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Development of a purity control strategy for pemetrexed disodium and validation of associated analytical methodology

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ABSTRACT

Stability-indicating reversed phase HPLC methods have been developed and validated for the determination of 13 potential process and degradation impurities in pemetrexed disodium drug substance (DS) and pemetrexed for injection drug product (DP). This paper describes the development of HPLC-UV impurity methods for drug substance and drug product. Relative response factors (RRF) have been determined using HPLC-UV in tandem with CAD or by NMR detection. Conditions for the generation of system suitability solutions are described and assure adequate chromatographic resolution and peak identification without the need for impurity reference standards. The methods were fully validated and demonstrated to have acceptable specificity, linearity, accuracy, repeatability, intermediate precision, detection/quantitation limit, and robustness.

1. Introduction

Pemetrexed is a synthetic compound used in the treatment of various cancers. The drug product is formulated as a lyophilized sterile powder of pemetrexed disodium that is reconstituted prior to intravenous administration. Key to assurance of patient safety and product quality is the development of a comprehensive understanding of the potential process and degradation impurities. An understanding of these degradation and process impurities is central to the development and understanding of the drug substance synthetic and drug product manufacturing processes and for the definition of the control strategy.

Accurate assessment of potential impurities is a critical development goal that requires highly selective, stability-indicating methods to determine which impurities are relevant in the drug substance and drug product commercial process. While there are literature reports on the determination of pemetrexed drug substance and its enantiomer [1–3], literature information on the determination of impurities in the drug substance is limited [4,5] and does not include a comprehensive evaluation of potential degradation impurities. In this paper we present stability indicating methodology for the determination of impurities in pemetrexed disodium drug substance (DS) and pemetrexed for injection drug product (DP).

During development, process modifications can result in changes to the impurity profile; this may cause project delays due to the need to identify new impurities and assure adequate selectivity and control. Therefore, the use of a MS-compatible method becomes an attractive tool for impurity identification and control especially for the drug substance process impurities.

The drug substance method development for pemetrexed disodium focused on delivering a method that was robust and compatible with mass spectroscopy and charged aerosol detectors in order to provide control for the process and degradation impurities, impurity identity verification and response factor determination capability. Drug product demonstrates no appreciable degradation during manufacture and shelf-life storage. To assure control of the drug product throughout shelf-life, the impurity method focus was on the development of a stability-indicating, robust, user-friendly, globally accessible HPLC method.

2. Experimental

2.1. Equipment

Chromatography was performed on Agilent 1100 systems equipped with variable wavelength UV detectors set at 250 nm and autosamplers set at 2–8 °C. For UV response factors determinations, an Agilent DD2 400 mHz NMR Spectrometer equipped with

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a 5-mm ATB probe was used as well as an ESA Corona Ultra™ charged aerosol detector in series with the UV detector. The NMR spectrum was acquired using a total relaxation delay of 30.1 s and 16 scans. Chromatographic data were collected using Millenium and Empower software (Waters Corporation, Milford, MA) or an in-house data acquisition system based on a Hewlet Packard HP1000 computer system. Gradient separations were carried out on Zorbax® SB-C8, 3.5 μm, 15 cm × 4.6 mm columns (Agilent Technologies) at ambient conditions for DS, and at 35 °C for DP.

2.2. Materials

Ammonium formate (Aldrich), 88% formic acid (Fluka), acetic acid (Mallinckrodt) and 50% sodium hydroxide (Mallinckrodt) were of reagent grade. Water was purified with a Millipore Milli-Q Plus purification system (Millipore, Billerica, MA) or de-ionized by the local system. Acetonitrile (Burdick & Jackson, OmniSolv) was HPLC grade. For NMR relative response factor determination, deuterated acetonitrile (acetonitrile-d3) and water were used (Cambridge Isotope Laboratories).

Vazo 52 (2,2′-azobis(2,4-dimethyl)valeronitrile) was obtained from Du Pont, and hydrogen peroxide (30%, ACS) was acquired from Fisher Scientific. A 0.2 or 0.45-μm filter (Life Science PTFE membrane Acrodisk CR 13 mm or 25 mm) was used to filter the DP system suitability sample preparation that contained Vaso 52 oxidizing agent. Pemetrexed disodium heptahydrate, reference standard and DS were obtained from Lilly Research Laboratories.

