Determination of Voriconazole Related Compound F in Voriconazole Using IC

Kassandra Oates, Brian De Borba, and Jeffrey Rohrer
Thermo Fisher Scientific, Sunnyvale, CA, USA

Application Note 1022

Key Words
Pharmaceuticals, Dionex OmniPac PAX-100 Column, Triazole, Antifungal

Introduction

Voriconazole (VFEND®) is a second-generation triazole antifungal approved by the U.S. FDA in 2002 for the treatment of invasive aspergilliosis and other serious fungal infections. Invasive aspergilliosis is a rapidly progressive and often fatal infection that occurs in severely immunosuppressed patients, recipients of bone marrow and organ transplants, and those undergoing chemotherapy. Underlying immunosuppression or use of corticosteroids (which suppress inflammatory responses) impair macrophage and neutrophil function, leaving these patients highly susceptible to bacterial and fungal infections.[1]

Voriconazole is among a class of novel antifungal agents—triazole and imidazole derivatives—responsible for the inhibition of fungal ergosterol biosynthesis.[2] The decrease of ergosterol (an essential component of the fungal cell membrane) inhibits production of a functioning cell membrane and fungal cellular growth.

When compared to its parent compound fluconazole (Diflucan™), voriconazole demonstrates increased potency, efficacy, and affinity for the target enzyme.[3] When compared to amphotericin B, a polyene antimycotic used to treat systemic fungal infections since the late 1950s, voriconazole exhibits significantly less adverse events and an increased survival rate of 71% at 12 weeks compared to 58% for amphotericin B.[4] Therefore, voriconazole has replaced amphotericin B as the first line of defense against invasive aspergilliosis and other serious fungal infections.

As with many active pharmaceutical ingredients (APIs), voriconazole's increased activity lies within the single enantiomer (2R, 3S).[5] Improvements in process development have increased the diastereoselectivity 12:1, favoring the (R*, S*) over the (R*, R*) isomers.[6] The final resolution of voriconazole (2R, 3S) from its enantiomer (2S, 3R) is achieved by a salt resolution process using (1*R*)-(–)-10-camphorsulfonic acid, also known as voriconazole related compound F.[5,6] Methods have been reported using high-performance liquid chromatography (HPLC) with UV detection for the determination of voriconazole isomers,[7] synthetic intermediates and impurities,[8] and degradation products.[9] However, voriconazole related compound F cannot be determined by UV detection due to the lack of a UV chromophore. Consequently, until recently there have been no published methods to determine voriconazole related compound F in voriconazole.[10]

As described by the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, the reporting threshold for organic impurities in a given API is 0.1% or 1.0 mg per day, whichever is lower.[11] In this work, the method proposed by the U.S. Pharmacopeia (USP)[10] was evaluated for the determination of voriconazole related compound F with a threshold amount of no more than 5 µg/mL (0.1%) in a 5 mg/mL solution of voriconazole, which is the final concentration amount for intravenous (IV) administration.[2] The USP method specifies the Thermo Scientific Dionex OmniPac PAX-100 Analytical Column (4 × 250 mm) with manually prepared eluent and chemically suppressed conductivity detection. The column chosen contains a solvent-compatible core support material that allows methanol to enhance the ion-exchange process and elute voriconazole related compound F and chloride in less than 12 min. The method was found to successfully determine voriconazole related compound F as required by the proposed USP method, provided proper attention was paid to eluent preparation and maintenance.

Thermo SCIENTIFIC

Goal

To evaluate the USP method for voriconazole related compound F by demonstrating system suitability, limits of detection, repeatability, and robustness

Equipment

- Thermo Scientific Dionex ICS-3000* system, including:
  - SP Single Pump or DP Dual Pump
  - DC Detector/Chromatography compartment
  - AS Autosampler**
  - Vial Kit, 1.5 mL Polypropylene with Caps and Septa (P/N 061696)
  - External Regenerant Installation Kit (P/N 038018)
- Thermo Scientific Dionex Chromeleon Chromatography Data System software version 7.1
- Helium or nitrogen; 4.5 grade (99.995%) or better (Praxair)

*A Dionex ICS-5000 system can also be used for this application.

