FORCED DEGRADATION STUDIES ON SELECTED DISEASE MODIFYING ANTI-RHEUMATIC DRUGS

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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 under which one minor degradation product was formed. However, it was not characterized due to very less content in the sample. MTX was found to degrade into five degradation products (I-V) under acid hydrolysis and to single major degradation product (V) under alkaline hydrolysis. All the five degradation products of MTX were characterized as 4-[(2,4-diamino-pteridin-6-ylmethyl)-methyl-amino]-benzamide (I), 4-[(2,4-Diamino-pteridin-6-ylmethyl)-methyl-amino]-benzamide (II), deaminatedhydroxylated MTX (III), a cyclic analog of MTX (IV) and deglutamyl MTX (V). 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\textsuperscript{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 and to one minor product under acid hydrolytic conditions. 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), 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.

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