Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTARY DATA

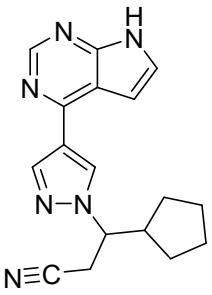


Figure S-I Structure of Ruxolitinib

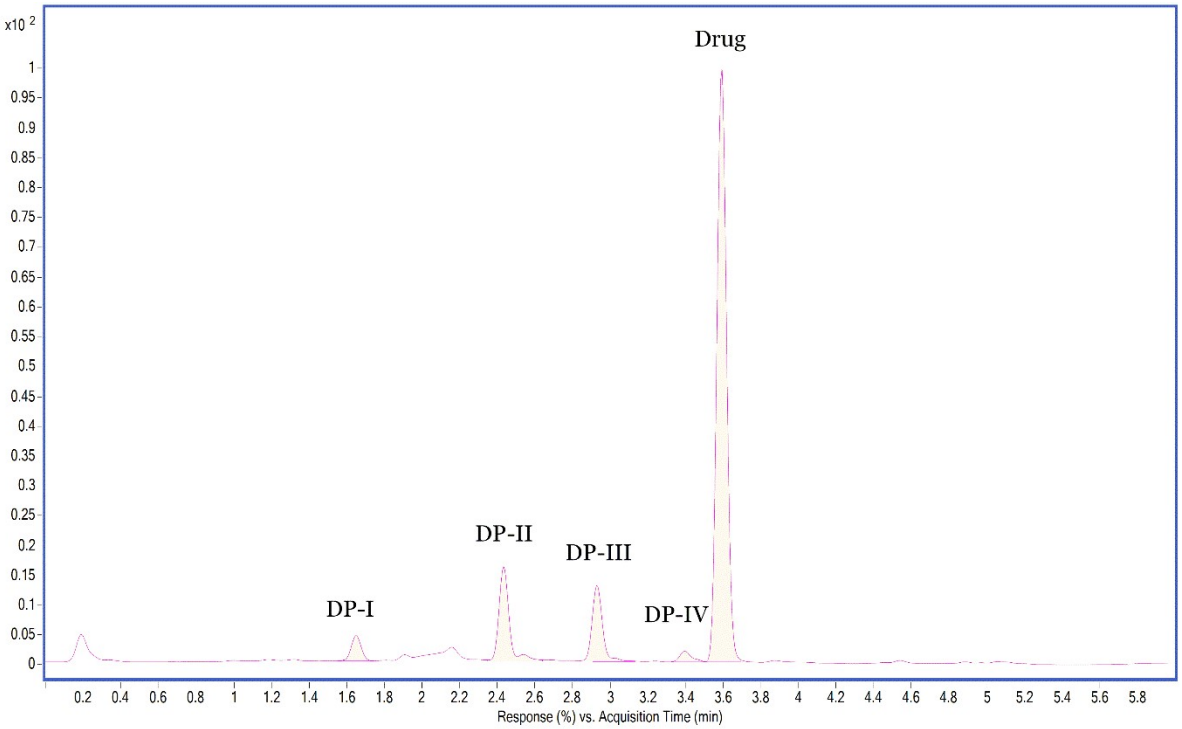
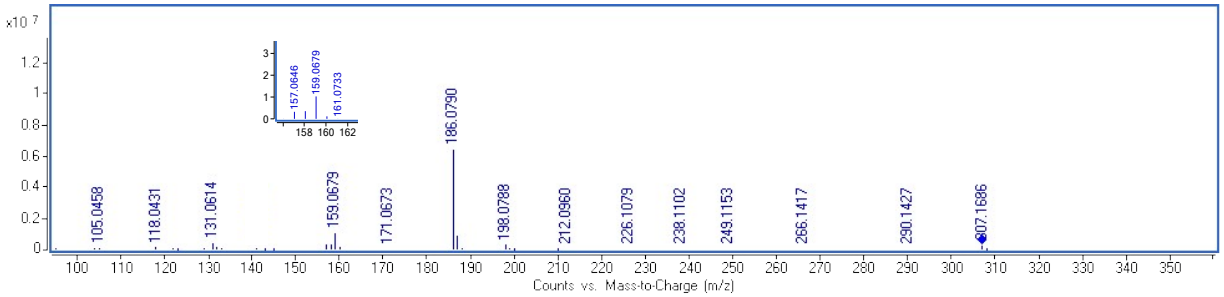