**An AS-AP Autosampler can also be used with this application.

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- Voriconazole, 4-Pyrimidineethanol, α-(2,4-difluorophenyl)-5-fluoro-β-methyl-α-(1H-1,2,4-triazol-1-ylmethyl)-, (αR,βS), USP RS (USP P/N 1718008)
- Voriconazole related compound F, {(1RS,4SR)-7,7-Dimethyl-2-oxobicyclo[2.2.1]-hept-1-yl} methanesulphonic acid, USP RS (USP P/N 1718063)
- Sulfuric acid (JT Baker P/N 9673-00)
- Sodium chloride crystal (JT Baker P/N 4058-05)
- Sodium hydroxide solution (50% w/w) (Fisher Scientific P/N SS254-1)
- Methanol, Optima™ (Fisher Scientific P/N A4544)

Conditions	
Columns:	Dionex OmniPac™ PAX-100 Analytical, 4 × 250 mm (P/N 042150) Dionex OmniPac PAX-100 Guard, 4 × 50 mm (P/N 042151)
Eluent:	1 mM NaOH/25% CH_3OH
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL (full loop)
Temperature:	40 °C (column compartment) 35 °C (detector compartment)
Detection:	Suppressed conductivity, Thermo Scientific Dionex AMMS 300 Anion MicroMembrane Suppressor, 4 mm (P/N 064558) in the chemical regeneration mode with 24 mN sulfuric acid at 2 mL/min
System Backpressure:	~1700 psi
Background Conductance:	<2.0 µS
Typical Noise:	<1.6 nS
Run Time:	12 min

Preparation of Solutions and Reagents

Mobile Phase: 1 mM NaOH/25% CH_3OH

In a well-ventilated hood, add 500 mL of methanol to a 500 mL volumetric flask, then carefully pour into a 2 L eluent bottle. Using both 500 mL and 1000 mL volumetric flasks, add 1500 mL of degassed DI water to the eluent bottle containing the methanol.

Degas this mixture by pulling a vacuum on the eluent bottle while sonicating for 5 s. Remove the bottle from the sonicator and continue to pull a vacuum on the eluent bottle for no more than 30 s. Add 105 µL of a 50% (w/w) NaOH solution using a calibrated pipette. Do not shake the 50% (w/w) NaOH solution and aspirate the 105 µL portion from the middle of the reagent bottle. Avoid introducing carbon dioxide from the air into the eluent by adding the hydroxide directly into the 25% CH_3OH in water solution with the pipette tip below the surface of the liquid. Cap the eluent bottle, and once under nitrogen or helium, swirl to mix.

Methanol and Mobile Phase (50:50)

Use a 100 mL graduated cylinder to add 100 mL of methanol followed by 100 mL of freshly prepared mobile phase to a 500 mL polypropylene eluent bottle and shake to mix. Allow the mixture to settle for at least 15 min before using.

Stock Solution: 1 N Sulfuric Acid

Weigh 486.4 g of DI water into a 1 L glass bottle. In a well-ventilated hood, slowly add 13.6 mL of concentrated sulfuric acid while gently swirling.

Suppressor Hydration Solution: 200 mN Sulfuric Acid

Weigh 80 g of DI water into a 300 mL glass bottle. Slowly add 20 mL of 1 N sulfuric acid, cap the bottle, and swirl to mix.

Suppressor Regenerant Solution: 24 mN Sulfuric Acid

Add 2.66 mL of concentrated sulfuric acid to 4 L of DI water in a 4 L pressurizable bottle (included in the external regenerant installation kit), cap the bottle, and swirl to mix.

Chloride Stock Solution: 85 µg/mL Sodium Chloride

Weigh 42.5 mg of sodium chloride and carefully transfer to a 500 mL polypropylene volumetric flask. Dilute to the mark with DI water and transfer the solution to a polypropylene bottle for storage.

Standard Stock Solution: 250 µg/mL USP Voriconazole Related Compound F RS

Accurately weigh 25 mg of voriconazole related compound F RS and transfer to a 100 mL polypropylene volumetric flask. Dissolve the powder in 50 mL methanol and dilute to the mark with freshly prepared mobile phase. Transfer the solution to a 125 mL polypropylene bottle and store at 4 °C.

Spiked Sample Solutions

To prepare a 10 mg/mL stock solution of voriconazole RS, accurately weigh 50 mg of the compound into a 20 mL polypropylene vial. Then add 2.5 mL of methanol and vortex to completely dissolve the powder. Add 2.5 mL of freshly prepared mobile phase, shake to mix, and store at 4 °C.

To prepare a 25 µg/mL stock solution of voriconazole related compound F, pipette 1 mL of the 250 µg/mL stock solution into a 10 mL volumetric flask. Fill to the mark with a premade solution of methanol and mobile phase (50:50). Transfer the solution to a 20 mL polypropylene vial and store at 4 °C.

