$^1$H and $^{19}$F NMR in drug stress testing: the case of voriconazole

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Supporting Information

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Voriconazole NMR spectra

Figure S1: Voriconazole structure – C_{16}H_{14}F_{3}N_{5}O, molar mass 349.12 g mol^{-1}.

All the chemical shifts of voriconazole were assigned, by $^1$H, $^{13}$C, $^{19}$F, and a set of 2D NMR experiments (Figs. S2-S12), before degradation testing.

Figure S2: $^1$H NMR spectrum using presaturation to suppress OH from solvent, and hydrogen atom assignments of voriconazole in CD_{3}OH.
**Figure S3:** $^{13}$C\{$^1$H} NMR spectrum of voriconazole in CD$_3$OH.

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Characterization of degradation products

The products of thermal degradation in DMSO-\textit{d}_6 were identified as described below. The products were conformed, by spiking, to be identical to those obtained in alkaline degradation. Interestingly, the kinetics of thermal degradation were irreproducible, varying between different DMSO-\textit{d}_6 batches.

A sample of voriconazole (24 mM) was prepared in DMSO-\textit{d}_6 using TMS as internal reference and was submitted to forced degradation at 60 °C, monitored through a series of $^{19}$F NMR measurements, $^1$H and $^{19}$F DOSY spectra of a partly degraded mixture confirm that there are two significant degradation products, both which contain fluorine atoms (Fig. S13 and S14).

Figure S13: $^1$H DOSY spectrum of thermally degraded voriconazole, acquired with a net diffusion-encoding gradient pulse width delta ($\delta$) of 1.8 ms, and a diffusion delay ($\Delta$) of 50 ms, in a 3 mm NMR tube.
Figure S14: $^{19}\text{F}\{^1\text{H}\}$ DOSY spectrum of thermally degraded voriconazole, acquired with a net diffusion-encoding gradient pulse width delta ($\delta$) of 1.8 ms, and a diffusion delay ($\Delta$) of 50 ms, in a 3 mm NMR tube.

To identify the reaction site involved in thermal degradation it is necessary to determine the structures of the degradation products. Product 2 shows a $\text{CH}_3-\text{CH}_2$ spin system in the COSY contour plot (Fig. S15), and a coupling between protons from the $\text{CH}_2$ group, and the $^{19}\text{F}$ atom of a pyrimidine ring.
The $^1$H NMR spectrum of degradation product 1 (Fig. S18) shows a singlet for CH$_2$ group, instead of the diastereotopic pattern observed for voriconazole. The site of reaction thus involves the formation of a new CH$_2$ group and eliminates an asymmetric carbon. This leads to the proposed degradation products (Fig. S16).

**Figure S16:** Proposed structures for two degradation products for voriconazole thermal degradation.
To support the identification of these two products, degradation product 1 is available commercially and was added to a sample at about 35% of thermal degradation (Fig. S17), the spectrum of the spiked sample confirming the identity of degradation product 1. Expansion of $^1$H signals related to each degradation product are shown in Figs. S18, and S19. Lastly, the presence of a carbon resonance at 189.2 ppm confirm the presence of a carbonyl group (Fig. S20).

**Figure S17**: $^1$H spectra of voriconazole at about 35% thermal degradation (bottom), and after addition of the commercially available degradation product 1 (top). The product 1 signals (highlighted in red) match perfectly.
Figure S18: $^1$H NMR spectrum of voriconazole thermal degradation, showing the expansion of the peaks related to degradation product 1.

Figure S19: $^1$H NMR spectrum of voriconazole thermal degradation showing the expansion of the peaks related to degradation product 2.
Voriconazole degradation product 1 assignment: $^1$H NMR (499.87 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.49 (1H, s, H3'''); 8.06-8.01 (1H, m, H6'); 8.02 (1H, s, H5'''); 7.53 (1H, ddd, $^3$J$_{H3'H5'} = 2.45$, $^3$J$_{H3'F2'/4'} = 7.55$ and $^3$J$_{H3'F2'/4'} = 11.70$ Hz, H3'); 7.31 (1H, m, H5') and 5.81 (2H, d, $^5$J$_{H1F2'} = 2.95$, H1).

Voriconazole degradation product 2 assignment: $^1$H NMR (499.87 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.96 (1H, d, $^5$J$_{H2''F} = 3.05$, H2''); 8.74 (1H, d, $^3$J$_{H4''F} = 2.15$, H6''); 2.84 (2H, qd, $^4$J$_{H3F} = 1.85$ and $^3$J$_{H3H4} = 7.55$ Hz, H3) and 1.24 (3H, t, H4).

Figure S20: $^{13}$C spectrum of voriconazole thermal degradation in DMSO-$d_6$.

Voriconazole acid stress test

Acid stress testing of voriconazole did not lead to any degradation. The only observable change is in the chemical shift of H3''' (an aromatic proton) in Figure S21, due to the protonation of N2''' (Figs. S22 and S23). The N2''' signal appears at 294.8 ppm under neutral conditions, and at 178.7 ppm in acid.
Figure S21: $^1$H NMR spectra of voriconazole in a) CD$_2$OH, b) CD$_3$OH with 0.45 mol L$^{-1}$ of DCl, and c) CD$_3$OH with 0.45 mol L$^{-1}$ of DCl after 2 h at 55 °C.
**Figure S22:** $^1$H-$^{15}$N HMBC contour plot of voriconazole in CD$_3$OH, optimised for a long range coupling constant (cnst13) of 2 Hz. The chemical shifts of nitrogens 1‴, 2‴, and 4‴ are highlighted.
Figure S23: $^1$H-$^{15}$N HMBC contour plot of voriconazole in CD$_3$OH with DCl, optimised for a long range coupling constant (cnst13) of 2 Hz. In the presence of acid a significant change in chemical shift is observed for N2''', but almost no change is observed for N4''', suggesting protonation at N2'''.
Figure S24: $^{19}$F{$^1$H} spectra of voriconazole after treatment with different concentrations of base (NaOH). Increasing the concentration of base increases the rate of degradation and shifts equilibrium to the enol form, but the same degradation products (P1, P2, x and y) are produced.