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Application Note

Seamless Method Transfer and Migration Between Instruments: Replicating an Aspirin and Related Substances Method on an Arc HPLC System

Margaret Maziarz, Paul D. Rainville

Waters Corporation

Abstract

Replicating results and demonstrating equivalent performance for same analysis are critical for a successful method migration to a different LC system or transfer between labs. In this work, seamless migration of a related substances of aspirin active pharmaceutical ingredient (API) method to an Arc HPLC System is presented by demonstrating equivalent method performance. The Arc HPLC System successfully replicated the quality of the chromatographic separation, system suitability and related substances assay results generated on other comparable HPLC Systems.

Benefits

- Seamless and easy migration of HPLC methods to an Arc HPLC System

- Robust, reliable, and reproducible performance of an Arc HPLC System
- Improve laboratory efficiency and maximize productivity

Introduction

Validated analytical methods are often transferred across laboratories and to partners such as CRO's and CMO's that are equipped with LC systems from different vendors. When methods are transferred, the receiving laboratory needs to generate equivalent results for the same analysis to assure quality control and compliance with the regulatory guidelines.[1] Additionally, if a new modern instrument is introduced into a laboratory, it must also be capable of replicating all the validated method's performance attributes.

Migration 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. This may produce different results for the same analysis generated across instruments.

In this work, migration of an HPLC method for related substances analysis of aspirin API to an Arc HPLC system is presented. The equivalent performance is demonstrated by examining chromatographic separation, system suitability results and related substances assay results. We show that the Arc HPLC System successfully replicates methods, producing equivalent chromatographic separation and analytical results generated by the method to the results obtained on the comparable LC systems.

The Arc HPLC System is a robust, reliable, and reproducible modern instrument suitable for routine testing that can replicate established HPLC methods.[2]

Experimental

Sample Description

Aspirin and impurities standard mixture

Separate stock solutions with related substances and aspirin API were prepared in diluent (60:40 water/acetonitrile with 0.1% formic acid) at 1.0 and 5.0 mg/mL, respectively. The API stock solution was diluted with diluent to 0.1 mg/mL and spiked with related substances at 10% level.

Aspirin and its related substances specified by the European Pharmacopeia[3] are listed in Table 1.

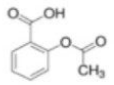
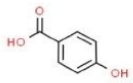
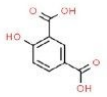
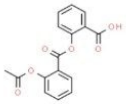
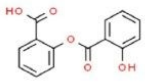
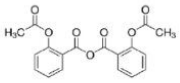
Compound	Name	Molecular formula	Monoisotopic mass (Da)	Structure
Aspirin API	2-Acetoxybenzoic acid, O-Acetylsalicylic acid	$C_9H_8O_4$	180.04	
Impurity A	p-Salicylic acid, 4-hydroxybenzoic acid	$C_7H_6O_3$	138.03	
Impurity B	4-Hydroxy-1,3-benzenedicarboxylic acid, 4-Hydroxyisophthalic acid	$C_8H_6O_5$	182.02	
Impurity C	Salicylic acid; 2-Hydroxybenzoic acid o-Hydroxybenzoic acid	$C_7H_6O_3$	138.03	
Impurity D	Acetylsalicylsalicylic acid, 2-(Acetyloxy) benzoic acid	$C_{16}H_{12}O_6$	300.06	
Impurity E	2-((2-hydroxybenzoyl)oxy) benzoic acid, salsalate	$C_{14}H_{10}O_5$	258.05	
Impurity F	2-Acetoxybenzoic anhydride, O-acetylsalicylic anhydride,	$C_{18}H_{14}O_7$	342.07	

Table 1. List of compounds for method development. Related substances (impurities) of aspirin API.

Aspirin drug tablets

Crushed tablets were dissolved in diluent (60:40 water/acetonitrile with 0.1% formic acid) at 1.6 mg/mL of aspirin by sonication for 10 minutes. After extraction, sample test solutions were centrifuged for 10 minutes at 3000 rpm and diluted to 0.5 mg/mL with diluent. Solutions were filtered through 0.2 µm nylon syringe (Waters p/n# WAT200524 <https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/wat200524-

acrodisc-syringe-filter-nylon-13-mm-02--m-aqueous-100-pk.html>) filter prior analysis.

