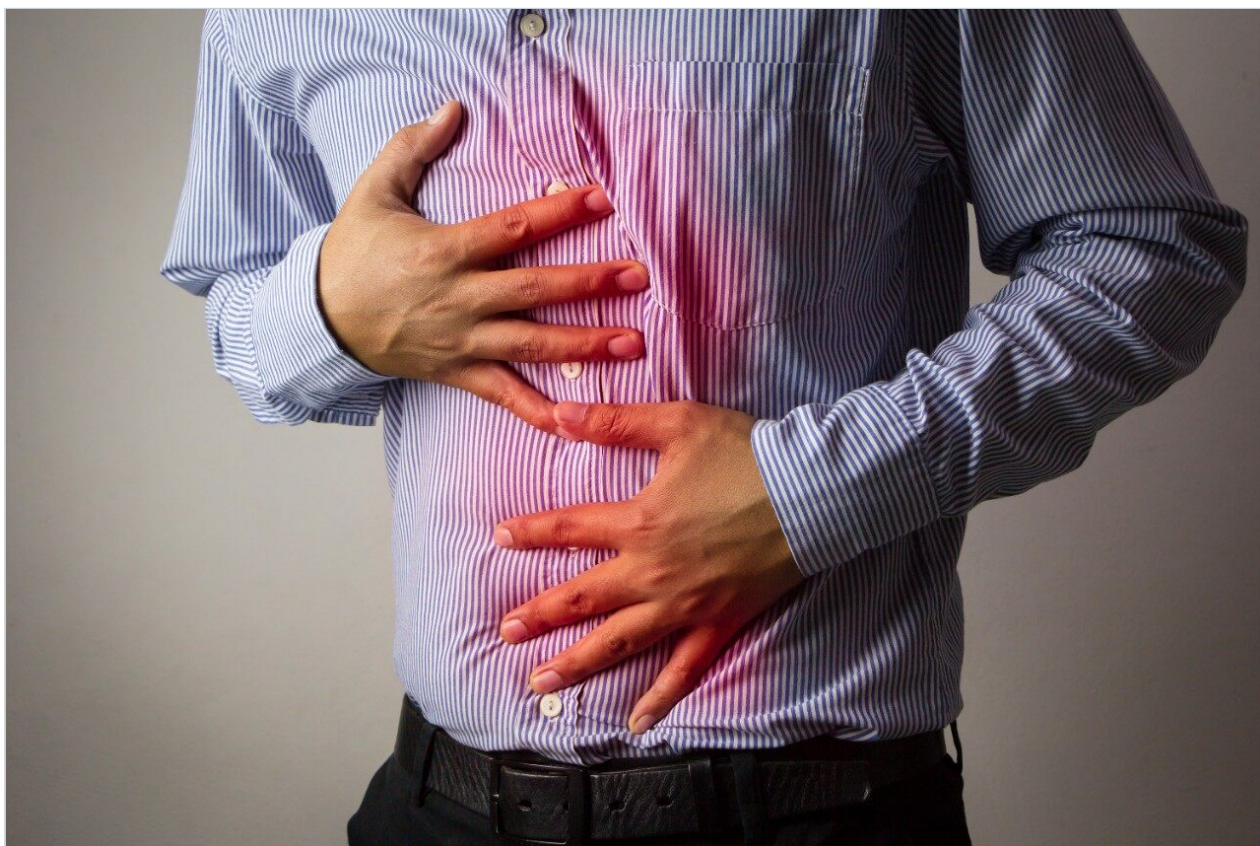


# Transferring an HPLC Method for Related Substances from Different LC Platforms to an ACQUITY Arc System

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## Abstract

This application note presents the transfer of an HPLC method for related substances of metoclopramide HCl from different LC systems to an ACQUITY Arc System.

## Benefits

- Efficient and easy transfer of HPLC and UHPLC applications from any LC platform to an ACQUITY Arc System using Arc Multi-flow path technology
- Improve laboratory efficiency and maximize productivity with ACQUITY Arc System by scaling to columns with smaller particles
- Accurate identification of components by mass detection with the ACQUITY QDa Detector

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## Introduction

Analytical methods used for testing of pharmaceutical raw materials and finished products are often transferred across organizations or to contract partners that utilize instruments from different vendors. It is therefore essential for the analytical laboratories to successfully transfer these methods between different instruments to ensure product consistency and compliance with the regulations. Effective method transfer generates equivalent results for the same analysis independent of the instrument, laboratory, or the resources. This is important to eliminate the need to revalidate the method, which is time-consuming and costly.

