A Validated RP-HPLC assay method for Tofacitinib in pharmaceutical drug products

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Figure S1: The structure of Tofacitinib, also known as 2-hydroxypropane-1,2,3-tricarboxylic acid; 3-[(3R,4R) 4 methyl 3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4l)amino]piperidin-1-yl] -3-xopropanenitrile, has a molecular formula of $C_{16}H_{20}N_6O$ and a molecular weight of 312.377 g/mol.

Figure S2: Chromatogram of mobile phase (A), placebo (B), TFC standard (C) and tablet sample (D) showing t_R 5.3 min approximately.

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0.50    Purity Curve                    Peak      mAU
        Zero Line
                                                  100.0
0.25
                                                  75.0
0.00
                                                  50.0
-0.25
                                                  25.0
-0.50                                             0.0
          6.0              6.5    min
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Impurity : Not Detected.
Peak purity index : 0.999998
Single point threshold : 0.999713
Minimum peak purity index : 285

Figure S3: UV spectrum of of Tofacitinib for peak purity assay

Figure S4: Calibration curve for TFC area of peak concentration of standard solution

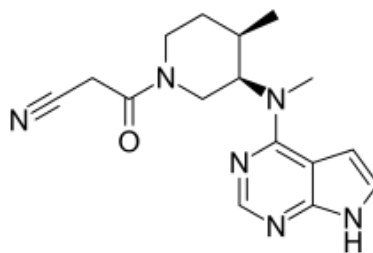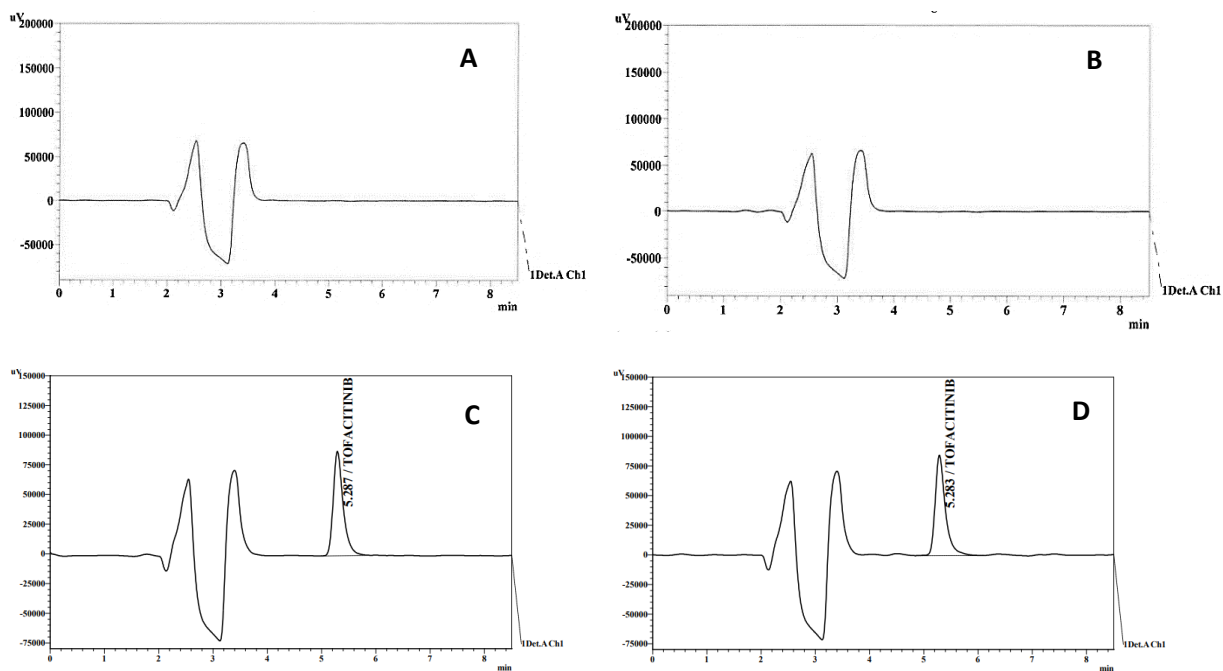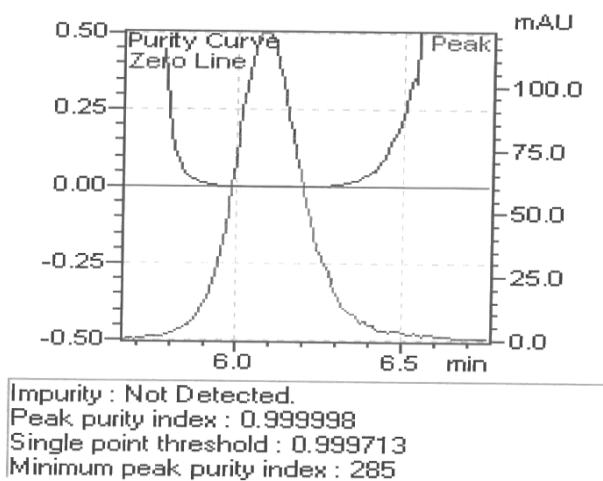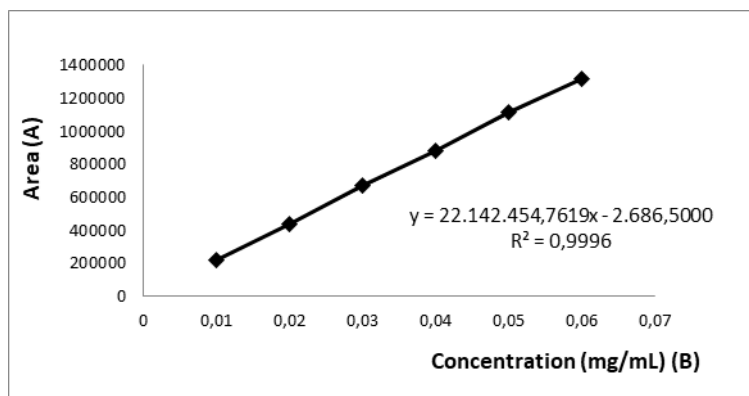**Appendix 1:** *Calculation of Uncertainty Budget of the Method*

First, uncertainty for standard was calculated by using the equation shown below.

$U_{standard} = 100 - \%Purity/\sqrt{3}$

Since the purity of the standard was reported as 98.0 (written in certificate of analysis), uncertainty of standard was found as;

$U_{standard} = (100-98)/\sqrt{3} = 1.15$

Second, uncertainty of calibration was calculated according to the equation shown below:

$U_{calibration} = (Standard\ Error\ of\ Slope*100)/Slope$

In order to calculate this parameter, first "standard error of slope" should be found. This value was also calculated by using the excel program called "LINEST", which was found as 214876.71. Moreover, the slope of linearity curve was found as 22142454.76.

$U_{calibration} = (214876,71*100)/22142454,76$

$U_{calibration} = 0.97$

Third, mean RSD values were used for uncertainty of recovery and repeatability parameters:

$U_{recovery} = RSD\ of\ recovery\ analysis$

$U_{recovery} = 0.02$

$U_{repeatability} = RSD\ of\ repeatability\ analysis$

$U_{repeatability} = 0.01$

Finally, combined uncertainty was calculated according to the equation given below.

$U_{combined} = \sqrt{((1.15)^2 + (0.97)^2 + (0.02)^2 + (0.01)^2)}$

$U_{combined} = \sqrt{2.26}$

$U_{combined} = 1.50$

$U_{expanded} = 3.01$