2.3. Sample preparation

Drug substance and drug product samples were prepared for analysis at 0.2 mg/mL pemetrexed (active component) in water. A detectability sample was prepared by making an appropriate dilution of the sample or standard in water to a pemetrexed concentration of 0.06 μg/mL (0.03%) for DS and 0.1 μg/mL (0.05%) for DP. A DS method system suitability resolution solution was prepared by heating a solution of drug substance at 3 mg/mL in NaOH (0.1 M) at 70 °C for 40 min. A 1:10 dilution in water of this degraded stock solution was used for analysis. The DP stock system suitability resolution solution was prepared by heating a solution of drug substance (2 mg/mL) and oxidizing agent Vazo 52 (2.8 mg/mL) in acetonitrile:water (1:1, v/v) at 70 °C for 30 min. Alternatively, the DP stock system suitability resolution solution was prepared by heating a solution of drug substance (2 mg/mL) in hydrogen peroxide (0.3%, v/v) at 75 °C for 2–5 h. A 1:10 dilution in water of one of the degraded stock solutions was used for analysis. Degradation impurities used for the specificity studies and relative response factor determinations were isolated from stressed material by reversed-phase preparative HPLC. Mixtures of the drug substance and the process impurities were prepared for the relative response factor determinations.

3. Results and discussion

3.1. Method development

3.1.1. DS and DP degradation summary

A comprehensive study of the degradation chemistry was performed which included stressing solution samples under various conditions of heat, light, oxidation, and pH (over the range 1–13) and solid samples under various conditions of heat, humidity, and light [6]. The stress testing studies indicated that the drug substance degrades in solution via two main degradation pathways: hydrolysis of the amide linkage at low pH and oxidation of the 5-member ring of the pyrrolopyrimidine moiety. Oxidation is also the primary

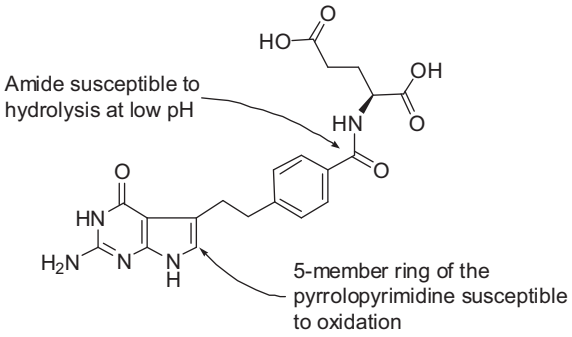

Fig. 1. Principal sites of degradation of pemetrexed.

degradation mechanism in the solid state. Fig. 1 illustrates the two principal reactive sites of the pemetrexed molecule. A total of seven significant degradation products were detected in the degradation studies, see Table 1. The degradation of pemetrexed to the des-glutamate also produces glutamic acid in and equi-molar amount which is not detectable at 250 nm, however, the des-glutamate peak is used as the indicator for the presence of both degradation products if they were to be present. Since these seven products were formed under stress conditions, they are potential degradation products and were used to validate the stability indicating power (i.e. specificity) of the drug substance and drug product analytical methods. While these compounds are potential impurities, only the oxidative dimers are seen in actual DS samples; in addition to the oxidative dimers, the lactam isomers are also seen in the DP at or above the ICH reporting level [7,8].

The potential impurities for pemetrexed disodium are shown in Table 1 and are shown as the free acid form. These include potential process impurities that can be present from the drug substance synthesis and drug product manufacture as well as the seven potential degradation products. For the drug product, no additional impurities resulting from interactions between the drug substance, the excipients, and the container closure system have been identified.

3.1.2. HPLC method parameter selection

The structures of the impurities are similar, as shown in Table 1, and as a result, their chromatographic behaviors are also similar and posed a challenge for method development to separate these analytes. A column/mobile phase screening study was performed to identify the column, aqueous buffer pH, and organic modifier composition that provided adequate selectivity for the impurities determination. The pH of the aqueous mobile phase was evaluated over the range from 2.1 to 8.2 with methanol (gradient range 4–65%) and with acetonitrile (gradient range 2–56%) as possible organic modifiers. Different column stationary phase types were used to evaluate a broad range of surface properties of which C8 showed the best selectivity. Further column screening was performed, evaluating several vendors of C8 columns, see Table 2. The retention time of impurities and pemetrexed was shown to be a function of pH. In order to optimize the chromatographic conditions and minimize run time, method development software tools such as DryLab™ were used to provide efficiency. Simulation and experimental data showed the critical pair to be the oxidative dimer peaks. Based on the data, a Zorbax® SB-C8, 15 cm × 4.6 mm, 3.5-μm particle size column was chosen for both drug substance and drug product method optimization. Both methanol and acetonitrile showed capability for the resolution of the related substances and acetonitrile was chosen because of the clean absorbance background it can afford.

The final DP method was developed to provide control of degradation impurities. Additional selectivity was required for the final DS method to provide selectivity for potential process impurities