Transfer of chromatographic methods between different LC systems, especially from different manufacturers, can be a challenging task. Often, these instruments have different system volumes, which may cause poor chromatographic separation and peak distortion in gradient methods. Therefore, system volume differences must be accounted for when transferring gradient methods between different LC systems to achieve the same separation.

In this work, we present the transfer of an HPLC method for related substances of metoclopramide HCl from different LC systems to an ACQUITY Arc System. Additionally, we will demonstrate method improvement by scaling this method to columns with a smaller particle size. The success of the method transfer will be measured by examining chromatographic data and the system suitability results against USP specifications.<sup>2,3</sup> Overall, we will show that the ACQUITY Arc System, enabled by its Arc Multi-flow path technology, can efficiently replicate an established method from any LC platform without altering the method's gradient table. The ACQUITY QDa Detector coupled to an ACQUITY Arc System provides quick peak identification of sample components by mass detection.

## Experimental

### Solutions preparation

The related compounds of metoclopramide HCl used in this study are listed in Table 1. Separate stock solutions were prepared in methanol at 1.0 mg/mL. A metoclopramide stock solution was diluted with water to 0.5 mg/mL and spiked with related substances at 1.0% level.

Compound	Common name	Monoisotopic mass (Da)	[M + H] (Da)
API	Metoclopramide	299.14	300.1
Impurity A	4-Acetamido-5-chloro-N-(2-(diethylamino)ethyl)-2-methoxybenzamide	341.15	342.0
Impurity B	Methyl 4-acetamido-5-chloro-2-methoxybenzoate	257.05	258.1
Impurity C	4-Amino-5-chloro-2-methoxybenzoic acid	201.02	202.0
Impurity D	Methyl 4-acetamido-2-methoxybenzoate	223.08	224.1
Impurity F	4-Amino-5-chloro-N-(2-(diethylamino)ethyl)-2-hydroxybenzamide	285.12	286.0
Impurity G	2-(4-Amino-5-chloro-2-hydroxybenzamido)-N,N-diethylethanamide oxide	315.14	316.0
Impurity H	4-Acetamido-2-hydroxybenzoic acid	195.05	196.0
Impurity 9	Methyl 4-amino-2-methoxybenzoate	181.07	182.0

*Table 1. List of USP-specified related substances of metoclopramide HCl for method transfer from different LC platforms to an ACQUITY Arc System.*

For a system suitability standard, each stock solution was transferred to one vial and diluted with standard diluent (50:50 methanol/water) to 0.05 mg/mL of each analyte. The mixture was then diluted with standard diluent (50:50 methanol/water) to 15 µg/mL for system suitability determination.

### Method conditions

LC systems for method transfer:

- Agilent 1100 Series LC System with quaternary pump and DAD Detector
- Agilent 1260 Infinity Quaternary LC System with low dispersion (3 µL) heat exchanger and DAD Detector
- Alliance HPLC System with 2998 PDA Detector
- ACQUITY Arc System with 2998 PDA and ACQUITY QDa Detectors, passive pre-heater, and flow path 1

The HPLC method for related substances of metoclopramide HCl was scaled to 3.5 and 2.5 µm particle size columns and then to UPLC using the Waters ACQUITY UPLC Columns Calculator.<sup>1</sup> The flow rate, injection volume, and gradient times were geometrically scaled to preserve separation integrity.

## HPLC conditions (5 µm)

Column:	XSelect CSH C <sub>18</sub> 4.6 x 150 mm, 5 µm
Flow rate:	2.9 mL/min
Injection volume:	10.0 µL
Separation:	Gradient

### Gradient:

Step	Time(minutes)	Solvent A(%)	Solvent B(%)
1	Initial	95.0	5.0
2	15.00	40.0	60.0
3	16.50	40.0	60.0
4	16.80	95.0	5.0
5	21.00	95.0	5.0

## Scaled Conditions (3.5 µm)

Column:	XSelect CSH C <sub>18</sub> 3.0 x 100 mm, 3.5 µm
Flow rate:	1.2 mL/min
Injection volume:	2.8 µL
Separation:	Gradient

