

Supplementary document for article “QbD-mediated RP-UPLC method development invoking an FMEA-based risk assessment to estimate nintedanib degradation products and their pathways”

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**Abbreviations**

%RSD – percentage relative standard deviation  
 2D – two dimensional  
 2FI – two factor interaction  
 3D – three dimensional  
 ANOVA – analysis of variance  
 AQbD - analytical quality by design  
 AR – analytical reagent  
 ATP - analytical target profile  
 CID - collision induced disassociation  
 CMA - critical material attributes  
 CMP - critical method parameters  
 CQA - critical quality attributes  
 DOE – design of experiments  
 DP – degradation product  
 ESI – electrospray ionisation  
 FD - forced degradation  
 FMEA - Failure mode effect analysis  
 FTN – flow through needle  
 ICH – international conference on harmonisation  
 IPF – idiopathic pulmonary fibrosis  
 LOQ – limit of quantification  
 MODS – method operable design space  
 MRM – multiple reaction monitoring  
 MS – mass spectrometry  
 NIN – nintedanib ,  
 PDA – photo diode array  
 pKa – dissociation constant  
 QbD – quality by design  
 QSM – quaternary solvent manager  
 QTMP – quality target method profile  
 QTP – quality target profile  
 QTPP – quality target product profile  
 RP-HPLC – reverse phase-high pressure liquid chromatography  
 RPN - risk priority number  
 RP-UPLC – reverse phase-ultra pressure liquid chromatography  
 RRT – relative retention time  
 Rt – retention time

Time	Mobile phase A (0.005 Ammonium acetate buffer 100%)	Mobile phase B (Acetonitrile 100%)
0.00	65.0	35.0
5.00	60.0	40.0
8.00	50.0	50.0
10.00	60.0	40.0
12.00	60.0	40.0

**Final gradient program**

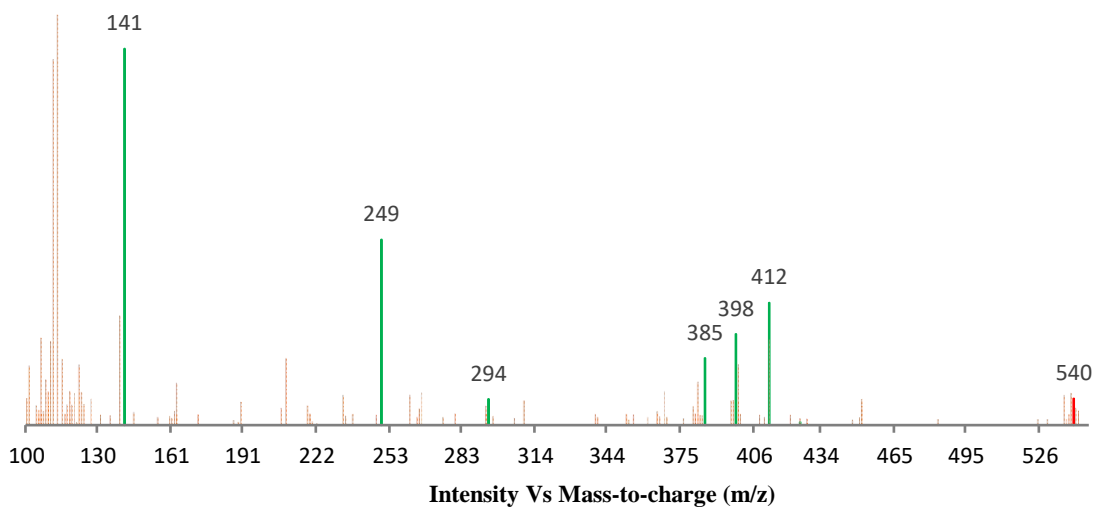
Mobile phase buffer	Remarks
pH 2.2 perchloric acid	Tailing factor of main analyte 1.5 with response about 0.6 AU
pH 4.5 Acetate buffer	Tailing factor of main analyte 1.6 with response about 0.3 AU
pH 6.8 phosphate buffer	Tailing factor of main analyte 3.0 with response about 0.2 AU
0.1% Trifluoroacetic acid	Tailing factor of main analyte 1.3 with response about <0.1 AU
0.1% Formic acid	Poor retention, peaks eluted before 2 mins.

