Supplementary Data: *Ex vivo* everted gut sac studies of TDF (at different time intervals)

In this study we performed *ex vivo* everted gut sac studies at different time intervals. The rats were fasted overnight and on the day of the experiment they were anesthetized (urethane 1.25 g/kg-ip) and dissected. After anaesthesia, laparotomy was done and the entire intestine was removed by cutting across the upper end of the duodenum and the lower end of the ileum and stripping the mesentery manually. The intestine was washed carefully with the normal saline (0.9% w/v NaCl) using a syringe equipped with blunt end. Intestinal segments (i.e. jejunal part of the intestine beginning from the ligament of Treitz) of 7.5 cm length was cut and carefully everted over a glass rod. The everted intestine was then slipped off the glass rod and tied at one end. From the other end, with the help of a blunt syringe, 750 µL of DPBS buffer was added and tied to form a sac. These everted sacs were kept in reservoirs containing 20 mM TDF in 50 mL of DPBS buffer maintained at 37 °C and oxygenated with O_2/CO_2 (95/5%). The samples were collected from both the donor and receiver chambers from all the reservoirs at 5, 15, 30 and 60 min of incubation. The samples were immediately stored at –20 °C until analysis and the study was done in triplicate.

To the 100 µL of the collected sample 10 µL of trifluoroacetic acid (13 M), 15 µL of 35% v/v perchloric acid and 400 µL of acetonitrile were added. This mixture was vortexed for 30 s and then centrifuged at 7800 × g at 4 °C for 15 min. The supernatant was collected and neutralized partially with 10 µL of ammonium hydroxide (14.8 M) and evaporated under a gentle stream of nitrogen gas at 40 °C. The dried residue was reconstituted with 100 µL of pH 4 ammonium acetate buffer, vortexed for 2 min and centrifuged at 11300 × g at 4 °C for 10 min. The HPLC system was stabilised for 1 h through baseline monitoring prior to the actual analysis. The supernatant was transferred into sample loading vials and injected into the HPLC system for analysis.

The collected samples were analysed 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 70:30 v/v for the quantitation of monoester form of TNF (TMF) and 50:50 v/v for the quantitation of TDF. Isocratic conditions were employed with a flow rate of 1 mL min^{-1} and the injection volume was set to 50 µL. TMF and TDF were monitored at a wavelength of 260 nm.

The concentrations of TDF and TMF in the receptor chamber is shown in table 2 and the corresponding chromatograms are shown in Figure 2 and Figure 3. It can be observed that when the samples of the receptor compartments were analysed detectable levels of TDF were not observed at 5 min, however, quantifiable levels of TMF was observed at all the time points.

The concentrations of TDF and TMF in the donor chamber is shown in table 3 and the corresponding chromatograms are shown in Figure 4 and Figure 5. It can be observed that quantifiable levels of TDF was present only at the 5^{th} min sample, but, quantifiable levels of TMF was observed at all the time points. Hence, the results of this study shows that metabolism of TDF to its monoester form takes place in the donor chamber and also within the gut wall even at shorter time points.

Table 2: Concentration of TDF and TMF in the receptor compartment

	TDF	**TMF**	
Time (min)	**Concentration (ng/mL)**	**Concentration (ng/mL) ± SD**	**%RSD**
5	---	348.62 ± 33	9.68
15	---	961.51 ± 108	11.26
30	---	1775.66 ± 105	5.95
60	---	2514.24 ± 318	12.54

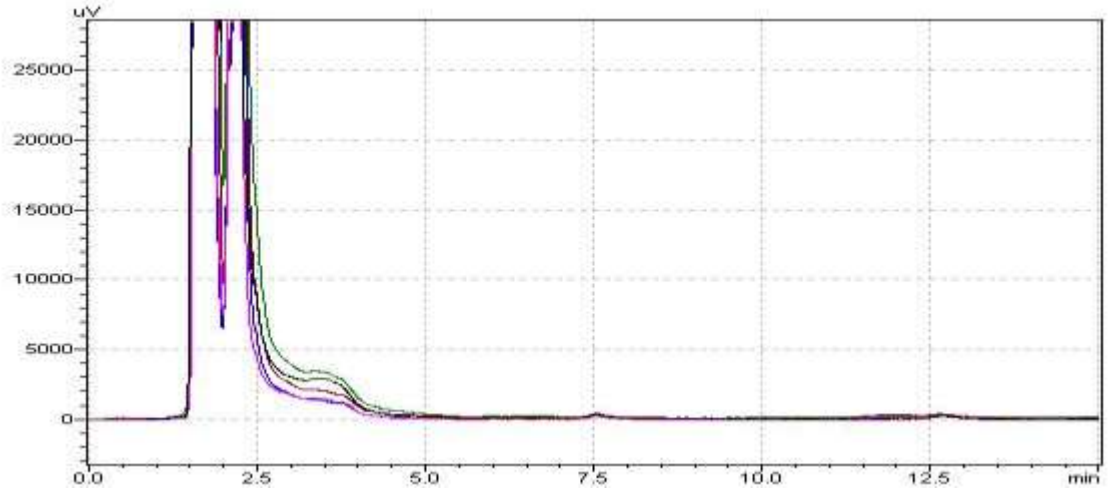

Fig 2: Overlay of chromatograms of the receptor compartment samples when analysed for TDF at 5, 15, 30 and 60 min (**Expected R_t: 8.6 min**).