In six 1.5 mL sample vials, pipette 500 µL of the 10 mg/mL stock solution of voriconazole RS into each vial. Using the 25 µg/mL stock solution of voriconazole related compound F, pipette 20, 40, 80, 200, 320, and 400 µL into their respective vials labeled for their final voriconazole related compound F concentrations (0.5, 1, 2, 5, 8, 10 µg/mL, respectively). Add the appropriate amount of a premade solution of methanol and mobile phase (50:50) to bring the total final volume to 1000 µL (480, 460, 420, 300, 180, and 100 µL, respectively). Cap the vials and vortex to mix. These are the spiked sample solutions and their preparation is summarized in Table 1.

Table 1. Spiked sample preparation.

Volume of Voriconazole RS 10 mg/mL (µL)	Volume of Voriconazole Related Compound F RS 25 µg/mL (µL)	Volume of CH_3OH: Mobile Phase 50:50 (µL)	Final Volume (µL)	Final Concentration Voriconazole RS (µg/mL)	Final Concentration Voriconazole Related Compound F RS (µg/mL)
500	20	480	1000	5000	0.5
500	40	460	1000	5000	1
500	80	420	1000	5000	2
500	200	300	1000	5000	5
500	320	180	1000	5000	8
500	400	100	1000	5000	10

System Suitability Solutions

Standard Solution: 5 µg/mL USP Voriconazole Related Compound F RS

Using a calibrated pipette, transfer 2 mL of the 250 µg/mL voriconazole related compound F standard stock solution into a 100 mL polypropylene volumetric flask and dilute to the mark with a premade solution of methanol and mobile phase (50:50). Transfer the solution to a 125 mL polypropylene bottle. This solution can be stored at 4 °C for up to three months.

System Suitability Solution A: 5 µg/mL USP Voriconazole Related Compound F RS and 1.7 µg/mL Sodium Chloride

Using a calibrated pipette, transfer 2 mL of the 250 µg/mL voriconazole related compound F standard stock solution into a 100 mL polypropylene volumetric flask. Add 2 mL of the 85 µg/mL sodium chloride stock solution and dilute to the mark with a premade solution of methanol and mobile phase (50:50). Transfer the solution to a 125 mL polypropylene bottle. This solution can be stored at 4 °C for up to three months.

System Suitability Solution B: 2.5 µg/mL USP Voriconazole Related Compound F RS

Using a 25 mL pipette, transfer 25 mL of the 5 µg/mL voriconazole related compound F standard solution into a 50 mL volumetric flask and dilute to the mark with mobile phase. Transfer the solution to a 125 mL polypropylene bottle. This solution can be stored at 4 °C for up to three months.

System Configuration

Prime the pump with the eluent and start the flow at 1 mL/min through the tubing leading to the column. Install a Dionex OmniPac PAX-100 (4 × 50 mm) Guard Column and flush for at least 10 min before connecting it to the Dionex OmniPac PAX-100 (4 × 250 mm) Analytical Column. Flush the guard and analytical columns for at least 60 min before connecting them to the suppressor. To maintain maximum column performance, ensure that any eluent passing through the Dionex OmniPac PAX columns contains at least 1% organic solvent.

Before installing the Dionex AMMS™ 300 suppressor, follow the QuickStart Guide instructions to hydrate the membranes for at least 20 min with a 200 mN sulfuric acid solution. Fill the 4 L reservior bottles supplied in the external regenerant installation kit with a 24 mN sulfuric acid solution. Follow the Dionex AMMS 300 suppressor product manual to configure the suppressor in the chemical regeneration mode. Adjust the pressure on the regenerant solution bottle to give a flow rate of 2–3 mL/min. Allow the system to equilibrate until the background falls below 2.0 µS.

Suppressor precautions: The Dionex AMMS 300 suppressor contains heat-sensitive components and must not reach temperatures above 40 °C. Adjust the cell-out tubing to achieve a backpressure of approximately 40 psi (add backpressure coils if more backpressure is needed but do not exceed 100 psi).

Eluent preparation precautions: The eluent must be mixed then degassed to avoid introducing bubbles into the pump. Do not degas or sparge excessively (no more than 30 s). The use of ultrahigh purity solvents typically will ensure that chromatography is not affected by ionic impurities. Each eluent batch (2 L) will last approximately 24 h. When switching to a new eluent batch, do not top off, and allow the system to re-equilibrate with the fresh eluent for at least 2 h before injecting samples or standards.