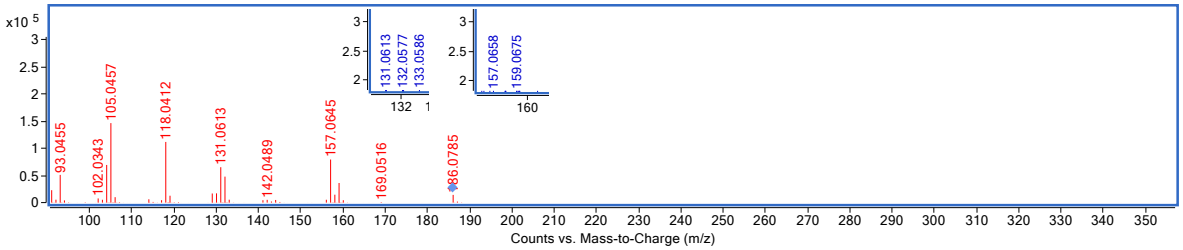
Figure S-II UHPLC chromatogram showing separation of Drug and DPs



307.1686 (Parent ion)	249.1153 (minor)	212.0960 (major)	171.0673 (minor)	159.0679 (major)	133.0609 (minor)	122.0973 (minor)
290.1427 (minor)	238.1102 (minor)	198.0788 (major)	169.0528 (minor)	157.0646 (major)	132.0578 (minor)	118.0431 (minor)
266.1417 (minor)	226.1079 (minor)	186.0790 (major)	161.0733 (major)	142.0522 (major)	131.0614 (major)	105.0458 (major)

Fragment List

Figure S-III Line spectra of drug obtained through LC-QTOF study

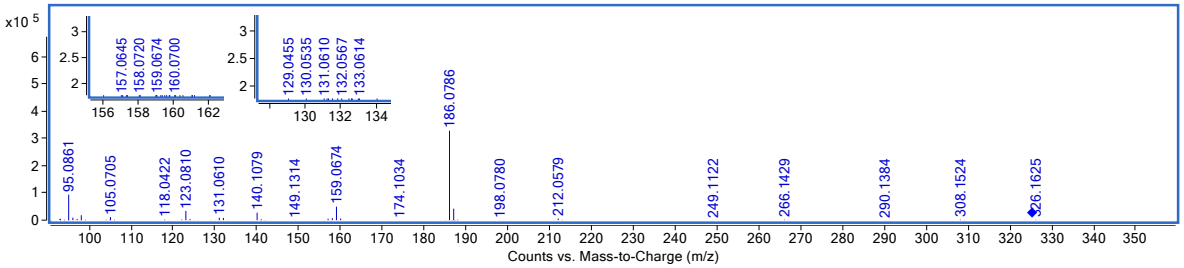


(M+H)

Fragment List

186.0785(Parent ion)	142.0489(major)	105.0457(major)
169.0516(major)	131.0613(major)	102.0343(major)
157.0645(major)	118.0412(major)	93.0455 (major)

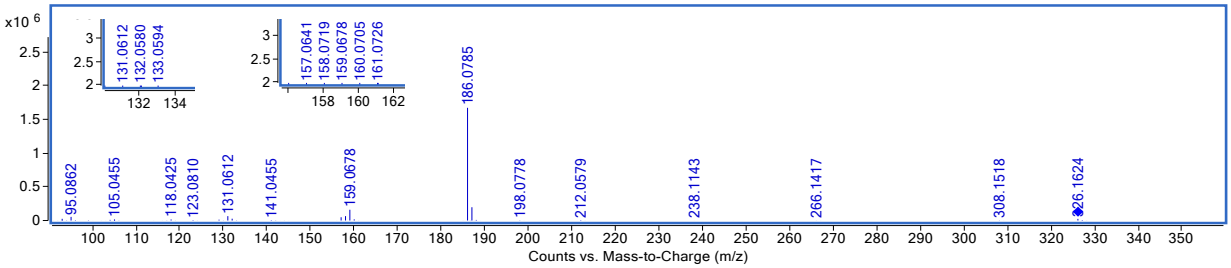
Figure S-IV MS/MS spectra of DP-I.



