[TECHNOLOGY BRIEF]

Method Transfer from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System with Gradient SmartStart Technology: Analysis of an Active Pharmaceutical Ingredient and Related Substances

Paula Hong and Patricia R. McConville

GOAL

To transfer a reversed-phase LC gradient method for an API and its related substances from an Agilent 1100 Series LC system to an ACQUITY UPLC® H-Class System.

BACKGROUND

The analysis of an active pharmaceutical ingredient (API) and its related substances by HPLC is often conducted throughout the life cycle of a drug to ensure safety and efficacy. These assays are typically performed in regulated laboratories, in which changes to the method are either limited[1] or not permitted and may result in the need for a complete revalidation. While many legacy methods were originally developed on traditional HPLC systems, there may be a desire to transfer the method to newer UHPLC instrumentation. This need may be driven by the available resources or by an overall drive to modernize. When transferring the method to a different HPLC/UHPLC instrument, the new instrumentation must typically produce the same separation and meet the system suitability requirements of the original method/instrument.[1] However, there are a number of instrument attributes that can affect the success of the method transfer. For gradient separations, the impact of dwell volume can be dramatic. The dwell volume, which is affected by the mixer, valves, and injector, varies from instrument to instrument whether the instrument is from the same or different manufacturers. For gradient method transfer, these instrument characteristics should be considered and compensated for in a gradient table.[1]

Facilitate methods transfer to an ACQUITY UPLC H-Class System using gradient SmartStart Technology.

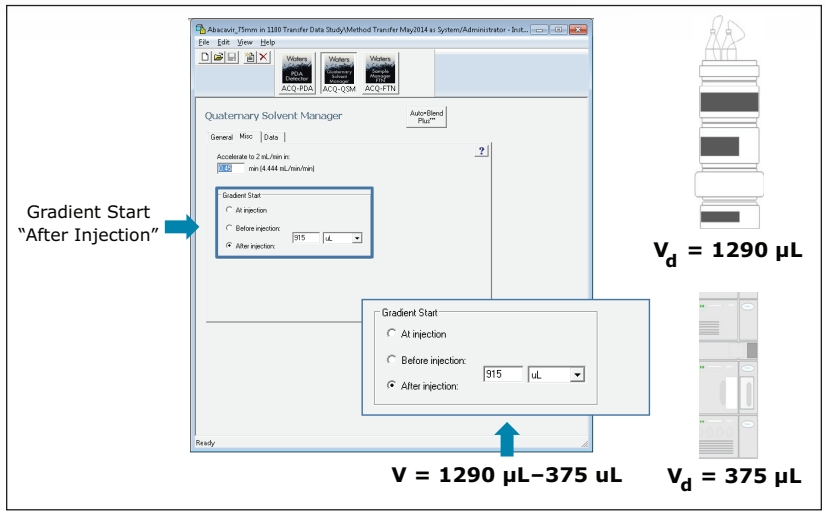

Figure 1. Instrument method editor for ACQUITY UPLC H-Class Quaternary Solvent Manager. To assist in methods transfer the instrument method allows the analyst to adjust the gradient start either before or after the injection using gradient SmartStart Technology. In this example, the system volumes for both instruments were measured and the difference was entered into ACQUITY UPLC H-Class System method.

Agilent 1100 Series LC System		ACQUITY UPLC H-Class System	
Module	Part Number	Module	Part Number
Degasser	G1322A		
Quaternary Pump	G1311A	Quaternary Solvent Manager	186015018
Autosampler	G1313A	Sample Manager FTN	186015017
Column Compartment	G1316A	Column Heater (CH-A)	186015042
DAD Detector	G1315B	PDA Detector	186015032

Table 1. System modules and part numbers. Chromatography Data System (both): Empower3 FR2.

THE SOLUTION

A previously published method for abacavir and related substances[2] was transferred from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System (Table 1). The Agilent 1100 Series LC System was configured with a passive mobile phase pre-heater (3 µL), while the ACQUITY UPLC H-Class System was configured with an active mobile phase pre-heater. To account for gradient delay differences, each instrument's dwell volume was measured.[3] The measured dwell volume was greater on the Agilent 1100 Series LC System. Therefore, to compensate for the differences in dwell volume between the Agilent 1100 Series LC System and the target ACQUITY UPLC H-Class System, a 915-µL "after injection" delay was used for the analysis on the ACQUITY UPLC H-Class System. This delay was entered directly in units of volume or µLs using gradient SmartStart Technology (Figure 1) in the instrument method.[4] This feature compensated for the differences in dwell volume in methods transfer, eliminating the need to make manual adjustments to the gradient table, an action which could trigger a full revalidation of the method. The volume entry into the gradient SmartStart Technology was the only adjustment to the method.

Both the Agilent 1100 Series LC System and the ACQUITY UPLC H-Class System produced comparable separations (Figure 2). Specifically, the retention times for abacavir and related compounds were all within 0.2 min or less than 3% deviation across the two instruments (Table 2). The relative retention times, which were calculated relative to the API, were all within 0.01% deviation for the related substances. The USP resolution for the critical pair (API and compound 3) was ≥2.5 on both systems, indicating no substantial loss of resolution in method transfer. The % area of the related substances and the API were within 0.2% on both instruments.