Once the system is equilibrated, make successive injections of System Suitability Solution A until retention times are within 2%. Day-to-day retention time variation is seen with retention time ranges of 6.1–6.9 min for voriconazole related compound F and 8.7–10.3 min for chloride (ranges may vary slightly from column to column). This interday variation is a result of the manually prepared low-concentration NaOH eluent that will routinely have a different amount of carbonate—a stronger eluent—from preparation to preparation. If retention times drift significantly (>10%) over a 24 h period with the same eluent batch, check all eluent reservoir fittings and, if necessary, switch to helium (nitrogen is soluble in the eluent and can cause contamination).

Results and Discussion

Separation and Detection

Separation of voriconazole related compound F and chloride was achieved using a Dionex OmniPac PAX-100 column in <12 min with manually prepared 1 mM NaOH/25% CH_3OH eluent and detected by suppressed conductivity detection. The Dionex OmniPac PAX-100 column is packed with an 8.5 µm diameter highly cross-linked (55%) microporous substrate bead core coated with 60 nm diameter functionalized Thermo Scientific Dionex MicroBead particles. The small particle size of the reactive surface allows excellent mass transfer, and thus high efficiency, while the highly cross-linked substrate bead eliminates swelling problems observed when changing organic solvents on columns with low cross-linking.

The addition of an organic solvent to the eluent enhances the ion-exchange process, efficiently eluting anions such as carboxylates and sulfonates (e.g., voriconazole related compound F) from the Dionex OmniPac PAX-100 column. Chemical anion suppression was performed using 24 mN sulfuric acid on a Dionex AMMS 300 suppressor optimized for chemical suppression with up to 40% organic solvents.

Figure 1 shows the separation of a 5 mg/mL voriconazole RS solution spiked with 5 µg/mL voriconazole related compound F using the conditions described in the proposed method. This sample represents the final concentration amount of voriconazole for IV administration and contains the threshold amount of voriconazole related compound F (0.1%). Retention times for voriconazole related compound F and chloride in Figure 1 are 6.4 and 9.8 min, respectively. Relative retention times of acetate, voriconazole related compound F, and chloride are 0.45, 1.0, and 1.5, which are similar to those in the proposed USP monograph (0.47, 1.0, and 1.5, respectively).

Columns:	Dionex OmniPac PAX-100 Guard (4 × 50 mm) Dionex OmniPac PAX-100 Analytical (4 × 250 mm)
Eluent:	1 mM NaOH/25% CH_3OH
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
Temperature:	40 °C (column compartment) 35 °C (detector compartment)
Detection:	Conductivity with chemical suppression, Dionex AMMS 300 suppressor (4 mm), 24 mN sulfuric acid, 2 mL/min
Trace A:	5 mg/mL voriconazole RS spiked with 5.0 µg/mL voriconazole related compound F RS
Trace B:	System Suitability Solution A: 5 µg/mL voriconazole related compound F RS, 1.7 µg/mL sodium chloride

Peaks:		A	B	
	1. Voriconazole related compound F	5.0	5.0	µg/mL
	2. Chloride	—	1.7	

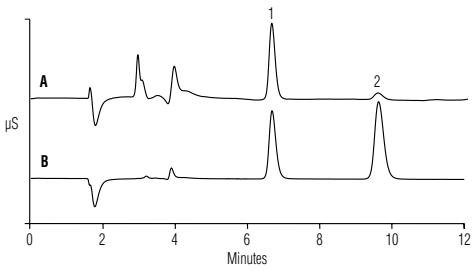

Figure 1. A) 5 mg/mL USP voriconazole RS solution spiked with 5 µg/mL USP voriconazole related compound F RS. B) System Suitability Solution A.

System Suitability

According to USP chapter <621>,[12] sample analysis is acceptable only if system suitability is demonstrated before the injection of samples and throughout the batch analysis at appropriate intervals. The USP specifies suitability parameters that this method must meet for resolution, peak area RSD, and tailing factor. This work shows that all three parameters specified by the USP are met using the proposed method conditions.

System Suitability Solution A (which contained 5 µg/mL voriconazole related compound F and 1.7 µg/mL sodium chloride) was used to evaluate the resolution between the two anions. Resolution was 9.9, which significantly exceeds the USP resolution specification of 3.5. System Suitability Solution B (2.5 µg/mL of voriconazole related compound F) was used to evaluate peak area precision and asymmetry of voriconazole related compound F. Peak area precision for seven replicate injections was 2.05%, well below the USP specification of 10%. Tailing factor, a measurement of peak asymmetry and an indication of the ability of the method to accurately quantify components in the sample, was 1.2, meeting the USP specification of ≤2. Table 2 summarizes the USP specifications and experimental values obtained and Figure 2 shows typical chromatograms of the two system suitability solutions.

