Supplementary Data: pH Stability Studies of TDF

In this study we evaluated the stability of TDF at pH 7. The pH 7 phosphate buffer was prepared as per the procedure given in United States pharmacopoeia. A 25 mL solution of 2 ppm of TDF was prepared (n = 3) in the pH 7 buffer and kept at 37 °C. A 500 µL of sample was collected at 0, 0.5, 1, 6, 12 and 24 hours and analysed immediately in HPLC-PDA. The samples were analysed for TDF with the help of Spincotech C18G enabled column (250 × 4.6 mm, 5 µm, Spinco Biotech Pvt Ltd, TN, India). The mobile phase used comprised of 10 mM ammonium acetate buffer (pH 4.0 ± 0.1, adjusted with glacial acetic acid) and methanol in the ratio of 50:50 v/v. The flow rate was set to 1 mL min^{-1}, the injection volume was 50 µL and the samples were monitored at a wavelength of 260 nm.

The observed concentrations of TDF and the %drug remaining at different time points are shown in table 1 and the corresponding chromatograms are shown in Figure 1. It can be observed that until 6 hours the level of degradation is not more than 5%. However after 24 hours a considerable amount of degradation was observed.

Table 1: Concentration of TDF in pH 7.0 buffer at different time points

Time (h)	**Concentration (ng/mL) ± SD**	**%RSD**	**%Bias**	**% Drug Remaining**
0	2014.13 ± 23	1.18	-0.60	100
0.5	2009.56 ± 24	1.22	1.72	99.7
1	1999.46 ± 49	2.49	-0.83	99.2
6	1917.78 ± 21	1.09	-4.86	95.2
12	1826.54 ± 20	1.14	-7.69	90.7
24	1727.56 ± 25	1.48	-13.37	85.7

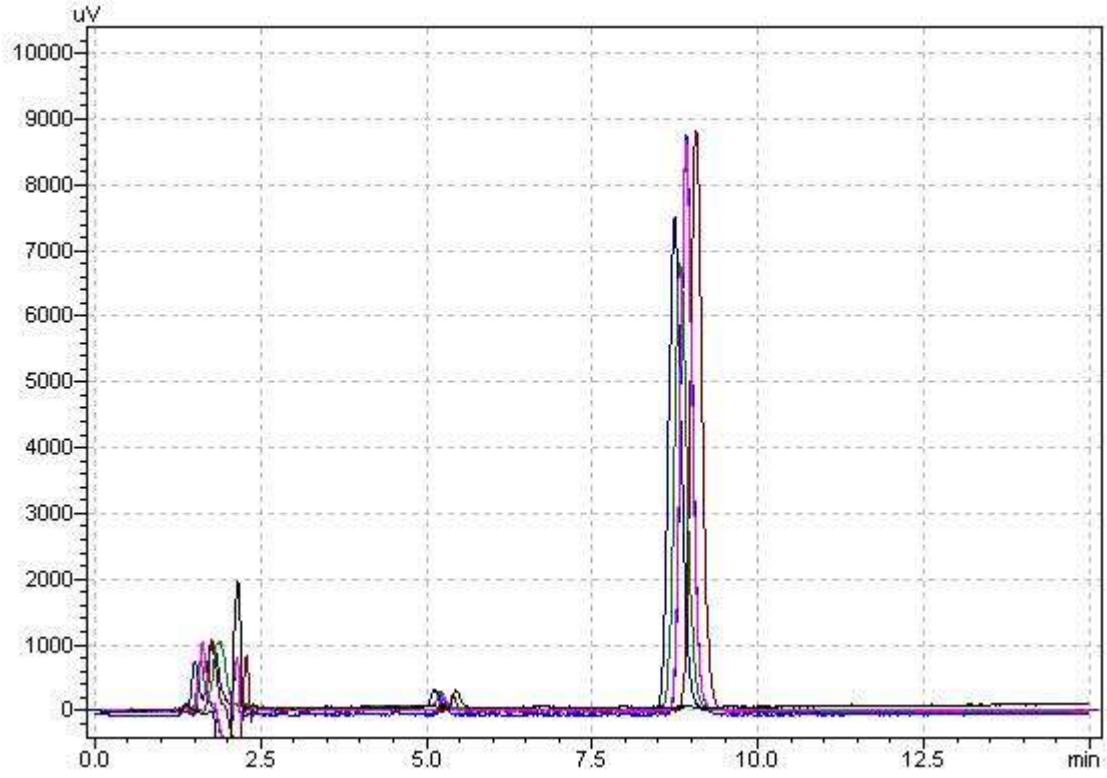

Figure 1: Overlay of chromatograms of TDF at all the time points with blank (**R_t: 8 min**)