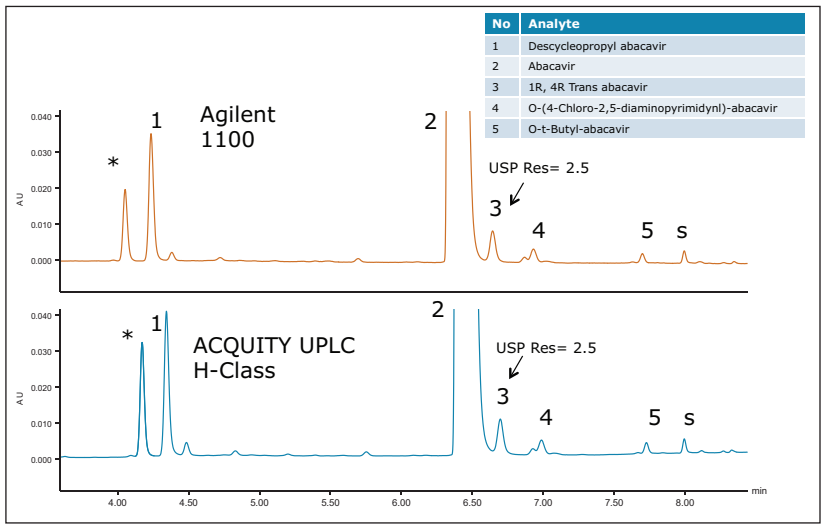

*Figure 2. Method transfer of the analysis of abacavir and related substances. The separation was performed on an Agilent 1100 Series LC System using a CORTECS® C_{18}, 2.7 µm, 4.6 x 75 mm column. Method transfer to an AQUITY UPLC H-Class resulted in a comparable separation and similar resolution for the API (2) and related compound (3). (s=solvent peak, *=unknown analyte)*

Compound	Retention time (min)			Relative retention time			% Area		
	Agilent 1100	ACQUITY UPLC H-Class	Percent Deviation	Agilent 1100	ACQUITY UPLC H-Class	Absolute Deviation	Agilent 1100	ACQUITY UPLC H-Class	Absolute Deviation
unknown	4.05	4.17	2.96	0.64	0.65	0.01	1.84	1.75	0.09
1	4.23	4.35	2.84	0.67	0.68	0.01	2.4	2.42	0.02
2-Abacavir	6.34	6.4	0.95	1.00	1.00	n/a	94.23	94.38	0.15
3	6.64	6.7	0.90	1.05	1.05	0.00	0.68	0.66	0.02
4	6.93	6.99	0.87	1.09	1.09	0.00	0.34	0.31	0.03
5	7.70	7.73	0.39	1.21	1.21	0.00	0.16	0.16	0.00

Table 2. Comparison of average retention times and peak areas for abacavir and related substances on an Agilent 1100 Series LC System and an ACQUITY UPLC H-Class System. Five replicate injections were performed. Retention times on both systems were within 3% deviation. Relative retention times and peak area percent (%) were within 0.2 absolute deviation.

Compound	Retention time				Peak area			
	Agilent 1100		ACQUITY UPLC H-Class		Agilent 1100		ACQUITY UPLC H-Class	
	Standard deviation	%RSD	Standard deviation	%RSD	Standard deviation	%RSD	Standard deviation	%RSD
1	4.64E-03	0.10	1.10E-03	0.03	2.22E+03	2.57	8.27E+02	0.88
2-Abacavir	6.12E-03	0.10	2.12E-03	0.03	5.25E+04	1.55	1.61E+03	0.04
3	5.81E-03	0.08	1.52E-03	0.02	4.17E+02	1.70	3.77E+01	0.15
4	5.54E-03	0.08	1.92E-03	0.03	2.49E+02	2.05	4.88E+01	0.40
5	4.34E-03	0.06	1.10E-03	0.01	1.06E+02	1.85	4.58E+01	0.72

Table 3. Comparison of retention time and peak area repeatability for analysis of abacavir and related substances on Agilent 1100 Series LC System and ACQUITY UPLC H-Class System. For five replicate injections, both systems produced relative standard deviations of less than 0.2% for retention time and less than 3% for peak area.

Many methods use retention time and relative retention time for identification purposes only.[5,6] When transferring a method from one manufacturer's system to another, a generally accepted criterion for retention time variance is within 3–5%.[7] The results obtained for the method transfer described meet this criterion.

To evaluate repeatability of the method, five replicate injections were performed on both instruments. The standard deviations and percent relative standard deviations (%RSD) were calculated (Table 3). On both systems, the retention time RSD's were less than 0.2% and the peak area RSD's were less than 3% for all the known analytes. In addition, the analysis on the ACQUITY UPLC H-Class System produced lower peak area RSD's for all the analytes, as compared to that run on the Agilent 1100 Series LC System. While the injection repeatability on both systems was acceptable (<3% RSD), the ACQUITY UPLC H-Class System had slightly lower injection-to-injection variability as measured by the peak area %RSD.

SUMMARY

An assay for the analysis of abacavir and related substances was successfully transferred from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System. The fidelity of the separation was preserved: the retention times were within 2%, and the relative retention times were within 2%. For this assay, the %RSD's for injection repeatability were lower on the ACQUITY UPLC H-Class System as compared to the Agilent 1100 LC Series System. In addition, by using gradient SmartStart Technology in the instrument method, the differences in system volume were factored into the method – without the need to make any manual adjustments to the gradient table. These results demonstrate how gradient method transfer from Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System can be performed with minimal adjustments to the method and/or the system.

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