### Gradient:

Step	Time(minutes)	Solvent A(%)	Solvent B(%)
1	Initial	95.0	5.0
2	10.00	40.0	60.0
3	11.00	40.0	60.0
4	11.20	95.0	5.0
5	14.00	95.0	5.0

### Scaled conditions (2.5 µm)

Column: XSelect CSH C<sub>18</sub> 3.0 x 75 mm, 2.5 µm

Flow rate: 1.2 mL/min

Injection volume: 2.1 µL

Separation: Gradient

### Gradient:

Step	Time(minutes)	Solvent A(%)	Solvent B(%)
1	Initial	95.0	5.0
2	7.50	40.0	60.0
3	8.25	40.0	60.0
4	8.40	95.0	5.0
5	10.50	95.0	5.0

Other LC parameters the same across systems

Solvent A:	0.1% formic acid in water
Solvent B:	0.1% formic acid in methanol
Purge/Sample wash:	50:50 water/methanol
Seal wash:	90:10 water/acetonitrile
UV detector:	$\lambda$ range: 210–400 nm, derived at 270 nm, sampling rate: 20 pts/sec
<b>MS conditions</b>	
Mass detector:	ACQUITY QDa Detector (Alliance, ACQUITY Arc, ACQUITY UPLC H-Class)
Ionization mode:	ESI+, ESIAcquisition
range:	100–440 <i>m/z</i>
Sampling rate:	10 pts/sec
Capillary voltage:	Pos: 0.8 kV, Neg: 0.8 kV
Cone voltage:	15 V
Probe temperature:	600 °C
Data:	Centroid
System Control, Data Acquisition, and Analysis:	Empower 3 FR2 CDS Software

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## Results and Discussion

### Method transfer between different LC platforms

For successful transfer of the gradient methods between different LC systems, the dwell volume of each system should be characterized and compensated for in efforts to maximize the success of the technology transfer. The ACQUITY Arc System is enabled by Arc Multi-flow path technology (Figure 1), which allows the user to select the fluidic path to emulate dwell volume and mixing behavior of different LC systems. By selecting path 1 or path 2, established LC methods can be easily replicated without any manual intervention or the need to alter the gradient table.

