 500 and 40 $\mu\text{g}/\text{mL}$ of pheniramine and naphazoline, respectively. The assay for related substances content (% impurity) was determined by comparing peak area of the detected related substance to the associated API, calculated using Empower Software. The assay results for APIs and related substances content generated by the ACQUITY Arc System were equivalent to the data obtained on the ACQUITY UPLC H-Class Plus System (Figures 5 and 6).

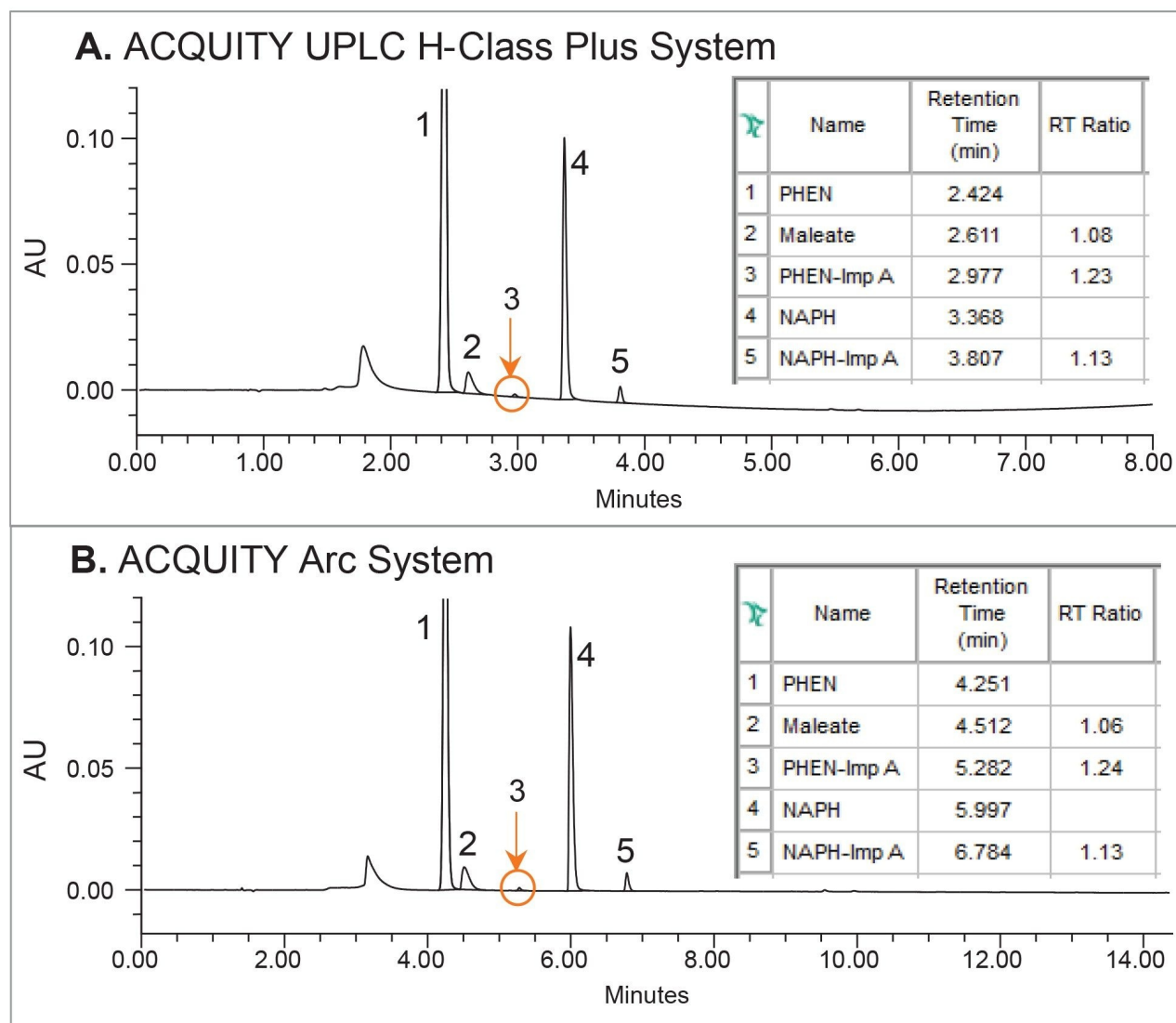


Figure 4. Representative chromatograms of ophthalmic sample solution containing 500 $\mu\text{g/mL}$ of pheniramine maleate and 40 $\mu\text{g/mL}$ of naphazoline HCl acquired using ACQUITY UPLC H-Class Plus System with 1.7 μm column (A) and ACQUITY Arc System with 2.5 μm column (B). RT Ratio: retention time of related compound to retention time of associated API. UV at 280 nm.