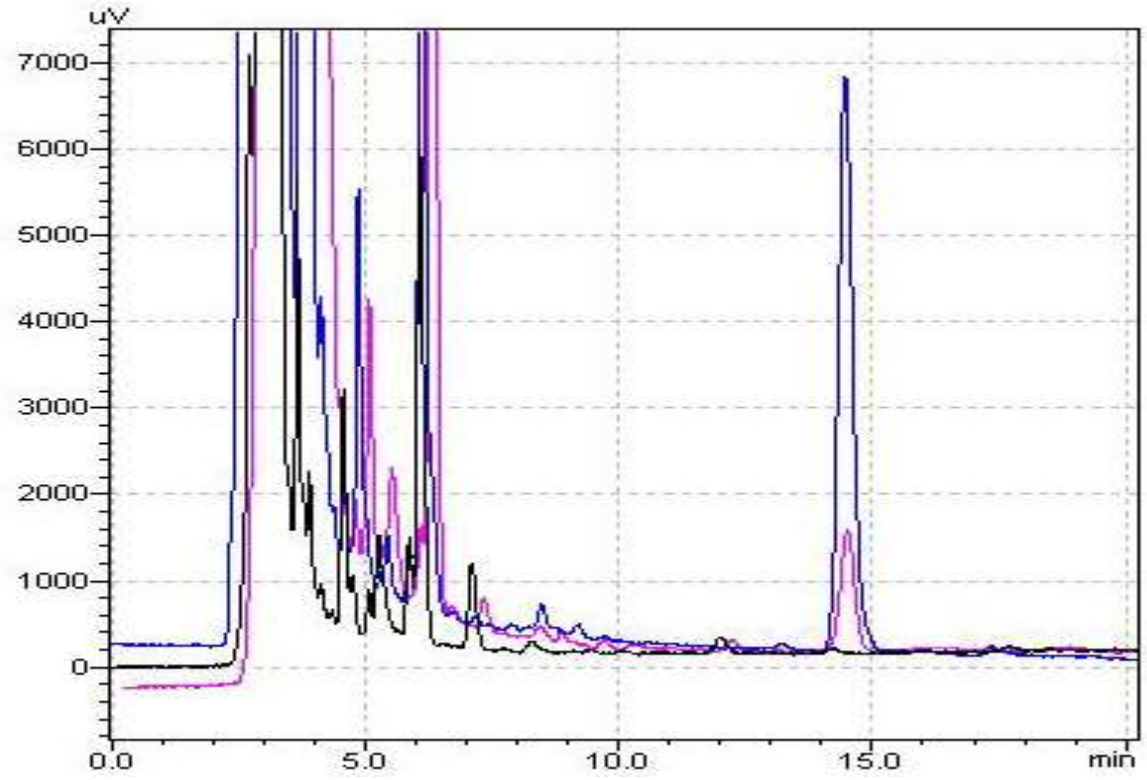

Fig 3: Overlay of chromatograms of the receptor compartment samples when analysed for TMF at 5 and 60 min with blank (**R_t: 14.6 min**).

Table 3: Concentration of TDF and TMF in the donor compartment

	TDF		TMF	
Time (min)	**Concentration (ng/mL)**	**%RSD**	**Concentration (ng/mL) ± SD**	**%RSD**
5	2397.41 ± 223	9.29	3956.44 ± 379	9.56
15	---	---	6212.10 ± 362	5.84
30	---	---	6659.92 ± 285	4.28
60	---	---	6957.13 ± 118	1.71