**Summary of results of preliminary method screening – Different aqueous phase of different pH.**

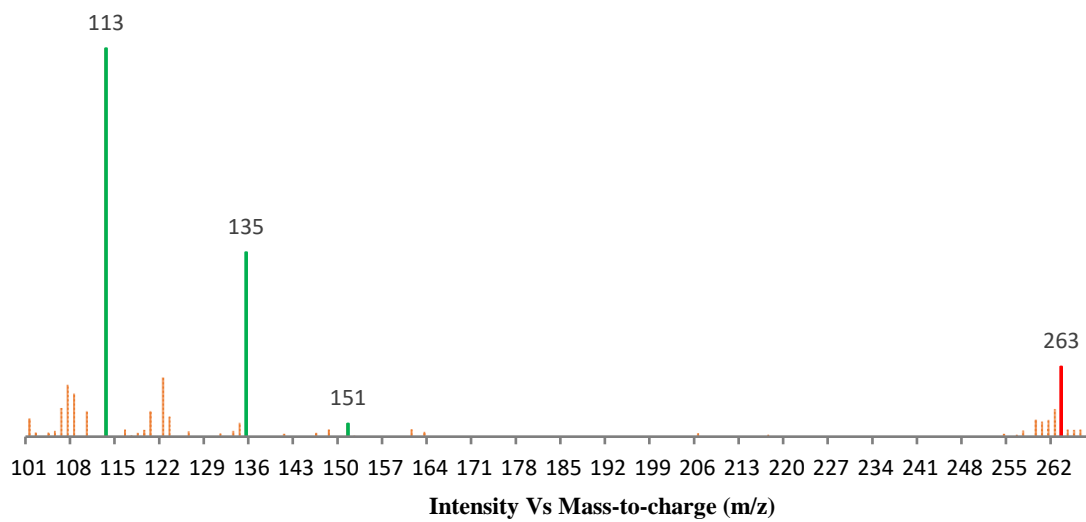
Determination of the overall risk using RPN.		
RPN	Overall Risk Level	Description
1-8	Low	Risk is considered acceptable and no further action required.
9-26	Medium	Risk is acceptable, further mitigation actions may be required in order to reduce to low level of risk.
27-64	High	Risk is unacceptable. To be resolved with definite actions.

Response	Predicted mean	Observed mean
R1	1.43	1.39
R2	1.82	1.76
R3	2.42	2.45

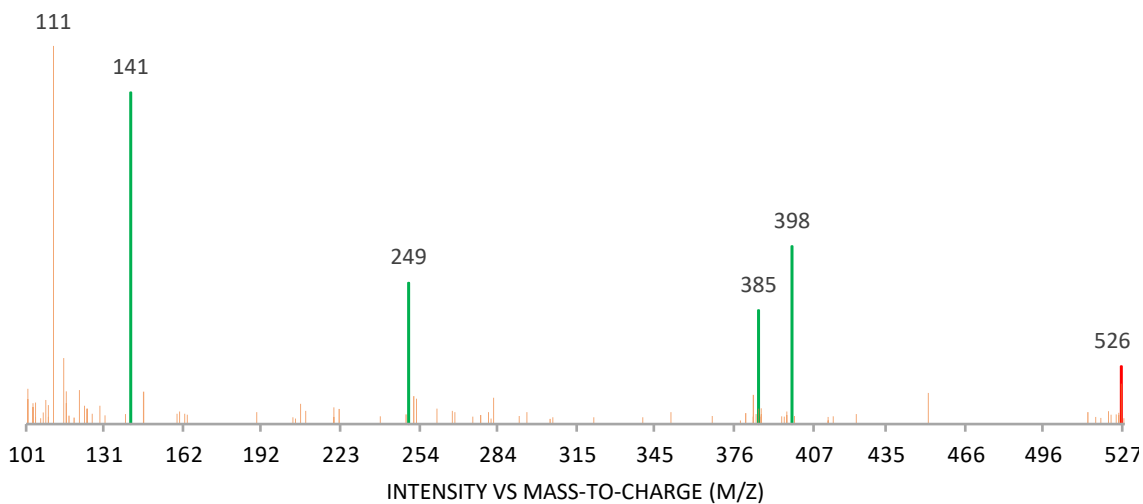
Predicted and observed values at highest desirability conditions.