Conditions

LC systems:	Agilent 1260 Infinity II LC System with DAD Detector Alliance e2695 HPLC System with 2998 PDA Detector, Column Heater/Cooler with Passive Pre-heater Arc HPLC System with 2998 PDA Detector, Column Heater/Cooler with Passive Pre-heater
Vials:	LCMS Maximum Recovery 2 mL volume, (p/n 600000670CV)
Column(s):	XSelect HSS T3, 4.6 x 150 mm, 3.5 µm (p/n 186004786)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	15 µL
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Wash solvents:	Purge/sample wash: 60:40 water/acetonitrile Seal wash: 90:10 water/acetonitrile
Detector settings:	PDA: 210–400 nm (derived at 237 nm)

Gradient

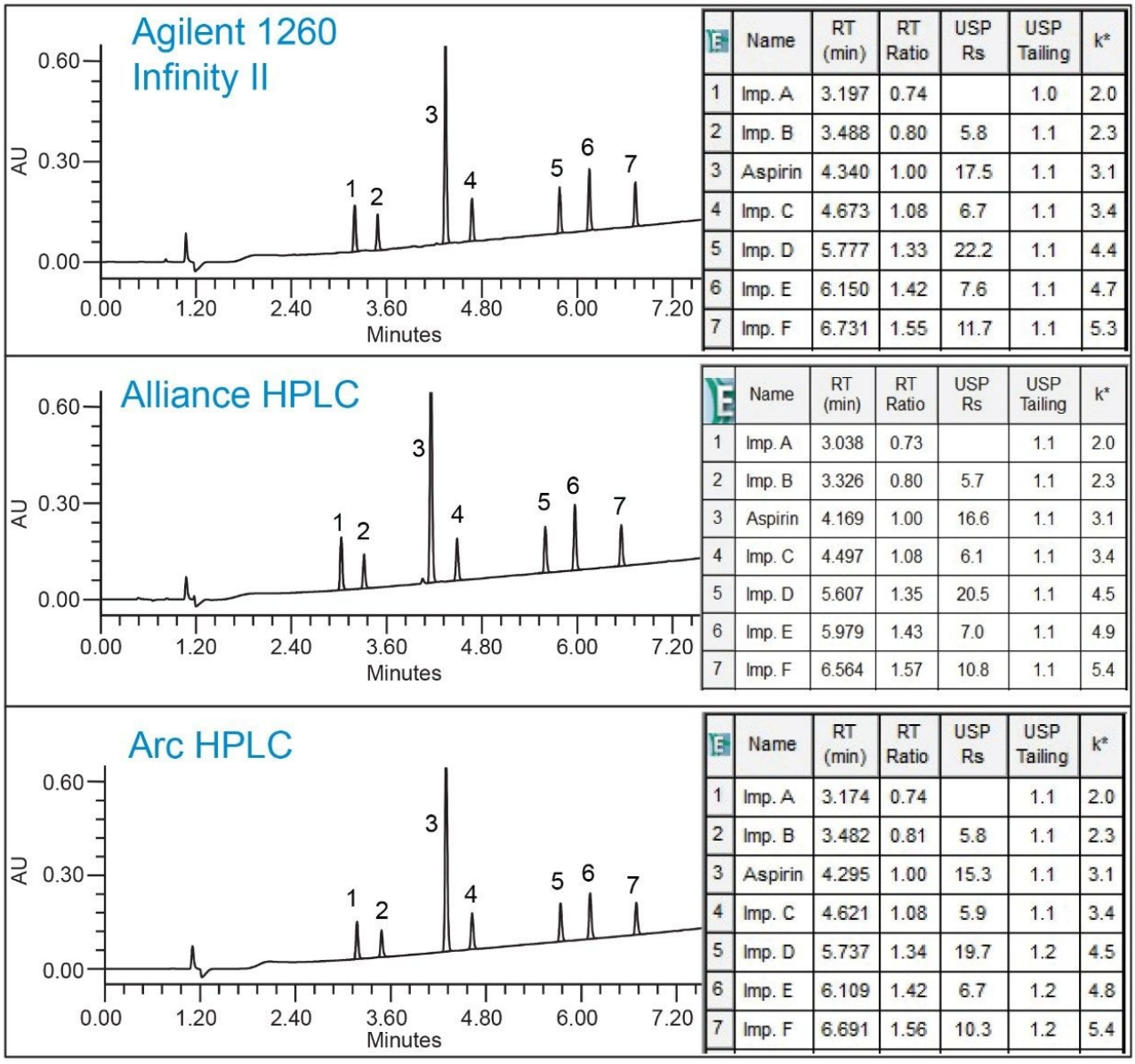
Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	1.8	95.0	5.0	6
0.10	1.8	95.0	5.0	6
7.60	1.8	5.0	95.0	6
9.20	1.8	5.0	95.0	6
9.30	1.8	95.0	5.0	6
13.00	1.8	95.0	5.0	6