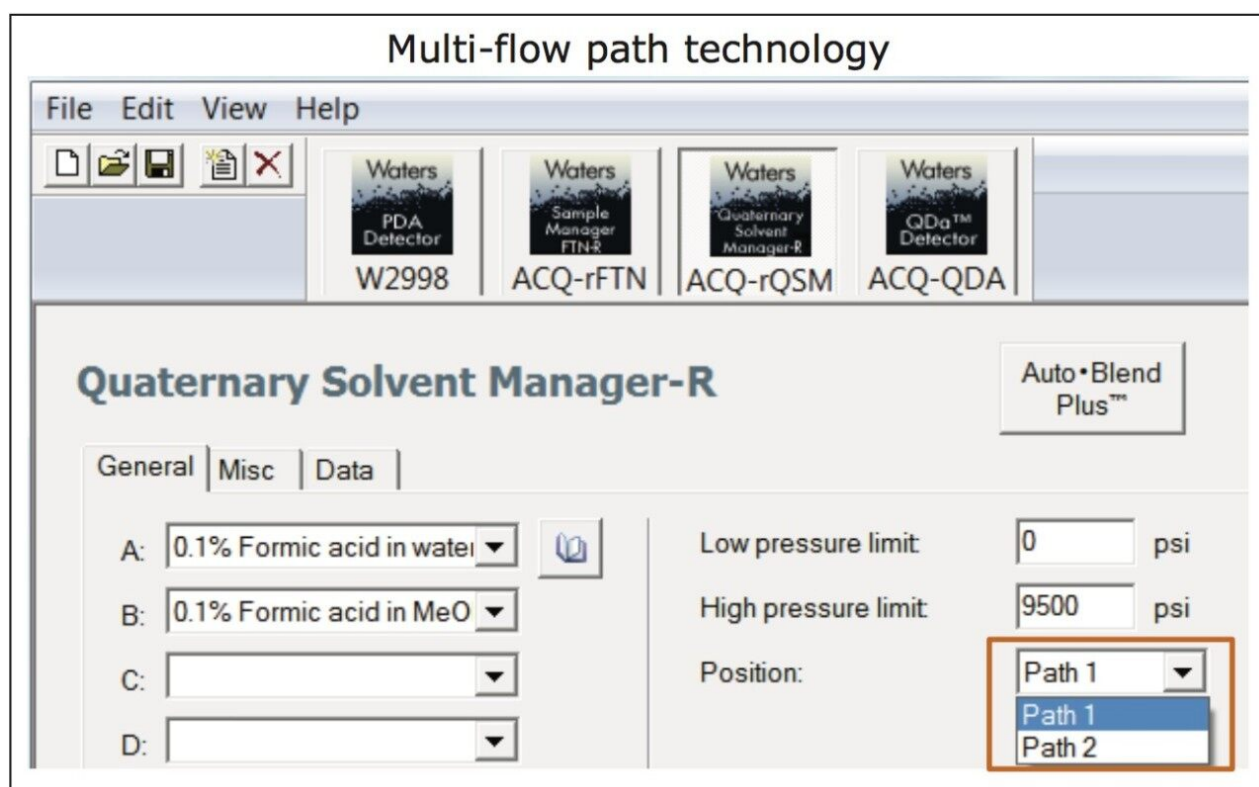


Figure 1. Instrument method editor of the ACQUITY Arc System. The multi-flow path technology gives users the ability to select fluidic path for HPLC and UHPLC applications.

In this study, the HPLC assay method for related substances of metoclopramide HCl was run on an Agilent 1100, Agilent 1260, Alliance HPLC Systems, and an ACQUITY Arc System using fluidic path 1. The chromatographic data of a sample containing metoclopramide API with 1.0% of related substances acquired on all four systems is shown in Figure 2. The chromatographic separation produced on an ACQUITY Arc System is comparable with the results obtained on the Agilent and Alliance HPLC Systems.