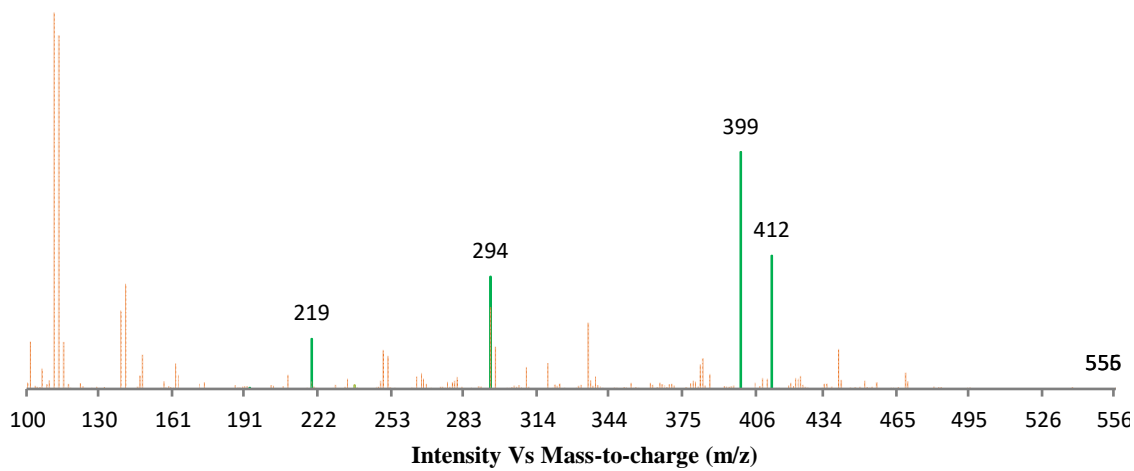
ESI/MS/MS spectrum of Nintedanib



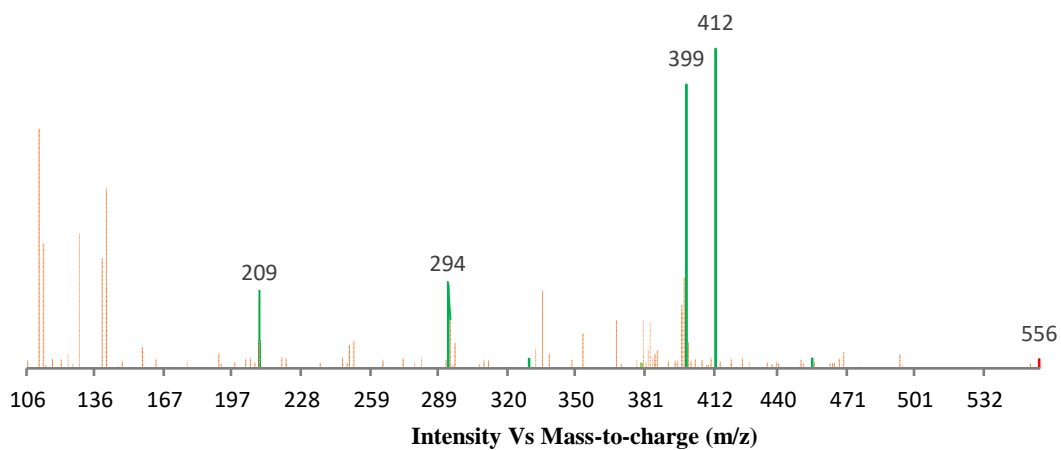
ESI/MS/MS spectrum of DP1



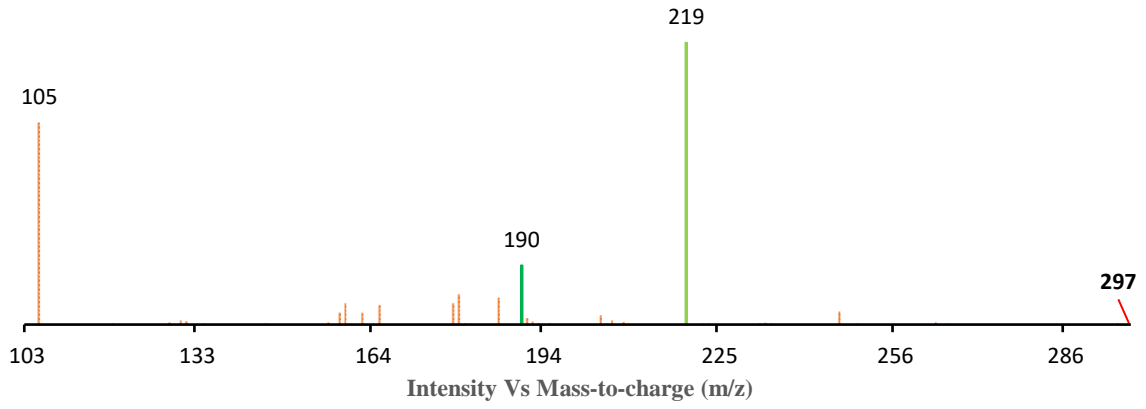
ESI/MS/MS spectrum of DP2



