**Table S1.** Preparation of calibrators and quality control samples for TD, CLR and LAN.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Prepared samples** | **Plasma volume** | **Adding 50 µL of mixed working standard solution (µg/mL)** | **Final volume** | **Final plasma concentration (µg/mL)** |
| **TZ** | **CLR** | **LAN** | **TZ** | **CLR** | **LAN** |
| **Calibrators** | **450 µL** | 5.0 | 5.0 | 2.5 | **500 µL** | 0.5 | 0.5 | 0.25 |
| 10.0 | 10.0 | 5.0 | 1.0 | 1.0 | 0.5 |
| 50.0 | 50.0 | 10.0 | 5.0 | 5.0 | 1.0 |
| 250.0 | 250.0 | 50.0 | 25.0 | 25.0 | 5.0 |
| -- | 500.0 | 250.0 | -- | 50.0 | 25.0 |
| 500.0 | 1000.0 | 500.0 | 50.0 | 100.0 | 50.0 |
| **LQC** | 15.0 | 15.0 | 7.5 | 1.5 | 1.5 | 0.75 |
| **MQC** | 200.0 | 400.0 | 200.0 | 20.0 | 40.0 | 20.0 |
| **HQC** | 400.0 | 800.0 | 400.0 | 40.0 | 80.0 | 40.0 |

**Table S2**. Statistical comparison of results obtained by proposed HPLC-DAD method and the official method for determination of TD, CLR and LAN in pure forms.

|  |  |  |
| --- | --- | --- |
| Parameter | Proposed HPLC-DAD | Official method [8] |
| **TD** | **CLR** | **LAN** | **TDa** | **CLRb** | **LANc** |
| Mean% | 99.37 | 99.42 | 100.79 | 99.54 | 100.21 | 100.89 |
| SD | 0.281 | 1.33 | 1.5 | 0.162 | 1.12 | 0.898 |
| Variance | 0.079 | 1.769 | 2.25 | 0.026 | 1.254 | 0.806 |
| t-test(2.12) | 1.58 | 1.36 | 0.17 |  |  |  |
| F(3.44) | 3.01 | 1.41 | 2.79 |  |  |  |
| n | 9 | 9 | 9 | 9 | 9 | 9 |

a Mobile phase: acetonitrile-methanol-water in a ratio of 10:20:70, by volume using C18 column at flow rate 0.5 mL/min and UV detection at 320 nm.

b Mobile phase: acetonitrile-potassium dihydrogen orthophosphate, pH 4.4 in a ratio of 45:55, v/v using C18 column at flow rate 1 mL/min and UV detection at 205 nm.

c Mobile phase: trimethylamine-water, pH 6.2-acetonitrile in a ratio of 1:39:60, by volume using C18 column at flow rate 1.2 mL/min and UV detection at 285 nm .



**Fig.S1**.The chemical structure of Metronidazole (MET).