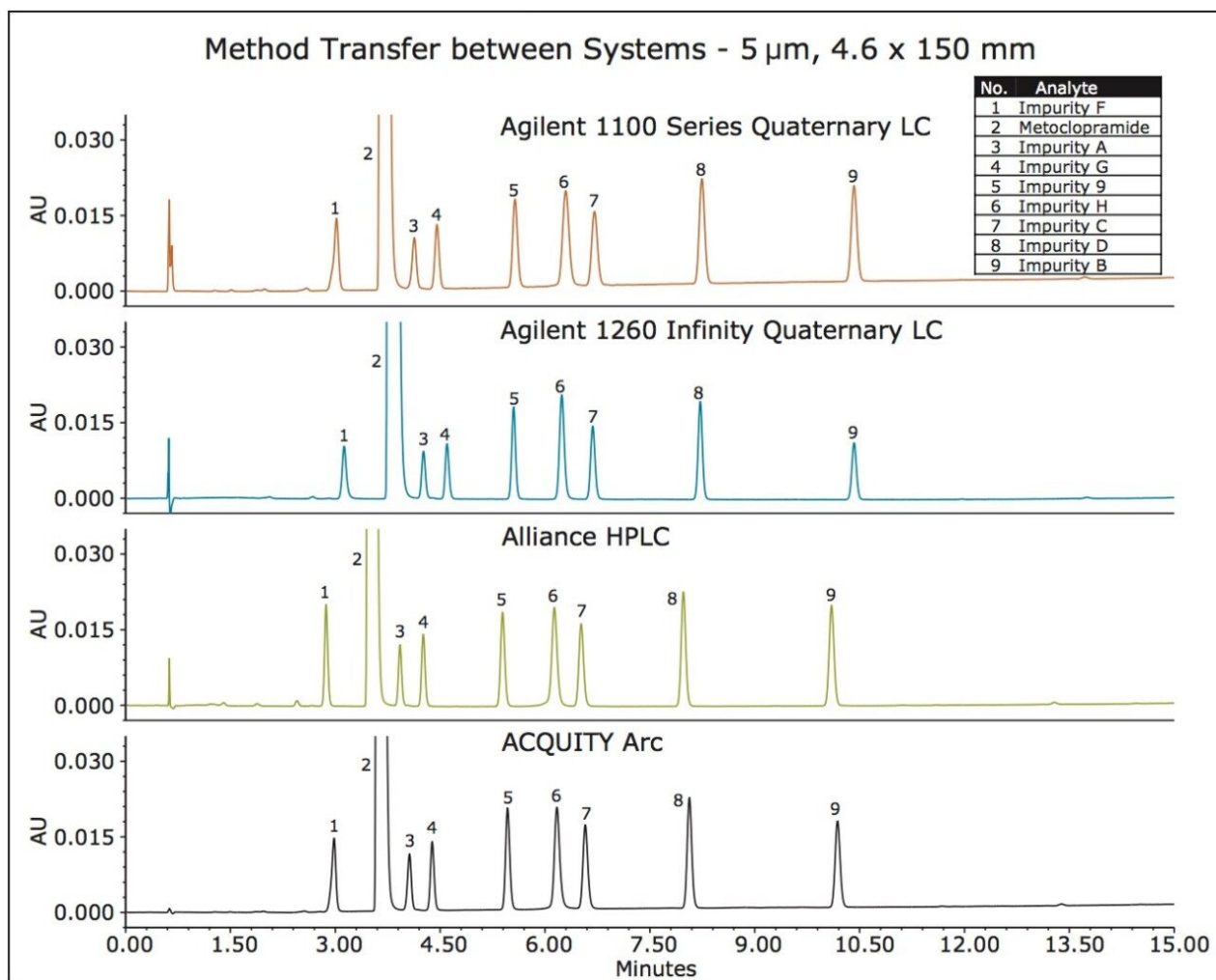


Figure 2. HPLC data of the metoclopramide API with 1.0% of related substances for the method transfer from an Agilent 1100 Series LC, Agilent 1260 Infinity Quaternary LC, and Alliance HPLC Systems to an ACQUITY Arc System.

#### Confirming peak identity

The mass spectra data acquired using an ACQUITY QDa Detector coupled with an ACQUITY Arc System was used to confirm the identity of metoclopramide and related substances by mass detection (Figure 3).



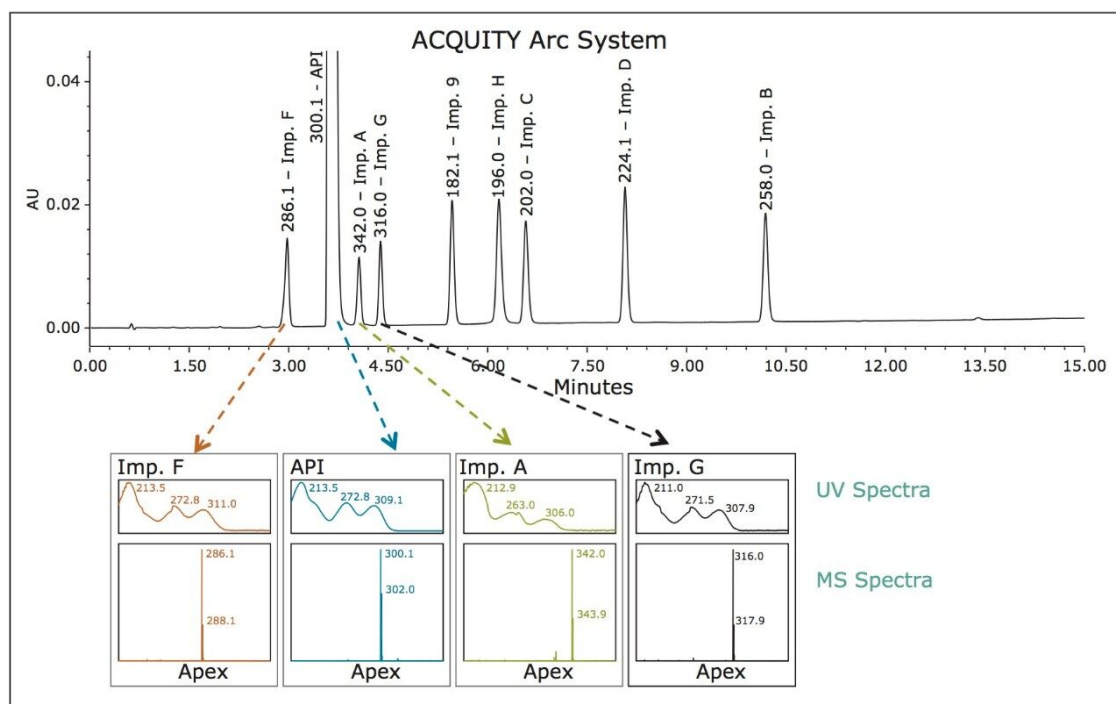


Figure 3. Confirming peak identity by mass detection using mass spectral data acquired using an ACQUITY QDa Detector. Mass analysis window from Empower 3 software shows UV and mass spectral plots. This data enabled quick identification of the sample components.