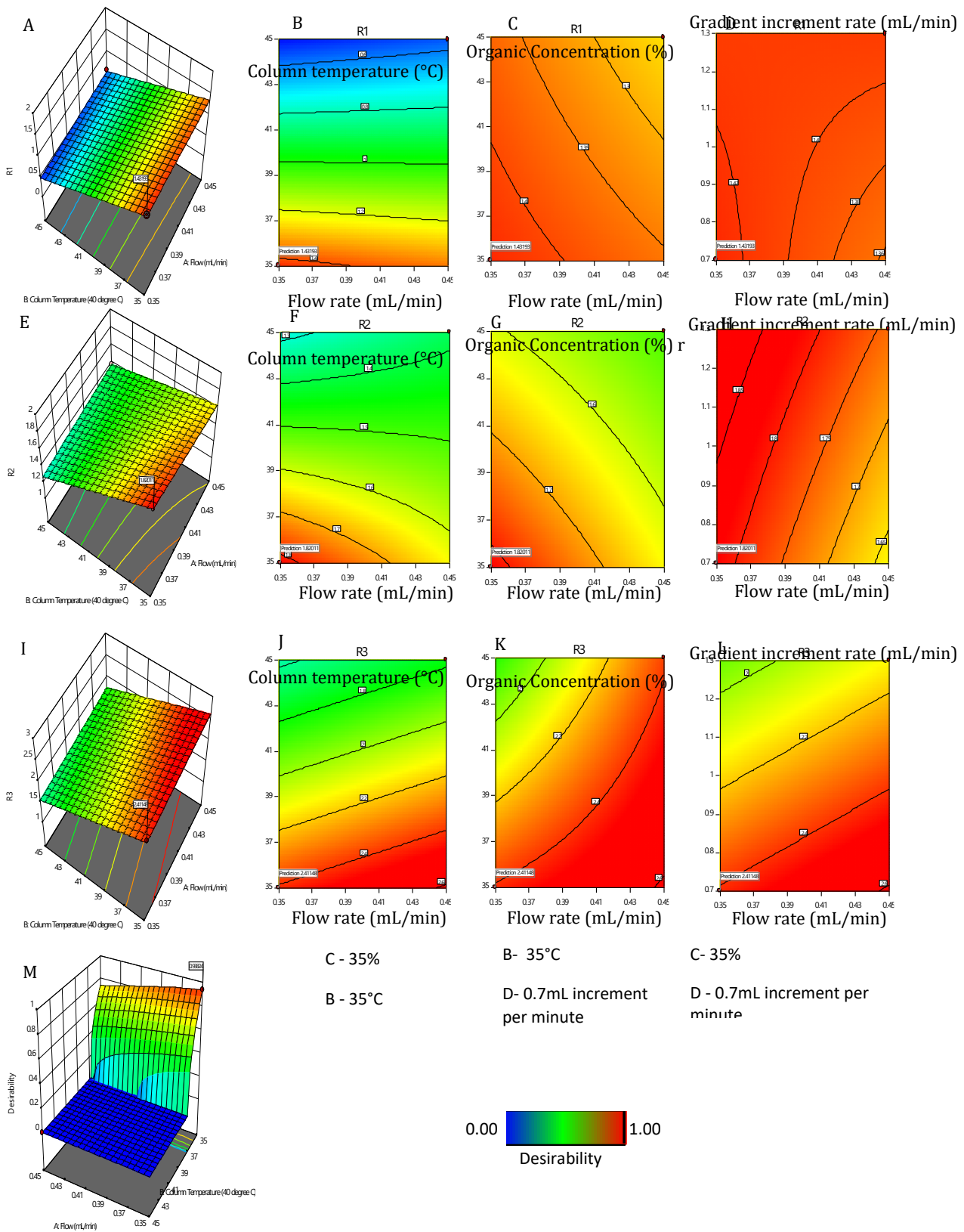
ESI/MS/MS spectrum of DP3



ESI/MS/MS spectrum of DP4



ESI/MS/MS spectrum of DP5



3D and 2D plots of CMA, namely (A – D) for R1, (E-H) from R2, (I-L) for R3 and M desirability plot.