Columns:	Dionex OmniPac PAX-100 Guard (4 × 50 mm) Dionex OmniPac PAX-100 Analytical (4 × 250 mm)
Eluent:	1 mM NaOH/25% CH_3OH
Flow Rate:	1.0 mL/min
Inj. Volume:	20 µL
Temperature:	40 °C (column compartment) 35 °C (detector compartment)
Detection:	Conductivity with chemical suppression, Dionex AMMS 300 suppressor (4 mm), 24 mN sulfuric acid, 2 mL/min
Trace A:	System Suitability Solution A: 5 µg/mL voriconazole related compound F RS, 1.7 µg/mL sodium chloride
Trace B:	System Suitability Solution B: 2.5 µg/mL voriconazole related compound F RS

Peaks:		A	B	
	1. Voriconazole related compound F	5.0	2.5	µg/mL
	2. Chloride	1.7	—	

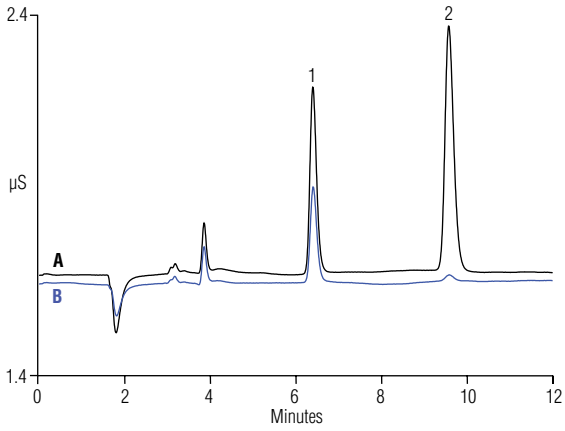

Figure 2. A) System Suitability Solution A, 5 µg/mL USP voriconazole related compound F RS and 1.7 µg/mL sodium chloride. B) System Suitability Solution B, 2.5 µg/mL USP voriconazole related compound F RS.

Table 2. Comparison of experimental results and USP specifications.

			Related Compound F		
(n = 7)	Average Retention Time, Voriconazole Related Compound F (min)	Average Retention Time, Chloride (min)	Average Resolution[1] (USP)	Average Tailing Factor[2]	Average Peak Area RSD[2]
USP Spec.	—	—	≥3.5	≤2.0	10.0
Experimental	6.4	9.9	10.2	1.2	2.0

[1]System Suitability Solution A

[2]System Suitability Solution B

Table 3. Limit of Detection (LOD) and Limit of Quantification (LOQ) for voriconazole related compound F.

(n = 6)	Average Noise (nS)	Average Signal (nS)	Sample Concentration (µg/mL)	Calculated LOD[1] (µg/mL)	Calculated LOQ[2] (µg/mL)
Sample	1.5	280	2.5	0.04	0.1
				Percent in a 5 mg/mL voriconazole sample	
				0.0008	0.002

[1]LOD calculated as 3 × S/N
[2]LOQ calculated as 10 × S/N

Limit of Detection and Limit of Quantification

Although not required by the USP monograph, LOD and LOQ were evaluated for this method. As described in USP chapter <1225>,[13] LOD is defined as three times the signal-to-noise ratio (S/N) and LOQ is defined as 10 times the S/N. The system baseline noise for the LOD and LOQ was determined to be 1.5 nS, based on measuring the peak-to-peak noise between 8.0–9.0 min over six consecutive injections of System Suitability Solution B. The estimated LOD and LOQ for voriconazole related compound F were determined to be 0.04 µg/mL and 0.1 µg/mL, respectively. This demonstrates that voriconazole related compound F can be determined in 5 mg/mL sample solutions containing as low as 0.002% voriconazole related compound F, which is well below the threshold amount of 0.1%. Table 3 summarizes the LOD and LOQ data.

Spiked Sample Analysis

A study was performed to evaluate method feasibility on samples containing 0.5–10 µg/mL (0.01–0.20%) voriconazole related compound F spiked into a solution containing 5 mg/mL voriconazole RS. Over this range, voriconazole related compound F in the spiked solutions demonstrated a linear response with a correlation coefficient >0.999, based on six concentrations injected five times each.

