6. CONCLUSIONS
ICH prescribed forced degradation studies on leflunomide (LLM), methotrexate (MTX), hydroxychloroquine (HCQ) and sulfasalzine (SSZ) were carried out in accordance with ICH guidelines Q1(R2). Different LC-MS compatible HPLC methods (isocratic and gradient) were developed for separation of all the degradation products from the drug peaks as well as from each other, and for studying the degradation behavior of each drug in different forced conditions. SSZ was found stable to all forced conditions except alkaline hydrolysis (5 N, 24 h, 80 °C) under which one minor degradation product was formed. However, it was not characterized due to very less content in the sample. HCQ was found to be stable under all conditions except alkaline photolytic conditions in which it degraded to six products (I-VI). Out of these five degradation products (I-V) were characterized through LC-MS-TOF studies, MS^n studies and PDA analysis. The product III was identified as N-de-ethylated HCQ which is a known impurity. It was also found to form in trace amounts under acidic and alkaline hydrolytic conditions. The products I, II, IV and V characterized as N-dehydroxyethylated-7-hydroxy HCQ, dechlorinated HCQ, N-dealkylated HCQ and N-oxide HCQ, respectively were identified as new degradation products of HCQ. The product VI was not characterized due to its trace levels. LLM was degraded to three degradation products under alkaline hydrolytic conditions (0.1 N, 8 h, 80 °C) and to one minor product under acid hydrolytic conditions (5 N, 8 h, 80 °C). The degradation behavior of LLM under alkaline conditions was observed to be similar to that under alkaline photolytic conditions though the extent of degradation was less in photolytic condition. Out of the three alkali degraded products, the major degradation product (IV), eluting at 36.2 min in alkali hydrolyzed drug solution was isolated through the column chromatography using dichloromethane and methanol in gradient elution mode and characterized through $^1$H NMR, IR and Mass spectral analysis. It was characterized as Impurity B reported in British Pharmacopoeia. MTX was found to degrade into five degradation products (I-V) under acid hydrolysis and to single major degradation product (V) under alkaline hydrolysis. Four degradation products of MTX were characterized through LC-MS-TOF studies and MS^n studies. I was proposed to be 4-[(2,4-diamino-pteridin-6-ylmethyl)-methyl-amino]-benzamide which was a pharmacopoeial impurity (impurity K). It was possible to form due to hydrolysis of the amide linkage in MTX. II was characterized as a reported process related impurity i.e. 4-[(2,4-Diamino-pteridin-6-ylmethyl)-methyl-amino]-benzamide. III was proposed to form due to substitution of an -NH$_2$ group on petridyl part of MTX with a -OH group and hence, it was characterized as
Conclusions

deeaminedhydroxylated MTX which is also a known pharmacopoeial impurity (Impurity C). IV 
was proposed to be a cyclic analog of MTX formed due to cyclization of glutamic part of MTX. 
V was proposed to be deglutamyl MTX formed due to acid catalyzed hydrolysis of amide 
linkage. It was also found to be a known BP impurity E. The mechanism of formation of 
characterized degradation products from each drug were proposed and discussed. In general, SSZ 
was found exceptionally stable drug. Each of other three drugs was susceptible to degradation in 
hydrolytic and/or photolytic conditions but stable to dry heat and light in dry state as well as to 
oxidative degradations. All the developed HPLC methods were validated for various validation 
parameters and found linear in the prescribed linearity range, highly accurate and precise and 
robust for the analysis for the specific drug analysis.