Fragment List

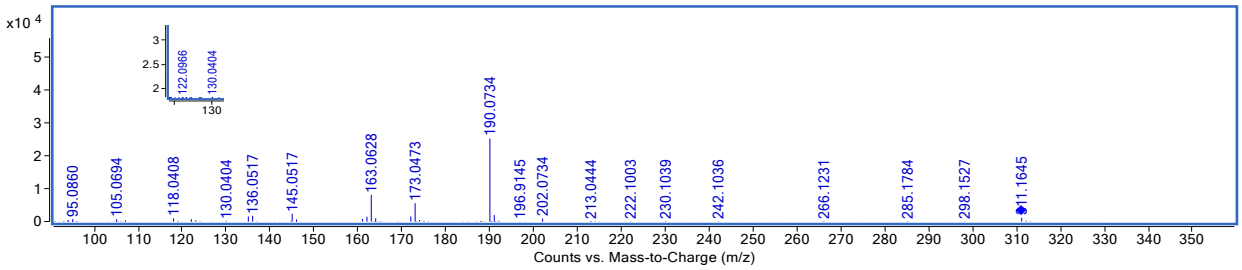
326.1625 (Parent ion) 308.1524 (major) 290.1384 (major)	266.1429 (major) 249.1122 (minor) 212.0579 (major)	198.0780 (major) 186.0786 (major) 174.1034 (major)	159.0674 (major) 149.1314(major) 140.1079 (major)	131.0610 (major) 123.0810 (major) 118.0422 (major)	105.0705 (major) 95.0861 (major)

Figure S-V MS/MS spectra of DP-II.


x10 6
2.5
2
1.5
1
0.5
0
95.0862
105.0455
118.0425
123.0810
131.0612
141.0455
159.0678
186.0785
198.0778
212.0579
238.1143
266.1417
308.1518
326.1624
100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350
Counts vs. Mass-to-Charge (m/z)
131.0612
132.0580
133.0594
132 134
157.0641
158.0719
159.0678
160.0705
161.0726
158 160 162

325.1771(Parent ion)	238.1143(major)	186.0785(major)	131.0612(major)	105.0455(minor)
308.1518(minor)	212.0579(major)	159.0678(major)	123.0810(minor)	95.0862(minor)
266.1417(major)	198.0778(minor)	141.0455(major)	118.0425(minor)	

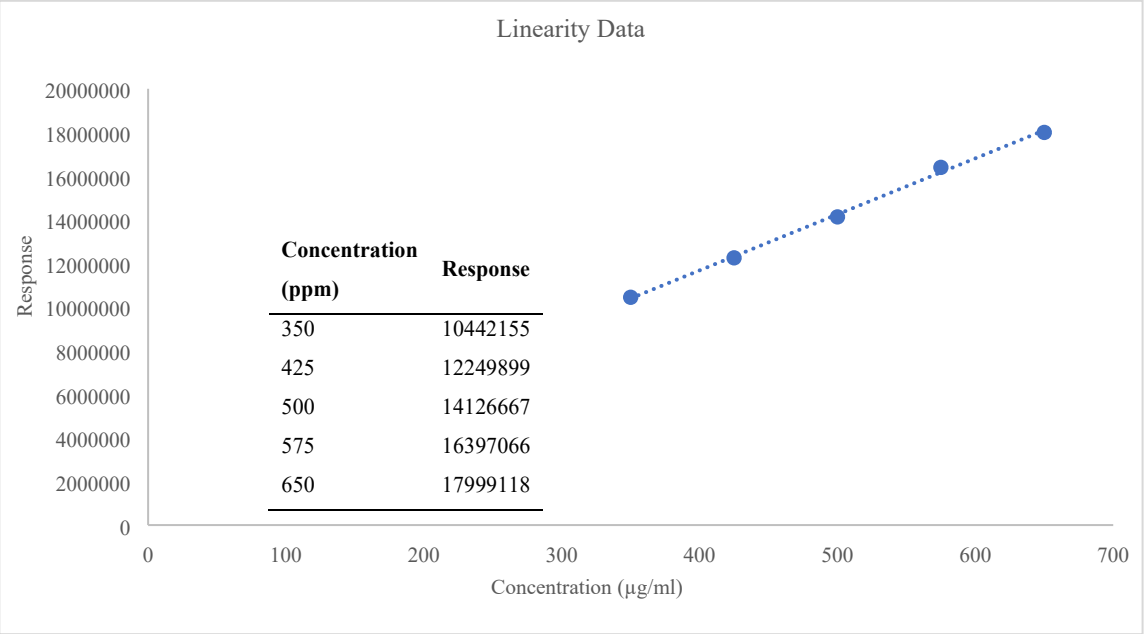
Figure S-VI MS/MS spectra of DP-III.