To determine the amount of voriconazole related compound F in the spiked samples, peak areas of the spiked samples were compared to that of the standard solution containing 5 µg/mL of voriconazole related compound F RS. The percent of voriconazole related compound F in the spiked samples was calculated using

Columns: Dionex OmniPac PAX-100 Guard (4 × 50 mm)
Dionex OmniPac PAX-100 Analytical (4 × 250 mm)
Eluent: 1 mM NaOH/25% CH_3OH
Flow Rate: 1.0 mL/min
Inj. Volume: 20 µL
Temperature: 40 °C (column compartment)
35 °C (detector compartment)
Detection: Conductivity with chemical suppression, Dionex AMMS 300 suppressor (4 mm), 24 mN sulfuric acid, 2 mL/min

Peaks: 1. Voriconazole related compound F 0.5 µg/mL

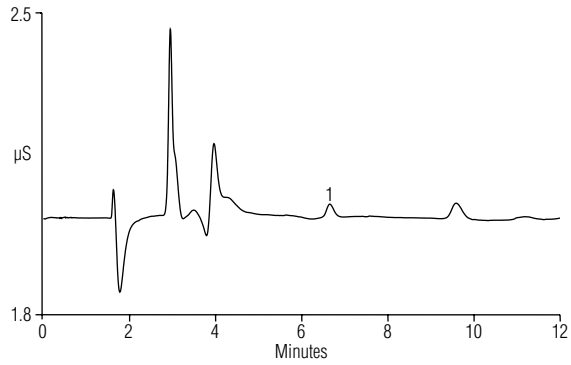

Figure 3. USP voriconazole RS solution, 5 mg/mL, spiked with 0.5 µg/mL USP voriconazole related compound F.

Equation 1 provided in the USP proposed monograph. The calculated amounts for voriconazole related compound F in the prepared solutions ranged from 0.01–0.22%. Table 4 summarizes the results. Figure 3 shows a 5 mg/mL voriconazole RS sample spiked with 0.5 µg/mL voriconazole related compound F (the lowest concentration tested). This study demonstrates that the method can detect and accurately quantify the percent of voriconazole related compound F in a 5 mg/mL solution of voriconazole.

Table 4. Spiked samples.

(n = 5)	C_U	r_U	r_S	
Spiked Amount Voriconazole Related Compound F RS (µg/mL)	Amount Voriconazole RS (µg/mL)	Average Peak Area Related Compound F, Spiked Sample (µS min)	Average Peak Area Voriconazole Related Compound F, Standard Solution[1] (µS min)	Calculated Amount Voriconazole Related Compound F, Spiked Sample[2] (%)
0.5	5000	0.0073	—	0.01
1	5000	0.0168	—	0.02
2	5000	0.0333	—	0.04
5	5000	0.0968	0.092	0.10
8	5000	0.1548	—	0.17
10	5000	0.2016	—	0.22

[1] 5 (µg/mL), C_S
[2] Calculated using Equation 1

Equation 1

The % of voriconazole related compound F in the portion of voriconazole taken:

$$(r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of voriconazole related compound F from the sample solution

r_S = peak response of voriconazole related compound F from the standard solution

C_S = concentration of USP voriconazole related compound F RS in the standard solution (µg/mL)

C_U = concentration of voriconazole in the sample solution (µg/mL)

Method Ruggedness

To evaluate repeatability of the method, separate preparations of mobile phase and standards were made over a three-day period. Each standard preparation was injected five times and the method was evaluated for changes in retention time, resolution, asymmetry, and peak area RSD.

During the three-day experiment, all parameters were reproducible and within USP specifications. Average resolution over the three days was 10.2, with a difference of no more than 0.1 from the mean on any single day. Average tailing factors for voriconazole related compound F over the three days were 1.21 for System Suitability Solution A and 1.18 for System Suitability Solution B, with deviations of no more than 0.03 from the mean on a given day. Peak area RSDs over the three days were 1.49% for System Suitability Solution A and 2.58% for System Suitability Solution B. Table 5 summarizes the data.

Although not required for this method, the retention times of both analytes were monitored to evaluate day-to-day eluent preparation reproducibility. Average retention times for voriconazole related compound F and chloride were 6.47 and 9.87 min, respectively, with retention time RSDs of no more than 0.66 and 0.47%, respectively. Although retention times in this study were reproducible, other studies performed indicate that retention times can shift by as much as 0.8 min for voriconazole related compound F and 1.6 min for chloride, yet still meet all USP specifications.

A second three-day repeatability study performed on another column from the same lot produced average retention times of 7.74 and 11.8 min, respectively, with retention time RSDs of 1.1 and 1.3%, respectively (System Suitability Solution A). When the eluent degassing time was increased to 2 min, retention time RSDs over three days doubled to 2.2 and 2.6% for voriconazole related compound F and chloride, respectively.