Software

Chromatography Data Software (CDS): Empower 3 FR4 SR2

Results and Discussion

The analysis for aspirin and its associated related substances was performed under MS compatible conditions, based on a previously developed method.[4] The method was scaled to 3.5 µm particle size column using columns calculator[5] and run on Agilent 1260 Infinity II, Alliance e2695, and Arc HPLC systems. The chromatographic separation produced on the Arc HPLC System was comparable with the data on the Agilent and Alliance HPLC systems (Figure 1). The Arc HPLC System resulted in a USP resolution of ≥5.8 for between all analytes, peak tailing of 1.1–1.2, and retentivity factor ≥2.0.



	Name	RT (min)	RT Ratio	USP Rs	USP Tailing	k*
1	Imp. A	3.197	0.74		1.0	2.0
2	Imp. B	3.488	0.80	5.8	1.1	2.3
3	Aspirin	4.340	1.00	17.5	1.1	3.1
4	Imp. C	4.673	1.08	6.7	1.1	3.4
5	Imp. D	5.777	1.33	22.2	1.1	4.4
6	Imp. E	6.150	1.42	7.6	1.1	4.7
7	Imp. F	6.731	1.55	11.7	1.1	5.3

	Name	RT (min)	RT Ratio	USP Rs	USP Tailing	k*
1	Imp. A	3.038	0.73		1.1	2.0
2	Imp. B	3.326	0.80	5.7	1.1	2.3
3	Aspirin	4.169	1.00	16.6	1.1	3.1
4	Imp. C	4.497	1.08	6.1	1.1	3.4
5	Imp. D	5.607	1.35	20.5	1.1	4.5
6	Imp. E	5.979	1.43	7.0	1.1	4.9
7	Imp. F	6.564	1.57	10.8	1.1	5.4

	Name	RT (min)	RT Ratio	USP Rs	USP Tailing	k*
1	Imp. A	3.174	0.74		1.1	2.0
2	Imp. B	3.482	0.81	5.8	1.1	2.3
3	Aspirin	4.295	1.00	15.3	1.1	3.1
4	Imp. C	4.621	1.08	5.9	1.1	3.4
5	Imp. D	5.737	1.34	19.7	1.2	4.5
6	Imp. E	6.109	1.42	6.7	1.2	4.8
7	Imp. F	6.691	1.56	10.3	1.2	5.4

Figure 1. Chromatographic separation for aspirin and its impurities for method transfer between systems. UV 237 nm.

Additionally, the relative retention times (RRT) of related substances were compared across the systems. The RRT values are often used to aid peak identification in chromatographic separation, therefore is it important that they are the same when performing transferring related substances assay method on another system. In this study, the RRT were calculated by comparing retention of each related substance to the aspirin retention time.

The data showed that the RRT values obtained on the Arc HPLC System were in an agreement with the results on the Agilent and Alliance systems (Figure 2). Overall, the Arc HPLC System successfully replicated the quality of the chromatographic separation without the alteration to the method.