Factor Coding: Actual

**All Responses**

**Actual Factors**

A: Flow = 0.35

B: Column Temperature = 35

C: Organic concentration = 35

D: Organic Flowrate = 0.7

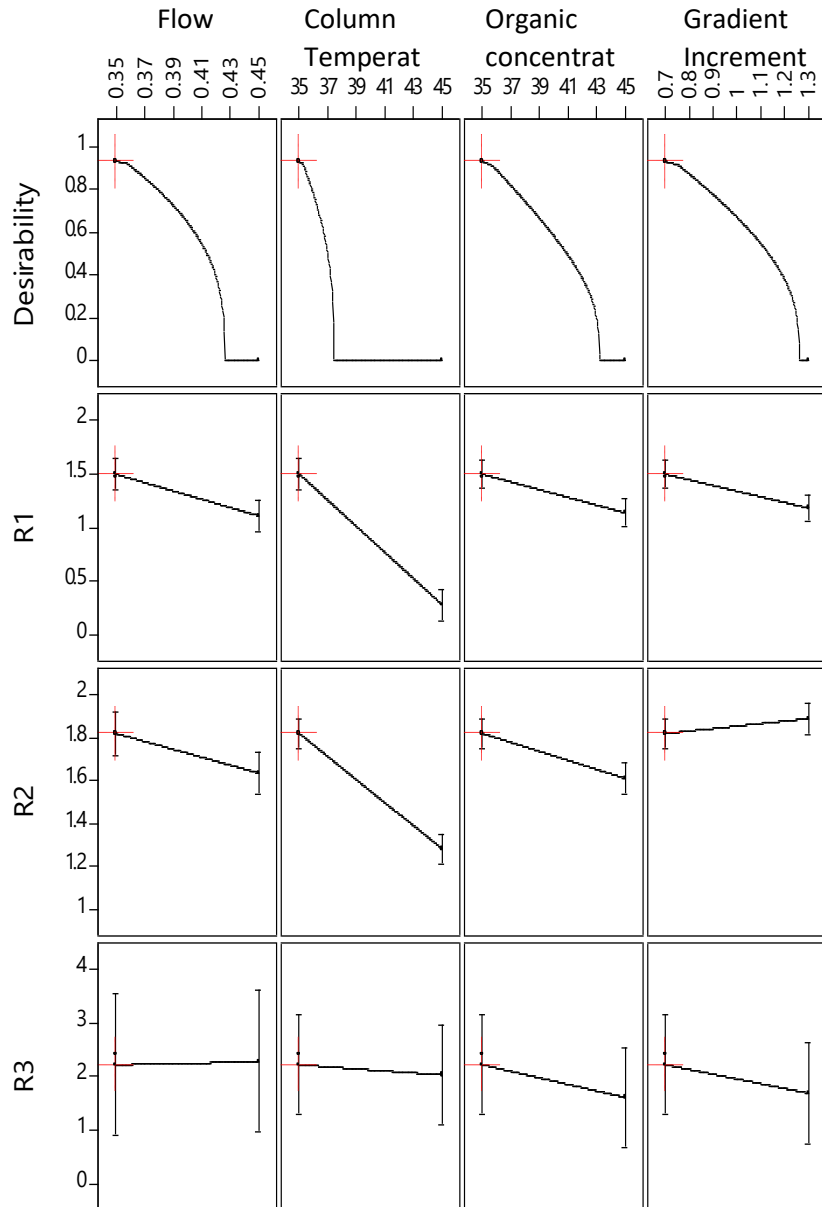