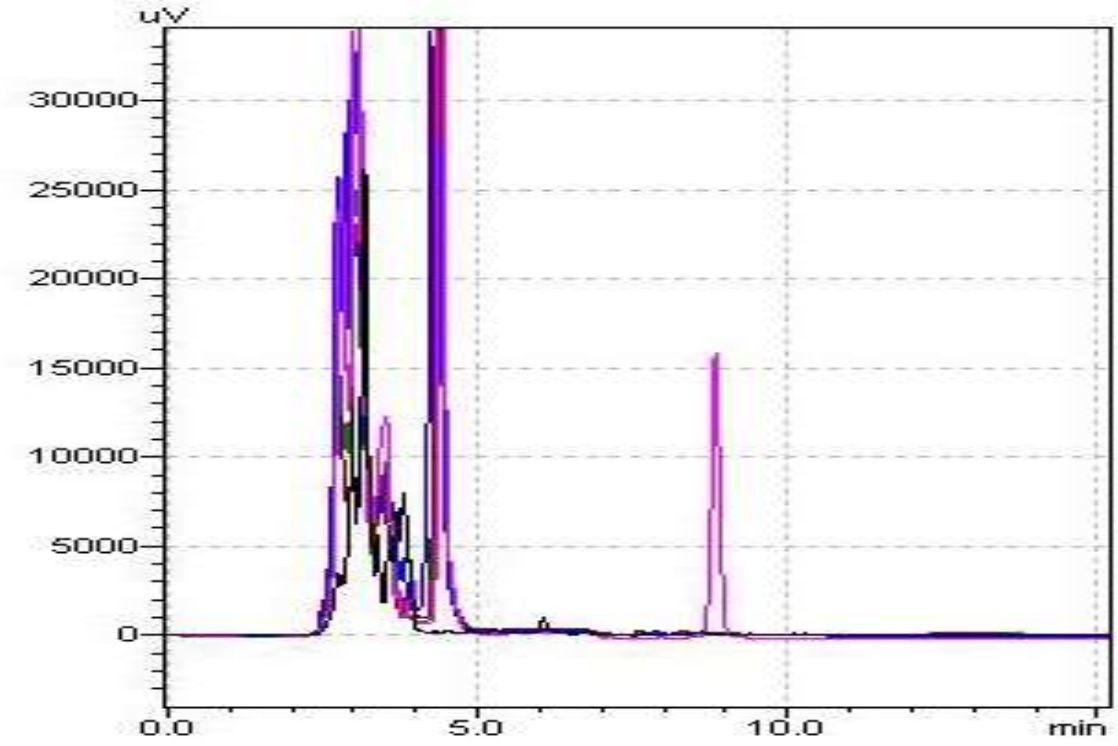

Fig 4: Overlay of chromatograms of the donor compartment samples when analysed for TDF at 5 and 60 min with blank (**Expected R_t: 8.6 min**).

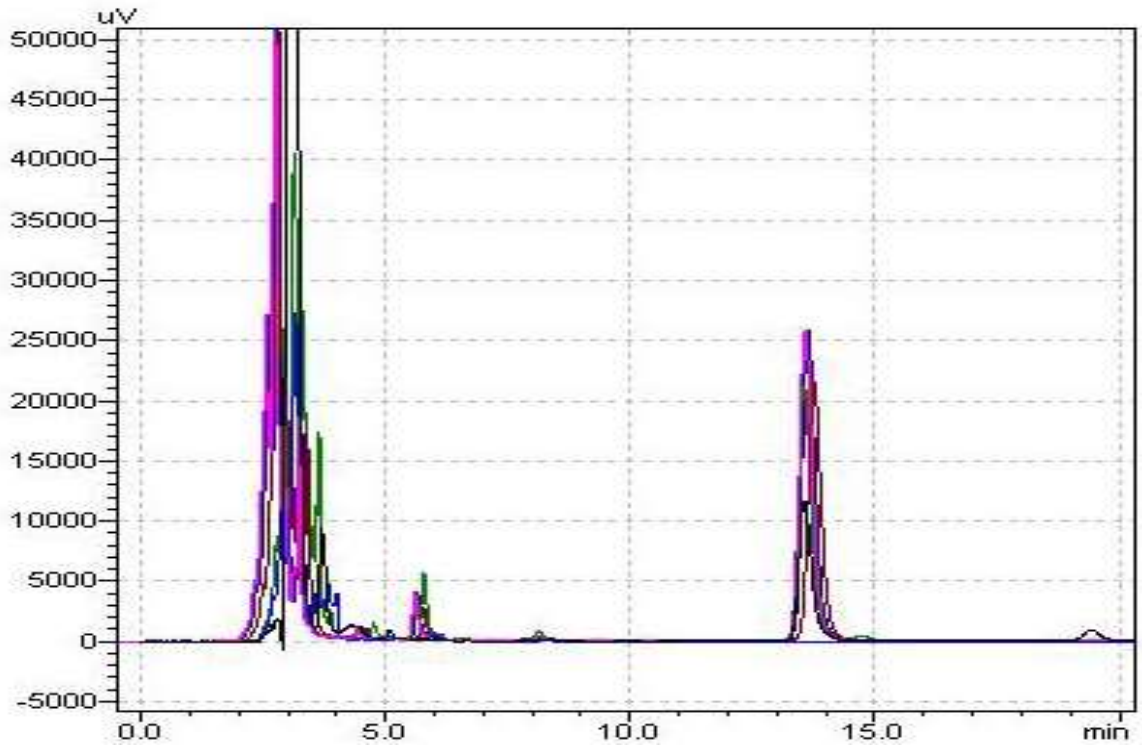

Fig 5: Overlay of chromatograms of the donor compartment samples when analysed for TMF at 5, 15, 30 and 60 min (**Expected R_t: 14.6 min**).