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アプリケーションノート

Performance and Verification of the USP Fluconazole Related Compounds Test on the Alliance HPLC System

Daniel S. Root

日本ウォーターズ株式会社

Abstract

This application note summarizes the performance and verification of the USP Fluconazole Related Compounds method on the Alliance HPLC System with a Waters 2998 Photodiode Array (PDA) Detector.

Introduction

Fluconazole is a synthetic bis-triazole fungicide. It works by inhibiting a fungal P450 enzyme involved in the synthesis of a specific membrane component, blocking its formation. The overall action of fluconazole is primarily fungistatic, though fungicidal activity is seen in some species of fungi at higher dosing levels. This increased dosing is the usual treatment path for systemic Candida spp. infections. As these higher doses of fluconazole are used in the treatment of systemic infections, the purity of the drug compound, already critically important, becomes paramount.

USP related compound analysis in the QC lab requires a sensitive and repeatable assay performed on a robust and reliable instrument.

Precise pumping, stable mixing, and accurate sample injection, among others, are important characteristics of an LC system in the QC lab. The Waters Alliance HPLC System delivers this high level of performance.

When a compendial assay is first adopted into a QC laboratory, it is customary and prudent to perform method verification. This is to achieve compliance with regulatory guidelines, but it is also sound science. Fulfilling the suitability/acceptance criteria of the USP monograph for the method is a good first step, but further documentation of selected, meaningful analytical parameters can provide a valuable understanding of the assay.

This application note summarizes the performance and verification of the USP Fluconazole Related Compounds method on the Alliance HPLC System with a Waters 2998 Photodiode Array (PDA) Detector.

Standards for this analysis were procured from the United Sates Pharmacopeia (USP). All suitability, standard, and test solutions for this analysis were made according to the USP.[1]

In this study, experiments were performed to verify and document the specificity, precision, and limit of quantitation (LOQ) of this HPLC method. These three parameters are of primary concern to those running related compounds methods and provide meaningful data for the evaluation of the assay. Only combined standards containing all three related compounds and fluconazole were used to determine assay characteristics.

