

Transfer of an Isocratic USP Assay from an Agilent 1100 Series LC System to a ACQUITY UPLC H-Class System: Analysis of Tioconazole and Related Impurities

Paula Hong and Patricia R. McConville
Waters Corporation, Milford, MA

GOAL

To transfer an HPLC USP method from an Agilent 1100 Series LC System to an ACQUITY UPLC® H-Class System, meeting all USP method criteria.

BACKGROUND

Many historical USP monographs were developed and designed for HPLC instrumentation and columns. However, with goals to modernize equipment, laboratories often need to ensure the transferability of established methods from existing HPLC instrumentation to newer or alternative UHPLC or UPLC® instrumentation. These HPLC methods typically require higher flow rates, larger column dimensions, and larger injection volumes than typical UPLC analyses. The USP monograph for tioconazole and its organic impurities is one such example.¹ This USP assay requires a 5 mm x 250 mm column, and a 20- μ L sample injection volume. While there is no specified flow rate, the retention time window for tioconazole is between 12–17 minutes, which is achievable using flow rates of between 0.5–1 mL/min. Although this method is typically run on HPLC instrumentation, the compatibility of these methods with UHPLC and UPLC instrumentation enables the laboratory to utilize new equipment with the goal of modernizing the laboratory.

USP methods can be successfully transferred from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System.

THE SOLUTION

The USP monograph for tioconazole and organic impurities¹ was performed using an Agilent 1100 Series LC System (Table 1). To meet the retention time criteria for the assay, a flow rate of 0.75 mL/min was used on a XBridge® C₁₈, 5 μ m, 4.6 mm x 250 mm Column. The assay was then transferred to an appropriately configured ACQUITY UPLC H-Class System with a 50- μ L extension loop (Table 1). Both systems were run with mobile phase temperature control. The Agilent 1100 Series LC System was configured with a passive pre-heater (3 μ L), while the ACQUITY UPLC H-Class System was configured with an active pre-heater in the standard configuration.

Agilent 1100 Series LC System		ACQUITY UPLC H-Class System	
Module	Part #	Module	Part #
Degasser	G1322A		
Quaternary pump	G1311A	Quaternary Solvent Manager	186015018
Autosampler	G1313A	Sample Manager FTN	186015017
Column compartment	G1316A	Column Heater (30 CH-A)	186015045
DAD detector	G1315B	PDA detector	186015032

Table 1. System modules and part numbers.

The USP monograph for tioconazole includes two HPLC tests: the assay, and the organic impurities analysis. Both tests were performed with standards purchased from USP (p/n 1667439). Figure 1 shows overlays of the standard chromatograms on each system. Both systems produced comparable separations for the assay and organic impurities analysis. For six replicate injections, the retention times and relative retention times varied by less than 2% deviation (Table 2). These values are within the generally accepted criterion for retention time variance of 3-5% for method transfer from one manufacturer's system to another.² Furthermore, equivalent USP resolution of 1.4 (n=6) was obtained for the critical pair (related compound C and related compound B). The retention time repeatability for each system was within 0.2% RSD, however, lower variability was observed on the ACQUITY UPLC H-Class System.

Although equivalency in methods transfer is desired, for USP methods, specific system suitability requirements must be met for each analysis. For the assay of tioconazole, system suitability includes column efficiency of not less than (NLT) 1000 and tailing of not more than (NMT).² Both sets of analyses met these criteria (Table 3). The relative standard deviation for USP tailing was less than 2.0% on each system.

Testing for organic impurities of tioconazole was also performed according to the USP monograph. The standard for organic impurities test contained a mixture of related compound A (USP p/n 1667450), related compound B (USP p/n 1667461), and related compound C (USP p/n 1667472).

The same standard was tested on both systems, and six replicate injections were performed. A representative chromatogram is shown in Figure 1, as previously described. For quantitation of a sample, the tioconazole standard was prepared according to the USP monograph. The results (Figure 2, Table 4) produced impurity percentages within 0.01% on both systems. According to USP guidelines, "In such tests the limit at or below which a peak is disregarded is generally 0.05%," therefore, Related compound A was disregarded (Table 4).³ Both related compound B

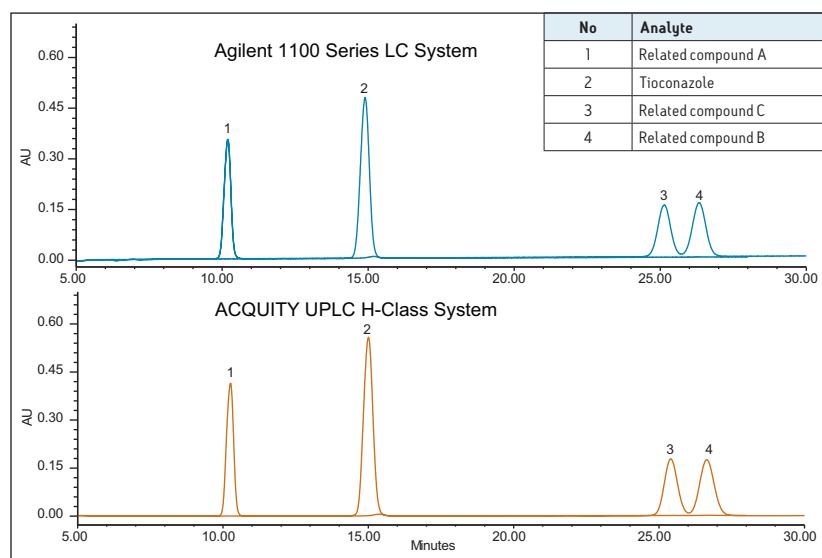


Figure 1. Assay and organic impurities analysis for tioconazole. The USP monograph was performed on both an Agilent 1100 Series LC System (top chromatogram) and an ACQUITY UPLC H-Class System (bottom chromatogram). Each chromatogram is an overlay of the standards for both the assay and organic impurities analysis.