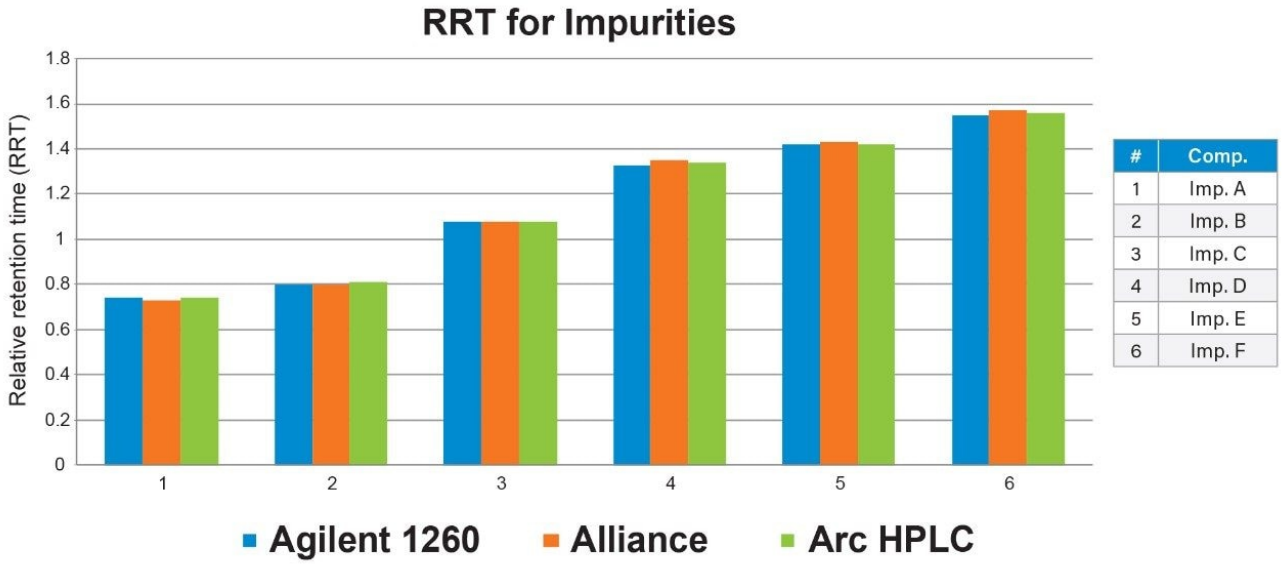
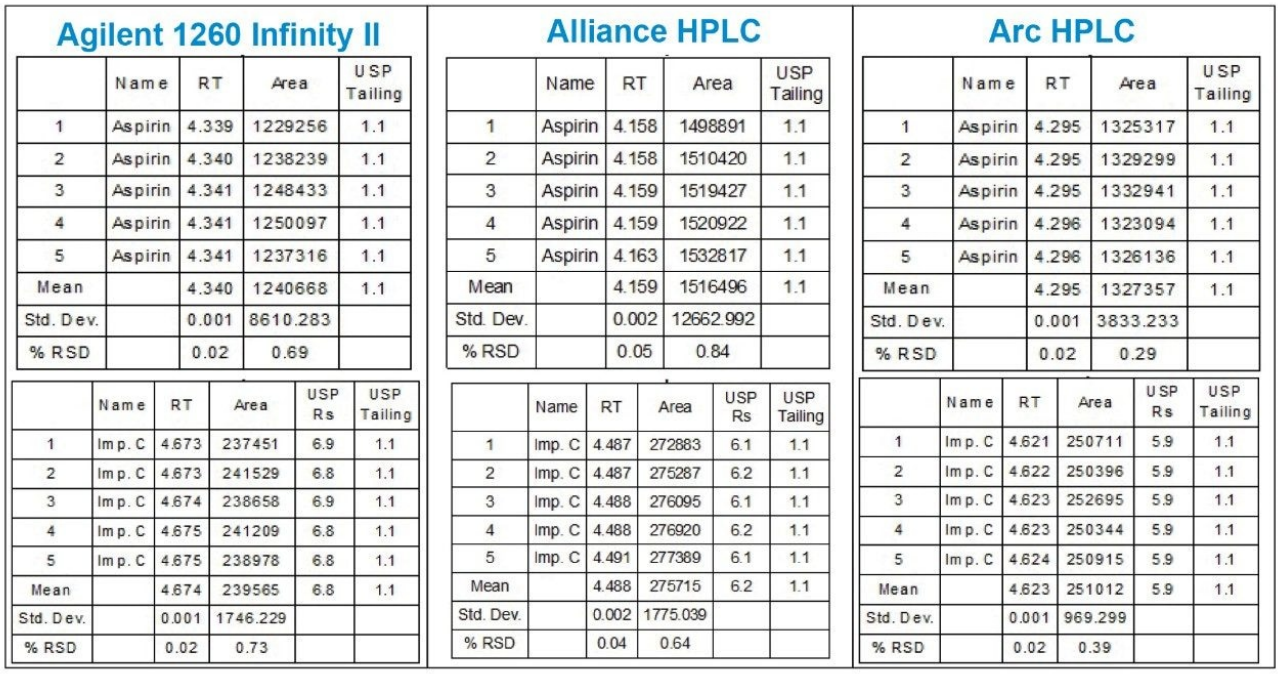


Figure 2. Relative retention times (RRT) ratios for related substances (impurities) with respect to the aspirin retention time.