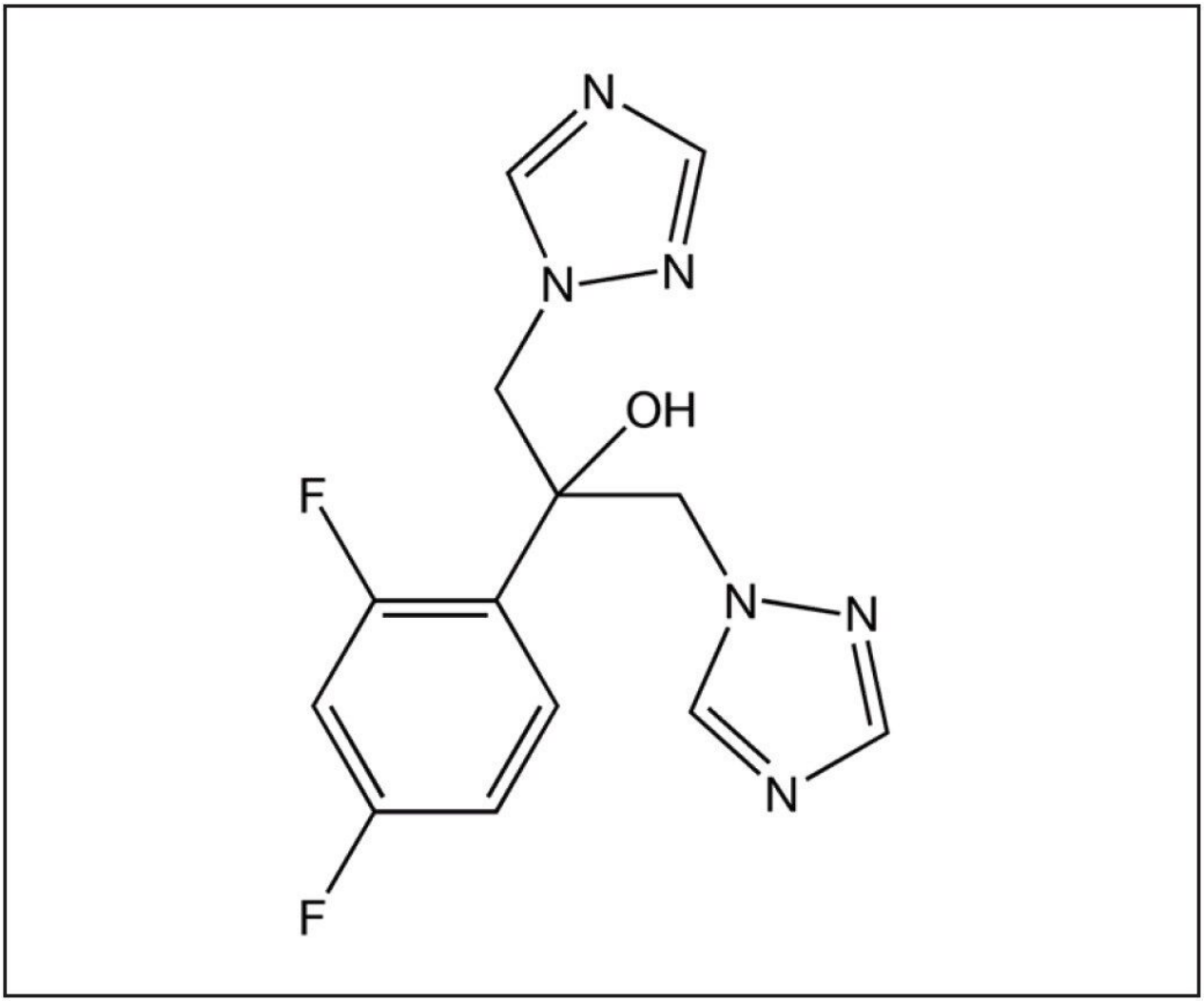

Figure 1. Structure of fluconazole.

Experimental

Method Conditions

LC System:	Alliance e2695 Separation Module
Column:	Atlantis dC_{18} 4.6 x 150 mm, 3 µm(Part No. 186001342)

Column temperature:	40 °C
Sample temperature:	15 °C
Injection volume:	20 µL
Mobile phase:	80/20 water/acetonitrile, isocratic with pump mixing
Flow rate:	0.5 mL/min
Run time:	15 min
Detection:	2998 PDA Detector
PDA wavelength:	260 nm at 1.2 nm
Date rate:	5 Hz, filter time constant:normal

Results and Discussion

The Fluconazole Related Compounds method was performed smoothly on the Alliance HPLC System and the Atlantis dC_{18} Column, which yielded a high quality separation, as shown in Figure 2. All USP criteria for this assay were easily exceeded, detailed in Table 1.

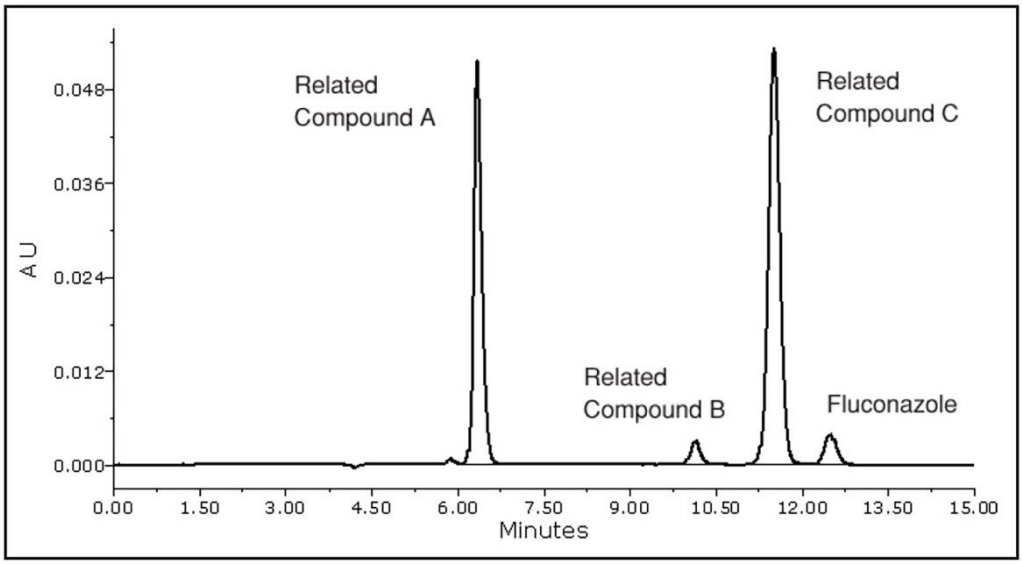

Figure 2. Example chromatogram of the atandard/auitability aolution for the USP Fluconazole Related Compounds analysis.

USP Acceptance Criterion	Alliance Fluconazole Assay
Resolution of critical pair, related compound B and C no less than 1.5	Critical pair resolution of 3.9
%RSD for area of all three related compounds and fluconazole no more than 5%	%RSD for all components 0.1 to 0.8%

Table 1. Assay results for this analysis compared to USP criteria.

Specificity is a measure of how well a method separates an analyte of interest from possible interferences. The primary specificity parameter of the fluconazole assay is the resolution between related compounds B and C. Adequate resolution for this critical pair implies adequate resolution throughout the assay. In this study, the resolution result between B and C was 3.9, which indicates ample resolution compared to the

USP criterion of 1.5. Table 2 gives the resolution results for all of the components of the suitability standard. Based upon these results, this assay demonstrates good specificity.