**Responses**

Desirability = 0.926278

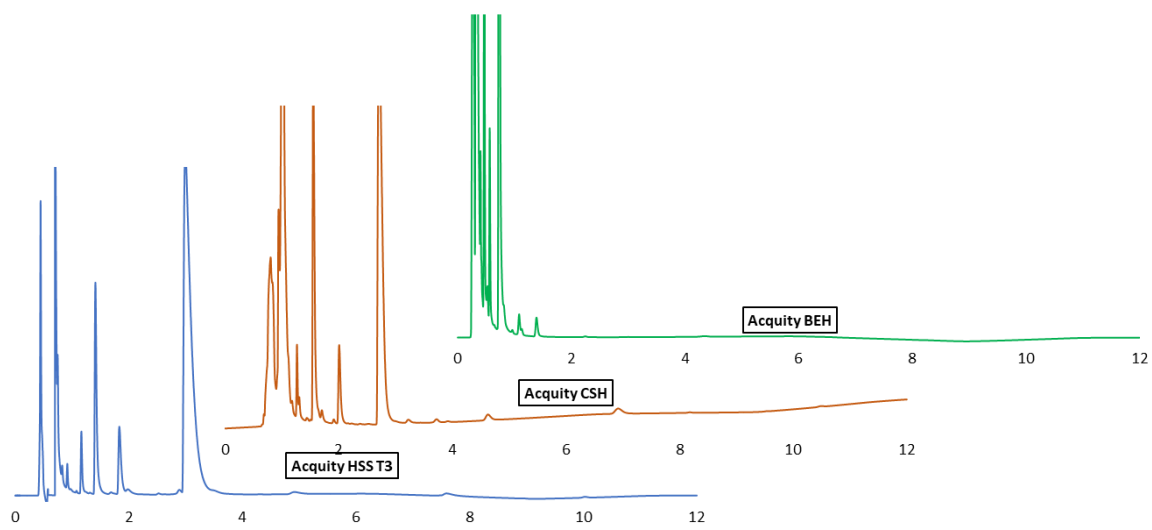
R1 = 1.49625

R2 = 1.82011

R3 = 2.22182



All response plots



Column screening chromatograms