Performance of the HPLC method run on the ACQUITY Arc System was verified by evaluating system suitability results of five replicate injections of the system suitability standard against the requirements listed in the USP Monograph for Metoclopramide Hydrochloride<sup>2</sup> and USP General Chapter, <621> Chromatography.<sup>3</sup> The system suitability results were also compared to the results obtained on the Agilent 1100, Agilent 1260, and Alliance HPLC Systems.

The recently updated USP Monograph for Metoclopramide Hydrochloride lists a method for analysis of several organic impurities (A, B and D).<sup>2</sup> The monograph states that the USP resolution between metoclopramide API and Imp. A must not be less than 3.0. The USP resolution of our method run on all four systems met the USP criteria (Table 2). Additionally, a USP resolution of  $\geq 3.1$  was observed for all peaks on the ACQUITY Arc System. The USP tailing factors were less than 1.5 and comparable across the systems (Table 2). The retention times and peak areas repeatability of the method run on the ACQUITY Arc System were substantially lower than the specifications defined in the General Chapter, <621> Chromatography of less than 2.0% RSD and comparable with the results acquired on other LC systems (Table 3).

Comp.	USP resolution					USP peak tailing				
	Agilent 1100	Agilent 1260	Alliance	ACQUITY Arc	USP criteria*	Agilent 1100	Agilent 1260	Alliance	ACQUITY Arc	USP criteria
Imp. F	n/a	n/a	n/a	n/a	n/a	0.8	1.2	1.1	0.9	1.5
API	6.1	7.0	7.8	6.5	3.0	1.0	1.2	1.2	1.1	
Imp. A	3.3	3.7	4.0	3.6	n/a	0.9	1.1	1.1	1.0	
Imp. G	2.8	3.4	3.7	3.3		1.1	1.1	1.1	1.1	
Imp. 9	9.1	9.4	11.1	10.3		1.1	1.1	1.1	1.1	
Imp. H	4.8	6.0	5.9	5.6		1.1	1.1	1.0	1.1	
Imp. C	2.5	3.6	2.9	3.1		1.1	1.1	1.1	1.1	
Imp. D	10.1	13.2	11.8	11.8		1.1	1.0	1.1	1.1	
Imp. B	14.3	18.4	16.8	16.5		1.1	1.0	1.1	1.1	

Table 2. System suitability. Comparative results of the USP resolution and peak tailing for the method transfer from an Agilent 1100, Agilent 1260, and Alliance HPLC Systems to an ACQUITY Arc System.

\*The USP Monograph for Metoclopramide Hydrochloride lists one system suitability requirement that the resolution for metoclopramide API and Imp. A is not less than 3.0.

Comp.	%RSD of retention times					%RSD of peak areas				
	Agilent 1100	Agilent 1260	Alliance	ACQUITY Arc	USP criteria	Agilent 1100	Agilent 1260	Alliance	ACQUITY Arc	USP criteria
Imp. F	0.02	0.05	0.24	0.09	2.0	0.26	0.26	0.17	0.04	2.0
API	0.01	0.04	0.24	0.05		0.28	0.11	0.17	0.10	
Imp. A	0.02	0.03	0.25	0.04		0.32	0.11	0.19	0.02	
Imp. G	0.02	0.03	0.22	0.03		0.35	0.12	0.18	0.13	
Imp. 9	0.01	0.00	0.14	0.03		0.45	0.08	0.16	0.18	
Imp. H	0.01	0.02	0.13	0.03		0.31	0.21	0.29	0.20	
Imp. C	0.01	0.01	0.11	0.02		0.34	0.18	0.17	0.36	
Imp. D	0.01	0.01	0.10	0.04		0.31	0.10	0.15	0.16	
Imp. B	0.01	0.02	0.09	0.03		0.66	0.14	0.22	0.22	

Table 3. System suitability. Comparative results of the retention times and peak areas repeatability for the method transfer from an Agilent 1100 Series LC, Agilent 1260 Infinity Quaternary LC, and Alliance HPLC Systems to an ACQUITY Arc System.