Performance of the method on the Arc HPLC System was assessed by measuring system suitability of five replicate injections of the standard mixture following the requirements listed in the USP monograph for aspirin tablets[5] and compared with other systems. The USP system suitability requirements included:

- Resolution: not less than (NLT) 2.0 between salicyclic acid (impurity C) and aspirin
- Relative standard deviation (RSD): not more than (NMT) 4.0%, salicyclic acid (impurity C)

The system suitability results generated on all three systems met the USP criteria (Figure 3). Additionally, the RSD of impurity C peak areas and retention times generated on the Arc HPLC System was lower than the USP requirement of NMT 4.0% and the results acquired on the other LC systems.



Agilent 1260 Infinity II

	Name	RT	Area	USP Tailing
1	Aspirin	4.339	1229256	1.1
2	Aspirin	4.340	1238239	1.1
3	Aspirin	4.341	1248433	1.1
4	Aspirin	4.341	1250097	1.1
5	Aspirin	4.341	1237316	1.1
Mean		4.340	1240668	1.1
Std. Dev.		0.001	8610.283	
% RSD		0.02	0.69	

	Name	RT	Area	USP Rs	USP Tailing
1	Imp. C	4.673	237451	6.9	1.1
2	Imp. C	4.673	241529	6.8	1.1
3	Imp. C	4.674	238658	6.9	1.1
4	Imp. C	4.675	241209	6.8	1.1
5	Imp. C	4.675	238978	6.8	1.1
Mean		4.674	239565	6.8	1.1
Std. Dev.		0.001	1746.229		
% RSD		0.02	0.73		

Alliance HPLC

	Name	RT	Area	USP Tailing
1	Aspirin	4.158	1498891	1.1
2	Aspirin	4.158	1510420	1.1
3	Aspirin	4.159	1519427	1.1
4	Aspirin	4.159	1520922	1.1
5	Aspirin	4.163	1532817	1.1
Mean		4.159	1516496	1.1
Std. Dev.		0.002	12662.992	
% RSD		0.05	0.84	

	Name	RT	Area	USP Rs	USP Tailing
1	Imp. C	4.487	272883	6.1	1.1
2	Imp. C	4.487	275287	6.2	1.1
3	Imp. C	4.488	276095	6.1	1.1
4	Imp. C	4.488	276920	6.2	1.1
5	Imp. C	4.491	277389	6.1	1.1
Mean		4.488	275715	6.2	1.1
Std. Dev.		0.002	1775.039		
% RSD		0.04	0.64		

Arc HPLC

	Name	RT	Area	USP Tailing
1	Aspirin	4.295	1325317	1.1
2	Aspirin	4.295	1329299	1.1
3	Aspirin	4.295	1332941	1.1
4	Aspirin	4.296	1323094	1.1
5	Aspirin	4.296	1326136	1.1
Mean		4.295	1327357	1.1
Std. Dev.		0.001	3833.233	
% RSD		0.02	0.29	

	Name	RT	Area	USP Rs	USP Tailing
1	Imp. C	4.621	250711	5.9	1.1
2	Imp. C	4.622	250396	5.9	1.1
3	Imp. C	4.623	252695	5.9	1.1
4	Imp. C	4.623	250344	5.9	1.1
5	Imp. C	4.624	250915	5.9	1.1
Mean		4.623	251012	5.9	1.1
Std. Dev.		0.001	969.299		
% RSD		0.02	0.39		

Figure 3. System suitability results for method transfer across systems.

Related substances assay results

The assay for related substances content (% impurity) was determined by comparing peak areas of each related substance to the aspirin peak area. Example of chromatographic data of the tablet sample solution analysis on Arc HPLC is shown in Figure 4. The assay results were compared against the criteria specified in the impurities procedure of the USP monograph for aspirin tablets.[5] The USP acceptance criteria for impurities in coated tablets includes not more than (NMT) 3.0% of salicyclic acid (impurity C). The related substances results generated on Agilent 1260, Alliance, and Arc HPLC systems met the USP criteria (Table 2).