Compound	Retention time (min)			Relative retention time		
	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	Percent deviation	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	Absolute deviation
Assay						
Tioconazole	14.91 (0.07)	15.01 (0.03)	0.67	N/A	N/A	N/A
Organic impurities						
Related compound A	10.18 (0.11)	10.26 (0.02)	0.79	0.683	0.684	0.001
Related compound C	25.11 (0.15)	25.42 (0.03)	1.23	1.684	1.694	0.010
Related compound B	26.3 (0.15)	26.65 (0.03)	1.33	1.764	1.775	0.011

Table 2. Comparison of average retention times and peak areas for tioconazole and related substances on an Agilent 1100 Series LC System and an ACQUITY UPLC H-Class System. Six replicate injections were performed. Retention times and relative retention times on both systems were within 2% deviation. %RSD for retention time of each analyte are in parentheses.

and related compound C were within USP acceptance criteria of NMT 1.0% of the API. While in this example, greater baseline noise was observed with the Agilent 1100 Series LC System, the disparity was consistent with instrument specifications^{4,5} and did not affect the analysis.

SUMMARY

For many laboratories, there is often a need to run existing HPLC methods on UHPLC or UPLC equipment while maintaining the separation and meeting system suitability requirements and acceptance criteria. This can be accomplished in method transfer from an Agilent 1100 LC Series System to an ACQUITY UPLC H-Class System. The USP assay for tioconazole, an isocratic separation, was successfully transferred across the systems from different manufacturers. Both systems met all acceptance and system suitability requirements for the USP monograph assay and organic impurities tests and demonstrated comparable separations. These results illustrate the ability to successfully transfer USP methods from HPLC instrumentation to properly configured ACQUITY UPLC H-Class System.

References

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4. 1100 Series DAD and MWD Reference Manual. Germany: Agilent Technologies; 2004.
5. ACQUITY UPLC Photodiode Array (PDA) Detector Instrument Specifications. Milford, MA: Waters Corporation; 2014.

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Compound	USP plate count			USP tailing		
	USP system suitability requirements	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	USP system suitability requirements	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System
Tioconazole	NLT 1000 Theoretical plates	10,780	10,009	NMT 2.0	0.998 (0.66)	1.02 (1.07)

Table 3. System suitability results for assay of tioconazole performed on an Agilent 1100 Series LC system and an ACQUITY UPLC H-Class System. Six replicate injections were performed. Testing performed on both systems met all system suitability criteria. Relative standard deviations in parentheses.

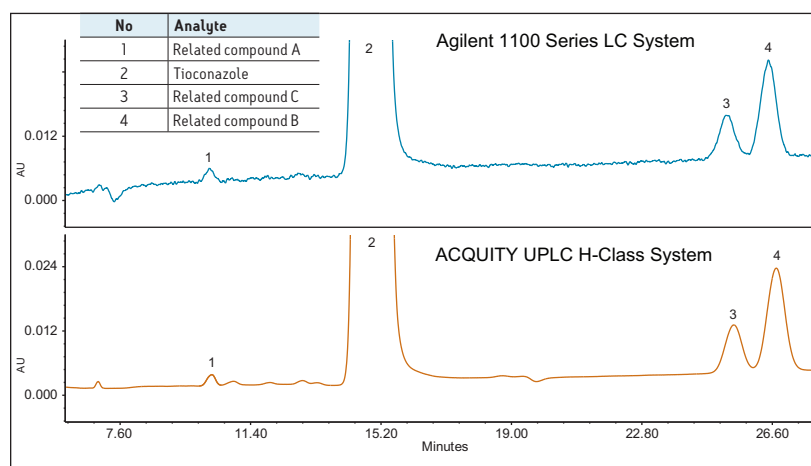


Figure 2. Organic impurities analysis for tioconazole. The USP monograph was performed on both an Agilent 1100 Series LC System (top chromatogram) and an ACQUITY UPLC H-Class System (bottom chromatogram).

Compound	% Impurity		
	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	Absolute deviation
Related compound A	0.01	0.005	0.005
Related compound C	0.06	0.05	0.01
Related compound B	0.12	0.11	0.01

Table 4. Results of USP monograph for organic impurities of tioconazole. The sample, a tioconazole standard, was purchased from USP and prepared according to the monograph. Six replicate injections were performed and the average was reported. All values (n=6) were within 0.01% on Agilent 1100 Series LC System and ACQUITY UPLC H-Class System. The calculated percent of each organic impurity was within the acceptance criteria of NMT 1.0%.

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com