## Method transfer to improve productivity

Method transfer to the columns with smaller particles results in shorter runs times and faster linear velocities that can be used to increase throughput and productivity. In order to properly transfer a method to a column with smaller particle size, the resolving power of the original method must be maintained. This is accomplished by using a column that has the same ratio of column length (L) to particle size (dp) as the original method. The flow rate, injection volume, and gradient times are scaled properly to maintain the

same separation integrity and can be achieved using the Waters ACQUITY UPLC Columns Calculator.

In this study, the HPLC method for related substances of metoclopramide HCl was scaled to the 3.5 and 2.5  $\mu\text{m}$  particle columns and run on the ACQUITY Arc System. Scaling the HPLC method to columns with smaller particles has resulted in a reduced run time and solvent consumption, while maintaining integrity of the chromatographic separation (Figure 4). Migration to a 3.5  $\mu\text{m}$  particle size column reduced analysis time by 33% and solvent consumption by 72%. Scaling to a 2.5  $\mu\text{m}$  particle size column reduced analysis time and solvent consumption by 50% and 79%, respectively.

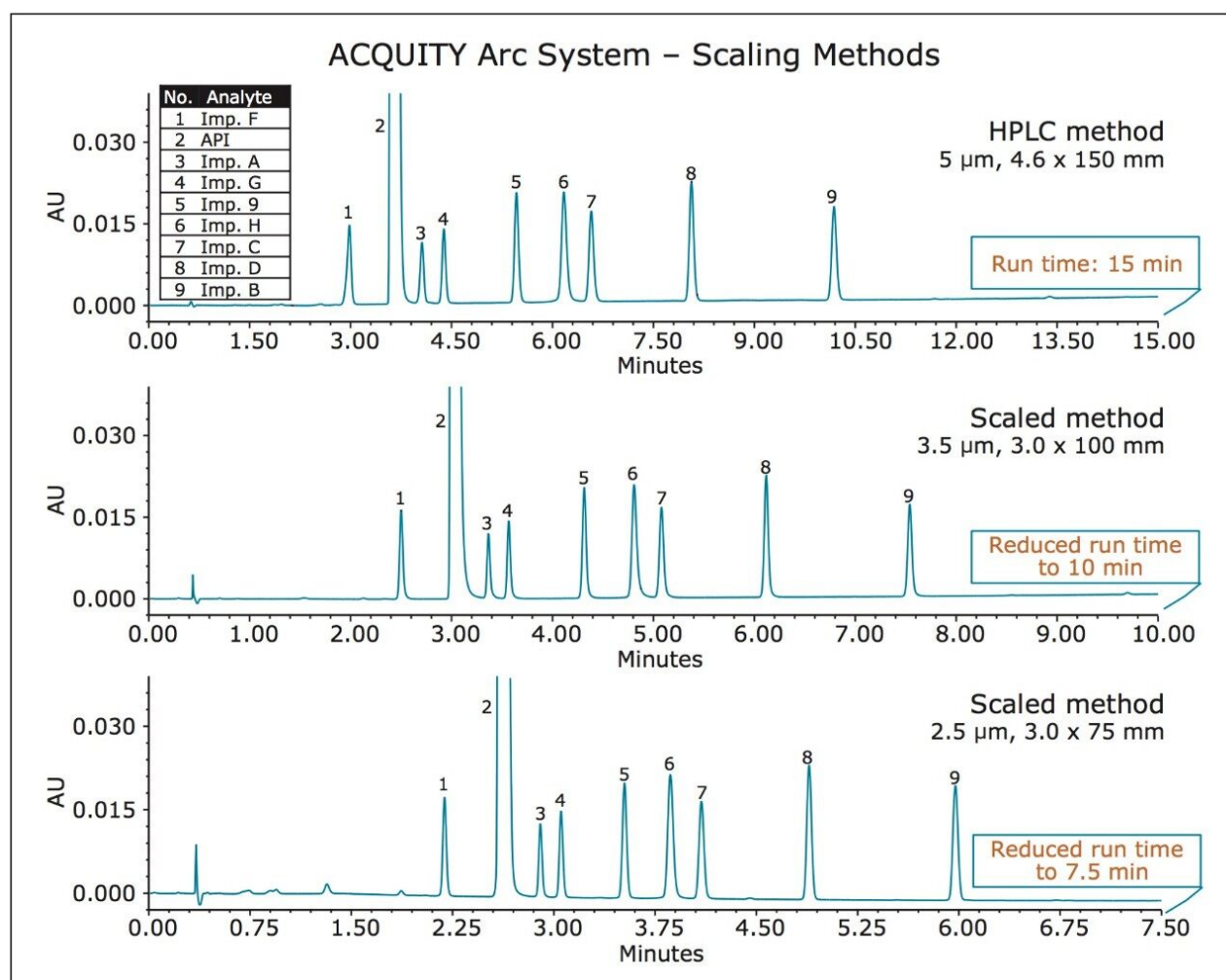


Figure 4. Chromatographic separation of metoclopramide and its related substances on three different columns with the same resolving power ( $L/pd$ ) ratio run on an ACQUITY Arc System.