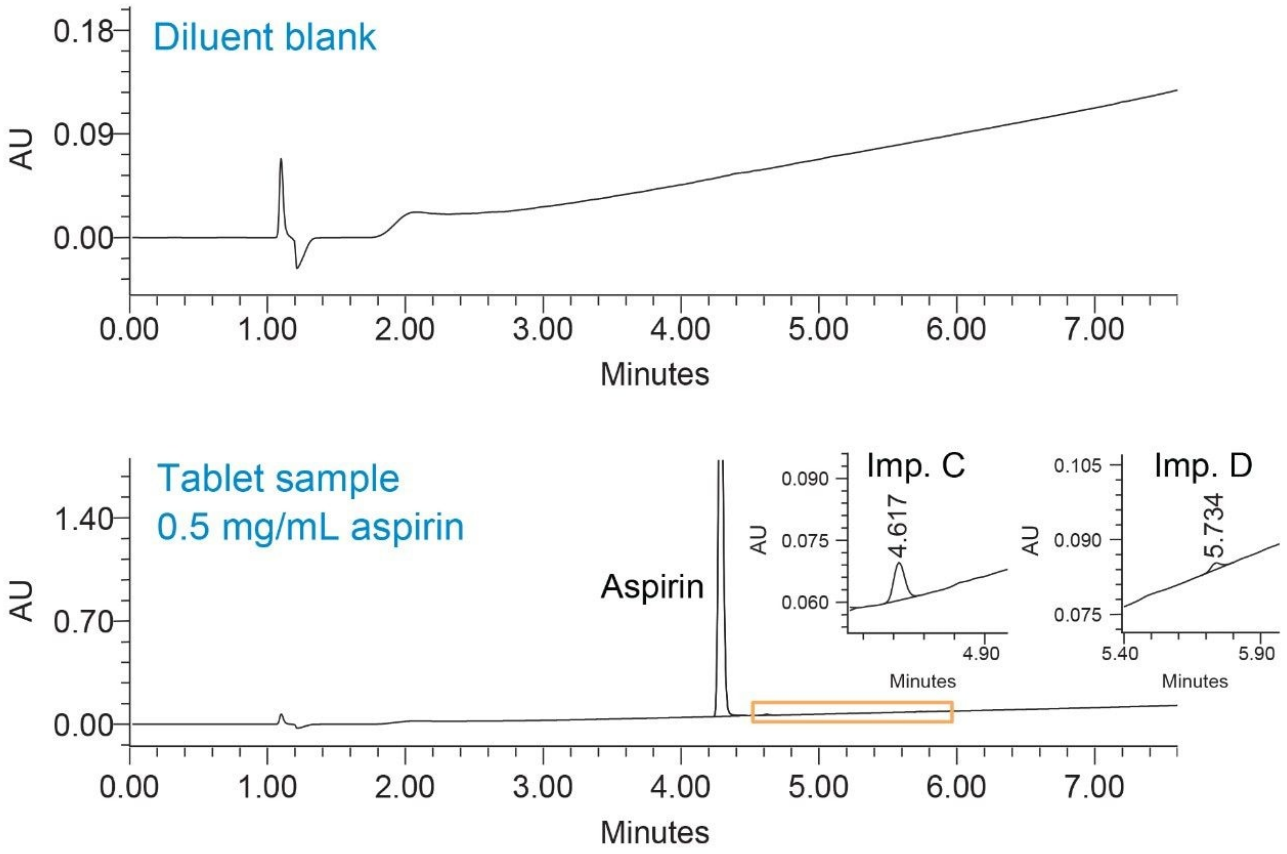


Figure 4. Analysis of tablet sample solution for related substances content. Samples at 0.5 mg/mL of aspirin on the Arc HPLC System. UV at 237 nm.

System	% Imp. C	% Imp. D
Agilent 1260 Infinity II	0.41	0.04
Alliance HPLC	0.37	0.05
Arc HPLC	0.38	0.04

Table 2. Assay results for related substances (%) in the tablet sample solution met the USP criteria of NMT 3.0% of salicyclic acid (impurity C).

Conclusion

The Arc HPLC System successfully replicated the assay method for related substances of aspirin active ingredient run on the Agilent 1260 Infinity II and Alliance HPLC systems. The chromatographic separation, relative retention times values, system suitability and related substances assay results produced on the Arc HPLC System met the acceptance criteria.

Overall, the Arc HPLC System easily accepts and replicates existing LC methods from a variety of platforms, producing equivalent test results without compromising method integrity. This eliminates the need to change and revalidate existing methods and remains in compliance with regulatory guidelines as the asset replacement does not require any adjustment which might be considered a method change. The Arc HPLC is a modern instrument that delivers powerful performance, high injection precision, low carryover, and high backpressure tolerance.

References

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6. USP Monograph for Aspirin Tablets. United States Pharmacopeia USP 43-NF 38, official 1 May 2020.

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