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Characterization of N-Acetyl-Tryptophan Degradation in Protein Therapeutic Formulations

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Abstract

N-Acetyl-tryptophan (NAT) is used as a stabilizer for preparations of human serum albumin and has more recently been demonstrated to provide oxidative protection for labile Trp residues in monoclonal antibodies. As a component in the formulations of protein therapeutics, NAT is sacrificially degraded; therefore understanding the identity and quantity of NAT degradants potentially formed in these drug products is essential to understanding the potential patient impact of this additive. Here we report a simple reversed phase chromatography approach that allows systematic investigation of NAT degradation in relevant formulations under stressed conditions. Screening a panel of NAT-containing samples following a variety of forced stress conditions led to a range of NAT degradation from minimal (3%) to significant (83%). NAT degradants were observed to be largely conserved between oxidative and thermal stress conditions. Online mass spectrometry and standard compound synthesis allowed for identification of the major degradants in the stressed sample panel. NAT degradation was minimal under recommended storage conditions and in relevant thermal stress conditions for a representative protein therapeutic drug product, suggesting that NAT is stable under normal manufacturing, storage, and handling conditions. This work supports the use of NAT as an antioxidant in liquid drug product formulations.

Keywords: HPLC (high-performance/pressure liquid chromatography), oxidation, excipients, chemical stability, formulation, analytical chemistry

Abbreviations

AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride), DiOia (dioxyindolylalanine), HSA (human serum albumin), ICH (International Conference on Harmonization), Kyn (kynurenine), mAb (monoclonal antibody), NAT (N-Acetyl-tryptophan), NFK (N-formyl-kynurenine), Oia (oxyindolylalanine), PIC (H, 1,2,3,3a,8,8a-hexahydro- 3a-hydroxypyrrolo [2,3-b]-indole 2-carboxylic acid), ROS (reactive oxygen species), RP (reversed phase), Trp (tryptophan).