Component	%RSD Retention Time	%RSD Area	Resolution
Related compound A	0.3	0.2	–
Related compound B	0.2	0.8	12.8
Related compound C	0.2	0.1	3.9
Fluconazole	0.2	0.4	2.5

Table 2. %RSD and resolution results for this assay.

The limit of quantitation was calculated from the residual standard deviations of the linear calibration curve for each related compound. The concentration range of the plots was 0.15 to 50.00 µg/mL, with each combined standard containing the test solution concentration of fluconazole. The plots were highly linear with all R^2 values above 0.999, as shown in Figure 3. Table 3 shows the LOQ for each related compound and its associated USP acceptance limit. The limits of quantitation determined from this experiment were much lower than the USP acceptance limits, which indicates the excellent sensitivity of this analysis. Analysis of a test solution of fluconazole containing spiked related compounds at their LOQs further confirms the ability of the assay to resolve and detect the fluconazole related compounds in the presence of interferences, as shown in Figure 4.

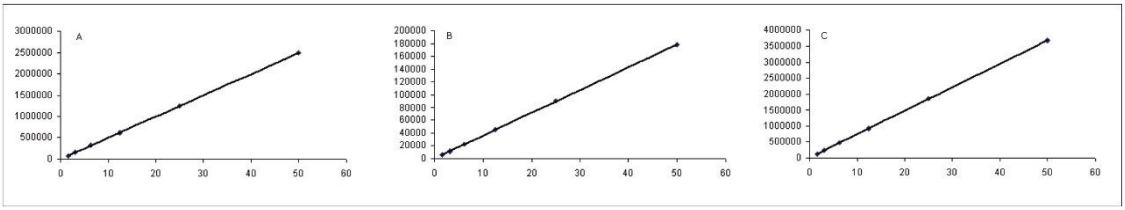

Figure 3. Calibration plots of fluconazole related compounds A, B, and C from a combined test solution standard. All R^2 were greater than 0.999.

Related Compound	LOQ µg/mL	USP Acceptance Limit µg/mL
A	0.186	6.0
B	0.228	3.0
C	0.163	6.0

Table 3. LOQ results in comparison to USP acceptance criteria.

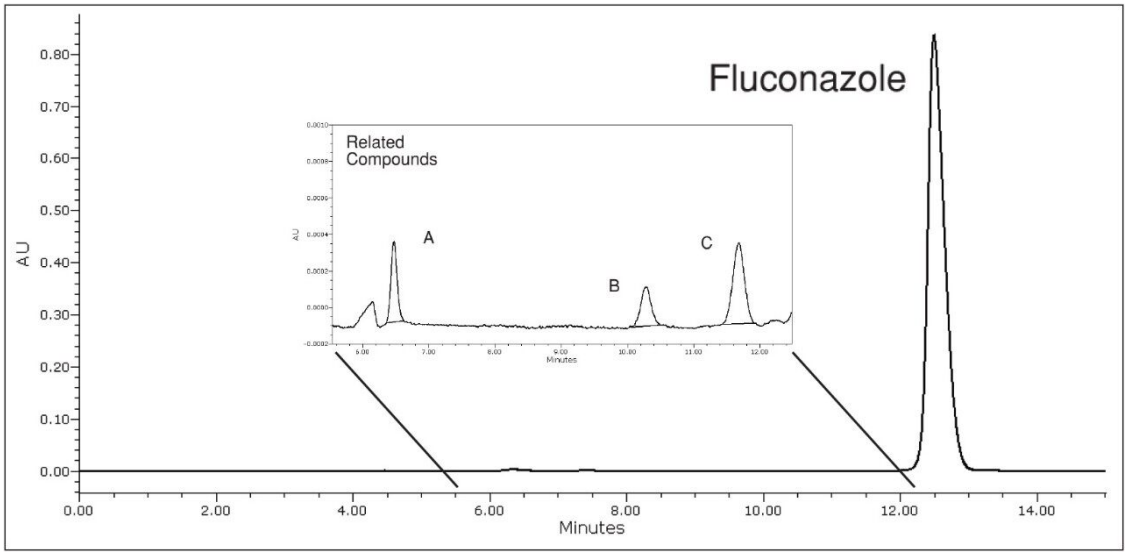

Figure 4. Chromatogram of the fluconazole and related compounds quantitation limit standard with zoomed inset.

Conclusion

- The high level performance of the Alliance HPLC System yielded superior results for precision and resolution.
- The assay was effectively verified with results far better than that of the USP acceptance criteria.
- Verfication testing with the high performance Alliance HPLC System was accomplished with minimum injections.
- The results of the assay and verification demonstrated that the Alliance HPLC System easily meets the

expectations of today’ s QC laboratory.

References

1. The United States Pharmacopeia USP 31, The National Formulary NF 26 United States Pharmacopeial Convention, Inc. 2008, pg 2171.

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Alliance HPLC System <https://www.waters.com/534293>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

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