These results indicate that retention time reproducibility is highly dependent on eluent preparation. The following steps will minimize day-to-day retention time RSDs when switching to a new eluent batch:

- Use volumetric flasks to measure the volumes of DI water and methanol added.
- Do not degas the mixture for more than 30 s.
- Keep delivery of the 50% hydroxide solution consistent by using the same pipette on a daily basis and ensuring no large drops are present on the outside of the pipette tip.
- Carbonate contamination is also an issue at such a low hydroxide concentration; therefore, minimize exposure to air and do not prepare intermediate NaOH dilutions as suggested by the proposed USP monograph.

Table 5. Between-day repeatability.

(n = 5)		Related Compound F						
System Suitability Solution	Day	Average Retention Time, Voriconazole Related Compound F (min)	RSD (%)	Average Retention Time, Chloride (min)	RSD (%)	Average Resolution (USP)	Average Tailing Factor (USP)	Average Peak Area RSD (%)
A	1	6.44	0.04	9.89	0.07	10.31	1.20	0.57
	2	6.50	0.09	9.92	0.06	10.23	1.18	0.42
	3	6.47	0.04	9.81	0.04	10.08	1.21	0.73
	Over All[1]	6.47	0.37	9.87	0.47	10.19	1.21	1.49
B	1	6.46	0.06	—	—	—	1.18	1.29
	2	6.52	0.05	—	—	—	1.17	0.75
	3	6.43	0.64	—	—	—	1.19	1.21
	Over All[1]	6.47	0.66	—	—	—	1.18	2.58

[1]15 injections (five each day)

Method Robustness

Variations in temperature, column, and eluent preparation were evaluated to test method robustness. Column temperature was varied ±10% from the normal operating temperature of 40 °C. Temperature variations did appear to have an impact on retention times; however, all USP requirements were met or exceeded.

Two columns were tested: Column A (both new and after 850 injections) and Column B (a new column from the same lot). A loss of chloride retention time in Column A after 850 injections resulted in a resolution decrease of almost 13%; however, the resolution still exceeded the USP requirement. Column B also had changes in retention times while still meeting all USP requirements.

Eluent preparation was evaluated by having a second chemist manually prepare the solution. Only minor differences were seen between the results from Chemist 1 and Chemist 2 (Table 6). A second eluent robustness test was performed to evaluate the accuracy of the hydroxide concentration (1 mM) in the manually prepared eluent. In this test, hydroxide generation mode was evaluated using a Reagent-Free™ IC (RFIC™) system to electrolytically generate the hydroxide concentration at 1 mM in a premade 25% methanol solution instead of manually preparing the 1 mM concentration.

Only slight differences were seen between manually prepared and electrolytically generated hydroxide (Table 6). Because retention times of the analytes are inversely proportional to the hydroxide concentration, retention time data can be compared between the two modes to conclude that the concentration of manually prepared hydroxide was accurate. Both eluent robustness tests gave comparable retention time results and met all USP requirements. In addition, due to the accurate and consistent generation of 1 mM hydroxide by the RFIC system, a decrease in the day-to-day retention time RSDs was observed (data not reported here). Method robustness testing is summarized in Table 6.

Table 6. Method robustness.

Parameter		Related Compound F		Chloride		Related Compound F		
		Average Retention Time (min)	% Difference	Average Retention Time (min)	% Difference	Average Resolution[1] (USP)	Average Tailing Factor[2] (USP)	Average Peak Area RSD[2] (%)
						USP spec.		
						≥3.5	≤2.0	10.0
Column Temperature	36 °C	6.50	0.9	9.92	0.4	10.1	1.20	1.6
	40 °C	6.44	—	9.88	—	10.4	1.18	1.6
	44 °C	6.37	1.1	9.77	1.1	10.5	1.19	2.6
Column[3]	A	6.47	—	9.81	—	10.2	1.20	2.0
	Column A after ~850 injections[4]	6.38	1.4	9.25	5.7	8.9	1.22	1.4
	B[4]	6.85	5.9	10.15	3.5	7.7	1.22	1.0
Chemist	1	6.47	—	9.81	—	10.3	1.21	—
	2[5]	6.52	0.8	9.85	0.4	10.0	1.21	—
Eluent Preparation Mode	Manual	6.47	—	9.81	—	10.2	1.20	2.0
	EG	6.63	2.5	9.73	1.8	9.5	1.24	0.4

[1]System Suitability Solution A
[2]System Suitability Solution B
[3]Same manufactured lot
[4]Same eluent batch
[5]Single data point

Conclusion

This work shows the determination of voriconazole related compound F using a Dionex OmniPac PAX-100 (4 × 250 mm) column with a manually prepared 1 mM NaOH/25% CH_3OH eluent solution and chemically suppressed conductivity detection. As summarized in Table 2, this method meets all requirements described by the USP. Although not required by the proposed USP monograph, the LOD and LOQ were evaluated for the method and were determined to be 0.04 and 0.1 µg/mL, respectively. This demonstrates that voriconazole related compound F can be determined in 5 mg/mL sample solutions containing as low as 0.002% voriconazole related compound F.

