1. INTRODUCTION
Introduction

Rheumatoid arthritis (RA) is an autoimmune and systemic chronic disease characterized by inflammation of multiple joints resulting in progressive and irreversible joints damage leading to cartilage destruction and deformity (Rindfleisch and Muller, 2005). It affects about 1% of the world as well as Indian population (Parle and Kaura, 2012). Non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), inhibitors of tumor necrosis factor (anti-TNF-α), and glucocorticoids are the main classes of the drugs, which are used for the treatment of RA. NSAIDs are used for the management of pain and inflammation while, DMARDs are used as first line treatment for RA (Gaffo et al., 2006). A long term use of these classes of drugs is required for treatment of RA (Tripathi, 2008). Presence of even trace amounts of impurities in these drugs may affect the efficacy and safety in long term treatment. Impurities can be formed during synthetic process of the drug substance (Process related impurities, PRI) or during formulation, transportation and storage of the drug product (degradation related impurities, DRI). International Conference on Harmonization (ICH) guidelines Q3A(R2) (2006) and Q3B(R2) (2006) require the identification of any impurity (PRI or DRI) above its identification threshold on the basis of sound scientific appraisal of potential degradation pathways arising from interaction of drug with impurities, excipients and/or immediate container closure system. ICH guidelines Q1A(R2) (2003) recommend conduct of forced degradation studies or stress testing on drug substance as well as on drug product. Forced degradation helps in generating all possible degradation products in sufficient amounts under different chemical environments to facilitate identification of unknown degradation products. Several other drugs regulatory guidelines also require stress testing of new as well as existing drug substances and/or drug products to study influence of various factors affecting quality, safety and/or efficacy of the drug substance, to validate stability-indicating assay method (SIAM) and to establish structures, physicochemical properties, mechanism and kinetics of formation of degradation products (ICH Q1B, 1996; FDA, 1987; FDA, 2000a; FDA, 2000b; WHO, 1996; CPMP, 1998; TPD, 2005).

Sulfasalazine (SSZ), leflunomide (LLM), hydroxychloroquine (HCQ) and methotrexate (MTX) are commonly used DMARDs for the treatment of RA (Schuna, 2005). These are official in different Pharmacopoeias. HCQ is an official drug substance in British Pharmacopoeia (BP) and United States Pharmacopeia (USP). No impurities or related substances are listed in both of its official monographs. LLM is official in BP where seven impurities (A-G) are listed in its
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MTX is an official drug substance in BP, USP and Indian Pharmacopoeia (IP) and nine impurities (A-L) are listed in its monograph. SSZ is an official drug substance in BP and IP with eight impurities (A-H) listed in its monograph. In addition to these impurities, certain DRIs are also expected to form from these drugs.

The presence of sulfonamide group, secondary and tertiary amines, carboxylic acid, carboxylic amide linkage, alkyl and aryl halide moieties in chemical structures of the selected DMARDs make the latter susceptible to degradation under varied chemical environments. The amide linkage is susceptible to cleavage under hydrolytic as well as in photolytic conditions to form carboxylic acid and amine products. Secondary or tertiary amines undergo N-dealkylation and N-oxidation under photolytic and oxidative conditions. The alkyl and aryl halide groups are prone to dehalogentation under different photolytic and oxidative conditions. Decarboxylation of drug molecules containing carboxylic acid group occurs under hydrolytic, photolytic and oxidative conditions. These chemical susceptibilities can lead to the formation of products which are termed as DRIs or degradation products. Some reports are available in literature which disclose some DRIs on the selected DMARDs. Tønnesen et al. (1988) have investigated the photochemical effect of light on aqueous solution of HCQ. They have isolated and characterized four decomposition products. Dongre et al. (2009) have identified and characterized two PRIs of HCQ. Zalipsky et al. (1978) have characterized PRIs in SSZ. Kher et al. (2012) have developed a stability indicating assay method for the determination of LLM in its pharmaceutical formulation. Chatterji and Gallelli (1978) have subjected MTX to thermal degradation in aqueous solution over a pH range of 8.3 to 12 and to photolysis using white fluorescent light and characterized its four degradation products. Chatterji et al. (1983) have also identified and quantified its two process related impurities. In addition, a number of analytical methods are reported for the analysis of the selected DMARDs in formulations, in biological fluids and in the presence of other therapeutic agents.

However, none of the reported studies on the selected DMARDs is carried out in accordance with the ICH guidelines for the characterization and quantification of selected DMARDs in the presence of their degradation products. The present study is designed to carry out ICH prescribed forced degradation studies on HCQ, SSZ, LLM and MTX, development of HPLC method(s) for separation of each drug and its degradation products, characterization of
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major degradation product(s) of each drug through spectral analyses (IR, NMR and/or Mass) or LC-MS studies and establishment of degradation pathways and inherent stability of the drugs.

SSZ was found stable to all forced conditions except alkaline hydrolysis (5 N NaOH, 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 stable under all conditions except alkaline photolytic conditions, in which it degraded to six products. Out of these, five products were characterized through LC-MS-TOF studies, MS^n studies and PDA analysis. LLM degraded to three degradation products under alkaline hydrolytic conditions (0.1 N NaOH, 8 h, 80 °C) and to one minor product under acid hydrolytic conditions (5 N HCl, 8 h, 80 °C). The major product formed in alkali hydrolyzed drug solution was isolated through the column chromatography and characterized through ^1^H NMR, IR and Mass spectral analysis. MTX was found to degrade to five degradation products under acid hydrolysis and to single major degradation product under alkaline hydrolysis. All degradation products of MTX were characterized through LC-MS-TOF studies and MS^n studies. All the developed HPLC methods were found to be stability-indicating, were validated for various validation parameters and found linear in the prescribed linearity range, highly accurate and precise and robust for the analysis of the specific drug. The validated stability-indicating methods were applied to stability studies of the commercially available tablets of the selected DMARDs.