A. ACQUITY UPLC H-Class Plus System

Empower 3 Sample Analysis
Sample Set Id: 7008
Result Set Id: 8770

% Recovery of API
Perc_Amount_SpleValue Summarized by Name

	SampleName	PHEN	NAPH
1	Sample (Phen/Naph 500/40)	104.21	93.03
2	Sample (Phen/Naph 500/40)	104.32	93.03
3	Sample (Phen/Naph 500/40)	104.27	93.03
4	Sample (Phen/Naph 500/40)	104.24	92.86
5	Sample (Phen/Naph 500/40)	104.17	93.01
6	Sample (Phen/Naph 500/40)	104.20	93.04
Mean		104.2	93.0
Std. Dev.		0.051	0.069
% RSD		0.05	0.07

B. ACQUITY Arc System

Empower 3 Sample Analysis
Sample Set Id: 7839
Result Set Id: 8821

% Recovery of API
Perc_Amount_SpleValue Summarized by Name

	SampleName	PHEN	NAPH
1	Sample (Phen/Naph 500/40)	104.08	92.73
2	Sample (Phen/Naph 500/40)	104.11	92.71
3	Sample (Phen/Naph 500/40)	104.04	92.76
4	Sample (Phen/Naph 500/40)	103.97	92.69
5	Sample (Phen/Naph 500/40)	104.00	92.73
6	Sample (Phen/Naph 500/40)	103.97	92.77
Mean		104.0	92.7
Std. Dev.		0.060	0.029
% RSD		0.06	0.03

Figure 5. Assay results (% recovery) of pheniramine (PHEN) and naphazoline (NAPH) from the ophthalmic sample solutions generated ACQUITY UPLC H-Class Plus System with 1.7 μm column (A) and ACQUITY Arc System with 2.5 μm column (B). UV at 280 nm.

A. ACQUITY UPLC H-Class Plus System

Empower 3	Sample Analysis
	Sample Set Id: 7008
	Result Set Id: 8770

% impurity			
Perc_ImpArea_RefPeakArea Summarized by Name			
	SampleName	PHEN-Imp A	NAPH-Imp A
1	Sample (Phen/Naph 500/40)	0.34	5.76
2	Sample (Phen/Naph 500/40)	0.34	5.73
3	Sample (Phen/Naph 500/40)	0.33	5.77
4	Sample (Phen/Naph 500/40)	0.34	5.82
5	Sample (Phen/Naph 500/40)	0.32	5.80
6	Sample (Phen/Naph 500/40)	0.34	5.74
Mean		0.33	5.77
Std. Dev.		0.007	0.037
% RSD		2.14	0.64

B. ACQUITY Arc System

Empower 3	Sample Analysis
	Sample Set Id: 7839
	Result Set Id: 8821

% impurity			
Perc_ImpArea_RefPeakArea Summarized by Name			
	SampleName	PHEN-Imp A	NAPH-Imp A
1	Sample (Phen/Naph 500/40)	0.33	5.92
2	Sample (Phen/Naph 500/40)	0.34	5.95
3	Sample (Phen/Naph 500/40)	0.34	5.92
4	Sample (Phen/Naph 500/40)	0.34	5.93
5	Sample (Phen/Naph 500/40)	0.35	5.92
6	Sample (Phen/Naph 500/40)	0.34	5.93
Mean		0.34	5.93
Std. Dev.		0.006	0.011
% RSD		1.64	0.19

Figure 6. Assay results for related substances content (% impurity) in the ophthalmic sample solutions generated using ACQUITY UPLC H-Class Plus System with 1.7 μm column (A) and ACQUITY Arc System with 2.5 μm column (B). UV at 280 nm.

Conclusion

A method for the analysis of pheniramine maleate and naphazoline HCl APIs and their associated related substances was successfully scaled from 1.7 μm to 2.5 μm particle size columns and migrated from an ACQUITY UPLC H-Class Plus System to an ACQUITY Arc System. The chromatographic separation, relative retention times (RRT) values, system suitability, and assay results produced on the ACQUITY Arc System were equivalent to the data obtained on ACQUITY UPLC H-Class Plus System.

The success of scaling and migrating methods across different LC platforms can be impacted by the system characteristics. Making appropriate adjustments to account for these differences increases the success of generating equivalent separation and performance.

References

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