Repeatability and ruggedness were also evaluated. During the three-day repeatability experiment, all parameters were reproducible and within USP specifications. Retention times were also evaluated, demonstrating that the retention time reproducibility is highly dependent on eluent preparation. For all parameters evaluated in the ruggedness testing, USP requirements were met or exceeded. The results obtained using an RFIC system to electrolytically generate the 1 mM hydroxide in a premade 25% CH_3OH solution show that the concentration of manually prepared hydroxide was accurate (based on retention time data) and day-to-day retention time RSDs were reduced using the RFIC system. The method described here is sensitive, repeatable, and accurate for the determination of voriconazole related compound F.

References

1. Harman, E.M. Aspergilliosis *Medscape Reference* [Online] **2012**, http://emedicine.medscape.com/article/296052-overview (accessed July 26, 2012).
2. Pfizer, VFEND® Package Insert. Revised 11/2011.
3. Sabo, J.; Abdel-Rahman, S. Voriconazole: A New Triazole Antifungal. *The Annals of Pharmacotherapy* **2000**, *34*, 1032–1043.
4. Herbrecht, R.; Denning, D.W.; Patterson, T.F.; et al. Voriconazole versus Amphotericin B for Primary Therapy of Invasive Aspergillosis. *New England Journal of Medicine* **2002**, *347*, 408–415.
5. Dickinson, R. P.; Bell, A. S.; Hitchcock, C. A.; Narayanaswami, S.; Ray, S. J.; Richardson, K.; Troke, P. F. Novel Antifungal 2-aryl-1-(1H-1,2,4-triazol-1-yl) butan-2-ol Derivatives with High Activity Against *Aspergillus fumigatus*. *Bioorganic and Medicinal Chemistry Letters* **1996**, *6*, 2031–2036.
6. Butters, M.; Ebbs, J.; Green, S. P.; MacRae, J.; Morland, M. C.; Murtiashaw, C. W.; Pettman, A. J. Process Development of Voriconazole: A Novel Broad-Spectrum Triazole Antifungal Agent. *Organic Process Research & Development* **2001**, *5*, 28–36.
7. Owens, P.; Fell, A.; Coleman, M.; Berridge, J. Separation of the Voriconazole Stereoisomers by Capillary Electrophoresis and Liquid Chromatography. *Enantiomer* **1999**, *4*, 79–90.
8. Ferretti, R.; Gallinella, B.; La Torre, F.; Zanitti, L. Direct Resolution of a New Antifungal Agent, Voriconazole (UK-109,496) and its Potential Impurities, by use of Coupled Achiral-Chiral High-Performance Liquid Chromatography. *Chromatographia* **1998**, *47*, 649–654.
9. Eldin, A. B.; Shalaby, A.; Maamly, M. Development and Validation of a HPLC Method for the Determination of Voriconazole and its Degradation Products in Pharmaceutical Formulation. *Acta Pharmaceutica Sciencia* **2010**, *52*, 229–238.
10. United States Pharmacopeia Forum 37(4) In-Process Revision: Voriconazole (2011).
11. *Impurities in New Drug Substances Q3A(R2)* ICH Harmonised Tripartite Guideline in International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH, **2006**, 1–11.
12. *Validation of Compendial Methods*, General Chapter 1225, The U.S. Pharmacopeia and National Formulary, USP 34 NF 29, Washington, DC, 2011, pp 778–782.
13. *Chromatography*, General Chapter 621, The U.S. Pharmacopeia and National Formulary, USP 34 NF 24, Washington, DC, 2011, pp 243–250.

Australia +61 3 9757 4486
Austria +43 1 616 51 25
Benelux +31 20 683 9768
+32 3 353 42 94
Brazil +55 11 3731 5140
China +852 2428 3282
Denmark +45 36 36 90 90
France +33 1 39 30 01 10
Germany +49 6126 991 0
India +91 22 2764 2735
Ireland +353 1 644 0064
Italy +39 02 51 62 1267
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Singapore +65 6289 1190
Sweden +46 8 473 3380
Switzerland +41 62 205 9966
Taiwan +886 2 8751 6655
UK +44 1276 691722
USA and Canada +847 295 7500

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

AN70191_E 08/16S