Artifacts Generated During Azoalkane Peroxy Radical Oxidative Stress Testing of Pharmaceuticals Containing Primary and Secondary Amines

MARCELA NEFLIU,[1] TODD ZELESKY,[2] PATRICK JANSEN,[3] GREGORY W. SLUGGETT,[2] CHRISTOPHER FOTI,[2] STEVEN W. BAERTSCHI,[3,4] PAUL A. HARMON[1]

[1]Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania 19486
[2]Pfizer Inc., Analytical Research & Development, Eastern Point Road, Groton, Connecticut 06340
[3]Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, Indiana 46285
[4]Baertschi Consulting, LLC, Carmel, Indiana 46033

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ABSTRACT: We report artifactual degradation of pharmaceutical compounds containing primary and secondary amines during peroxy radical-mediated oxidative stress carried out using azoalkane initiators. Two degradation products were detected when model drug compounds dissolved in methanol/water were heated to 40°C with radical initiators such as 2,2′-azobis(2-methylpropionitrile) (AIBN). The primary artifact was identified as an α-aminonitrile generated from the reaction of the amine group of the model drug with formaldehyde and hydrogen cyanide, generated as byproducts of the stress reaction. A minor artifact was generated from the reaction between the amine group and isocyanic acid, also a byproduct of the stress reaction. We report the effects of pH, initiator/drug molar ratio, and type of azoalkane initiator on the formation of these artifacts. Mass spectrometry and nuclear magnetic resonance were used for structure elucidation, whereas mechanistic studies, including stable isotope labeling experiments, cyanide analysis, and experiments exploring the effects of butylated hydroxyanisole addition, were employed to support the degradation pathways.

Keywords: artifacts; stress testing; forced degradation; oxidation; autoxidation; free radicals; chemical stability; strecker reaction; degradation products; mass spectrometry; stability

INTRODUCTION

A key component of pharmaceutical stress testing is the execution of simple solution-based experiments to reveal intrinsic hydrolytic and oxidative susceptibilities of new drug compounds.[1] Stress testing is used to support various aspects of drug development including development and optimization of stability-indicating methods and relevant control strategies. Identification of potential drug degradation products from solution-based experiments may also facilitate the risk assessment process for the evaluation of degradation products for potential mutagenicity (ICH M7).[2] It is therefore desirable that solution stress testing conditions trigger degradation pathways that are relevant not only to the drug substance, but also to the associated drug product dosage forms. Primary hydrolytic drug degradation products are often predicted via solution-phase chemistry,[3] however, evaluation of propensity of drugs to oxidize is more complex.[4–6] Peroxy radical mediated oxidation, a type of autoxidation, is induced by trace impurities (e.g., in various excipients) and is considered the most common route of drug oxidation.[6] The solution stress test involves peroxy radical formation via thermal decomposition of azo compounds and is designed to accelerate oxidative kinetics so as to induce in a short period of time an observable response for molecules susceptible to autoxidation induced by peroxy radical attack.[7] Because of the complexity of the oxidative system, the test has historically suffered from some selectivity issues in creating the peroxy radical activity, allowing artifactual degradation to be observed.[8] The current study represents another step in refining the test conditions and understanding its limitations.

Peroxy radicals, known to selectively abstract weakly-bound H atoms (bond dissociation enthalpy $< \sim$90 kcal/mol),[4,9] can be generated for the purpose of the stress test through thermal decomposition of radical initiators such as 2,2′-azobis(2-methylpropionitrile) (AIBN)[10,11] or 4,4′-azobis(4-cyanovaleric acid) (ACVA).[7,11] The subsequent, rapid reaction with dissolved oxygen generates the desired peroxy radicals as shown for AIBN in Scheme 1. Usually, the experiment is carried out in dilute drug solutions at 20 mol% or greater of the radical initiator.[12] The long-lived azoalkane-derived peroxy radicals may therefore encounter drug molecules from which they may abstract H atoms, but may also readily undergo recombination/decomposition as shown in Scheme 2. Characteristic to *tertiary* peroxy radicals, the recombination/decomposition of the azoalkane peroxy radicals leads to the undesired formation of alkoxy radicals, estimated to react 10^4–10^5 times faster with organic substrates, than the corresponding peroxy radicals.[13] As a result, the selectivity of the test for weakly bound H atoms is lost, as the drug may preferentially react with alkoxy radicals at a variety of sites within the drug molecule. For drug

Correspondence to: Christopher Foti (Telephone: +650-378-2135; Fax: +650-522-5472; E-mail: chris.foti@gilead.com)

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