Comparison of the system suitability results of five replicate injections of the system suitability standard for the HPLC and scaled methods run on an ACQUITY Arc System are shown in Table 4. The retention times and peak areas repeatability of the scaled methods were substantially lower than the USP specifications of

less than 2.0% RSD and comparable to the HPLC method. The USP resolution values and the USP peak tailing factors were also comparable.

Comp.	%RSD of retention times			%RSD of peak areas			USP resolution			USP peak tailing		
	5 $\mu$ m	3.5 $\mu$ m	2.5 $\mu$ m	5 $\mu$ m	3.5 $\mu$ m	2.5 $\mu$ m	5 $\mu$ m	3.5 $\mu$ m	2.5 $\mu$ m	5 $\mu$ m	3.5 $\mu$ m	2.5 $\mu$ m
Imp. F	0.09	0.10	0.05	0.04	0.20	0.12	n/a	n/a	n/a	0.9	1.2	1.1
API	0.05	0.08	0.05	0.10	0.05	0.20	6.5	9.4	9.4	1.1	1.3	1.2
Imp. A	0.04	0.06	0.05	0.02	0.10	0.12	3.6	5.3	5.0	1.0	1.3	1.2
Imp. G	0.03	0.07	0.05	0.13	0.10	0.08	3.3	3.6	3.2	1.1	1.2	1.1
Imp. 9	0.03	0.06	0.04	0.18	0.12	0.16	10.3	11.9	8.6	1.1	1.1	1.1
Imp. H	0.03	0.10	0.04	0.20	0.07	0.21	5.6	6.4	5.1	1.1	1.3	1.1
Imp. C	0.02	0.07	0.04	0.36	0.07	0.15	3.1	3.3	3.2	1.1	1.2	1.1
Imp. D	0.04	0.05	0.03	0.16	0.12	0.11	11.8	13.7	11.7	1.1	1.1	1.1
Imp. B	0.03	0.04	0.04	0.22	0.05	0.13	16.5	18.7	15.7	1.1	1.1	1.0

*Table 4. System suitability for HPLC and scaled methods run on an ACQUITY Arc System. Results show comparable performance for both HPLC and scaled methods.*

Overall, the data shows that the ACQUITY Arc System easily accepts and improves methods to increase efficiency and productivity. It accommodates columns with 2.5 to 5.0  $\mu$ m particles. This provides the flexibility to support a wide-range of LC applications.

## Conclusion

The ACQUITY Arc System successfully replicated the assay method for related substances of metoclopramide HCl run on an Agilent 1100 Series LC, Agilent 1260 Infinity Quaternary LC, and Alliance HPLC Systems. The ACQUITY QDa Detector coupled to an ACQUITY Arc System enabled quick peak identification using mass spectral data. Migration from 5.0 to 3.5 and 2.5  $\mu$ m particle size columns reduced analysis time and solvent consumption. The ACQUITY QDa Detector coupled to an ACQUITY Arc System enabled quick peak identification using mass spectral data.

Enabled by a unique Multi-flow path technology, the ACQUITY Arc System easily accepts and replicates methods from a variety of platforms without compromising method integrity. The ACQUITY Arc System provides powerful LC performance and secures the lab's investment by ensuring integration with new technologies such as the ACQUITY QDa Detector and Empower Chromatography Data System (CDS) software. Overall, the ACQUITY Arc System allows efficient method transfer from any LC platform, as well as flexible method optimization.

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## References

1. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Corp. 2010: 720003721en.
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4. ACQUITY Arc System, [http://www.waters.com/waters/en\\_US/ACQUITY-Arc-System/nav.htm?cid=134844390](http://www.waters.com/waters/en_US/ACQUITY-Arc-System/nav.htm?cid=134844390)

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