Fragment List

311.1645(Parent ion)	266.1231(major)	222.1003(minor)	196.9145(minor)	163.0628(major)	130.0404(major)	95.0860(major)
298.1527(minor)	242.1036(major)	213.0444(minor)	190.0734(major)	145.0517(major)	118.0408(major)	
285.1784(minor)	230.1039(major)	202.0734(major)	173.0473(major)	136.0517(major)	105.0694(major)	

Figure S-VII MS/MS spectra of DP-IV.



Concentration (ppm)	Response
350	10442155
425	12249899
500	14126667
575	16397066
650	17999118

Figure S-VIII Linearity of Ruxolitinb

Table S-I Recovery data for accuracy studies

Spiked concentration (µg/ml)	Calculated spiked concentration (µg/ml)	Recovery (%)
250	252.7695	101.11
500	497.7357	99.55
650	647.3528	99.59

Table S-II Inter-day& Intra-day precision

S.No	Area-Day1 (Repeatability)	Area-Day2 (Intraday Reproducibility)
1	21743617	18461460
2	21816750	18663374
3	21855636	18913057
4	21827007	18683340
5	21832683	18804814
6	21819140	18708382
Mean	**21815806**	**18705738**
SD	**34689.05**	**138306.6**
%RSD	**0.16**	**0.74**

Table S-III In silico toxicity prediction profile of drug and DPs through webservers (a) preADMET and (b) PROTOX-II.

Toxicity Description ID		Predicted Toxicity Value

	Ruxolitinib	Value for comparison with impurity[#]	DP-I	DP-II	DP-III	DP-IV
Ames_test [a]	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen
Hepatotoxicity [b]	Inactive	Inactive	Active	Active	Active	Inactive
Carcinogenicity [b]	Inactive	Inactive	Inactive	Active	Inactive	Active
Immunotoxicity [b]	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Mutagenicity [b]	Inactive	Inactive	Inactive	Inactive	Inactive	Active
Cytotoxicity [b]	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Carcino_Mouse [a]	Negative	Negative	Negative	Negative	Negative	Negative
Carcino_Rat [a]	Negative	Negative	Negative	Negative	Negative	Negative
PPAR-Gamma [b]	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
ATAD5 [b]	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
hERG_inhibition [a]	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk	Medium risk
TA100_10RLI [a]	Negative	Negative	Negative	Positive	Negative	Negative
TA100_NA [a]	Negative	Negative	Negative	Negative	Negative	Negative
TA1535_10RLI [a]	Negative	Negative	Positive	Negative	Negative	Positive
TA1535_NA [a]	Positive	Positive	Positive	Positive	Positive	Positive
Predicted LD_{50} [b]	800 mg/kg	-	1000 mg/kg	800 mg/kg	800 mg/kg	839 mg/kg

[#] As per the ICH guideline the identification threshold and qualification threshold is coming 0.1% and 0.15%, respectively based on dose of Ruxolitinib. Thus, it is assumed that no degradation product would cross the specification >0.5%. The impurity level would be 200 times lower than the API level and the correction with 200 times was applied for comparative qualitative data.

According to PreADMET it was interpreted that the impurities have almost similar safety profile as compared to API except TA1535_10RLI and TA100_10RLI.

While mutagenicity according to PreADMET could not be compared, as the drug and DPs both were shown to be a mutagen. Hence PROTOX-II was used, in which DP-IV was found to be mutagenic.