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RESEARCH ARTICLE

Development of a Reversed-Phase HPLC Impurity Method for a UV Variable Isomeric Mixture of a CRF Drug Substance Intermediate with the Assistance of Corona CAD

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Abstract The compound studied, an antagonist of corticotropin-releasing factor (CRF) receptor 1, was under development for the treatment of major depressive disorder and generalized anxiety disorder. To control the quality of intermediate compounds in the synthetic pathway, the preference is to develop a single HPLC method that could determine impurities of the sequential and structurally similar intermediates. However, the chemical nature of one of those intermediates, Compound C, posed a number of challenges to the development of a suitably rugged impurity method for this isolated intermediate. In the solid state, Compound C is a stable mixture of its *trans*-isomer and semicarbazone forms. In most solution states, Compound C interchanges between the *trans* and semicarbazone forms as well as degrades rapidly to impurity X, resulting in sample solution instability. Furthermore, the *trans*-isomers and semicarbazone exhibited different UV response factors, but reference standards of the two forms were not available to determine the relative response factor. Consequently, irreproducibility was observed during the HPLC analysis when conventional UV detection was applied directly. This article describes the following approaches that were employed to meet these challenges: (1) diluent screening was performed to select a \solvent that afforded adequate sample solution stability; (2) appropriate chromatographic conditions were selected to eliminate or minimize any on-column degradation; (3) alternate HPLC detection, Corona charged aerosol detection, in conjunction with UV analysis, was used to determine relative amounts of the isomeric forms which subsequently permitted the use of conventional UV detection for routine analysis. The validation data of this reversed-phase liquid chromatographic method are also discussed.

Keywords Corticotropin-releasing factor (CRF) receptor · Chromatography · HPLC · Corona charged aerosol detection · Impurity/degradation product · Validation

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Introduction

The compound "API-CRF", an antagonist of corticotropin-releasing factor (CRF) receptor 1, was under development for the treatment of major depressive disorder and generalized anxiety disorder [1]. A robust process for the synthesis of API-CRF (active pharmaceutical ingredient, API) was achieved, proceeding through six steps (Compounds A through F to API) with the isolation of only two intermediates, Compounds C and E (Fig. 1). It was critical to determine the quality of the intermediate compounds produced throughout this synthetic pathway to control the impact on downstream chemistry. Therefore, it was necessary to develop HPLC method(s) that could analyze the value of assay (purity based on % *w*/*w* versus a reference standard) and impurities (relative peak area percent) of the sequential and structurally similar intermediates. However,