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

Anne Warner*, Irina Piraner, Heather Weimer, Kevin White

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, United States

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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

* Corresponding author. Tel.: +1 317 276 0141.
E-mail address: warner_anne_m@lilly.com (A. Warner).

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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

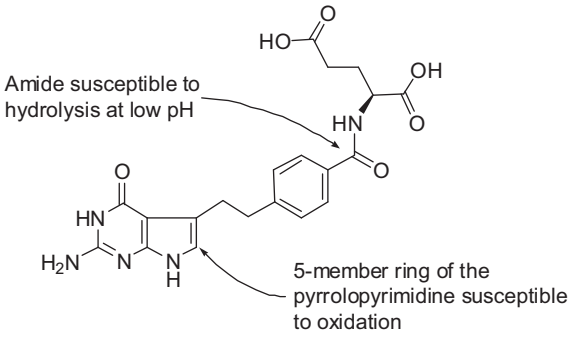


Fig. 1. Principal sites of degradation of pemetrexed.

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

Table 1
Pemetrexed structure and impurity information.

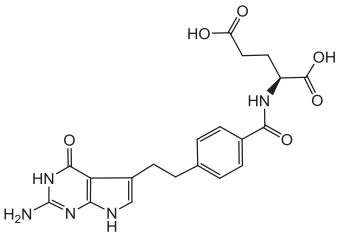
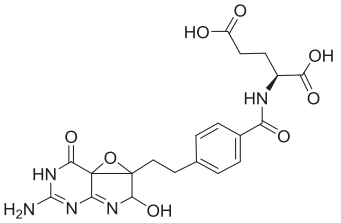
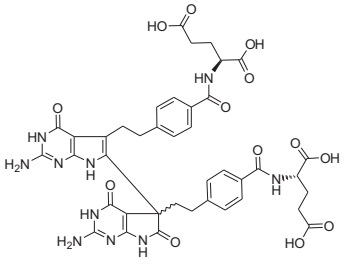
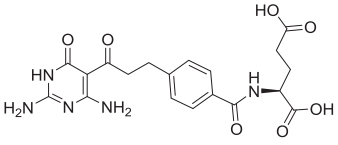
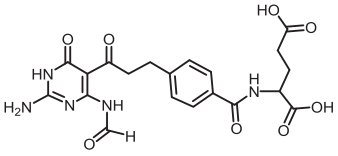
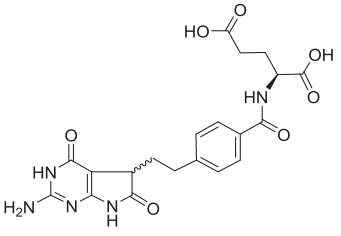
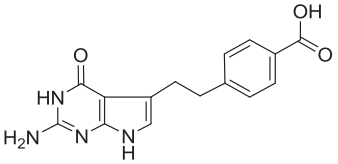
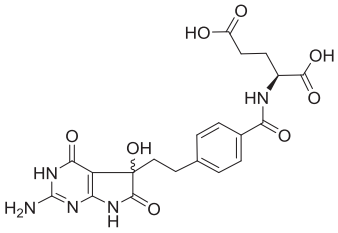
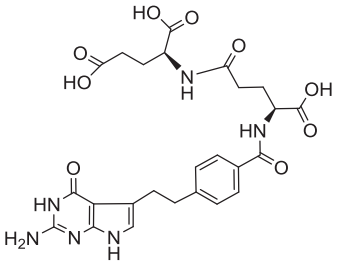
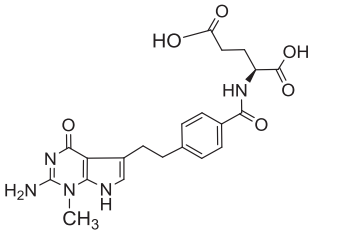
Common name	Structure	Source	Drug substance method relative retention time	Drug product method relative retention time
Pemetrexed			1.00	1.00
Epoxy hemiaminal		Potential degradation impurity	0.23	0.08
Oxidative dimers, isomers 1 and 2		Drug substance and drug product main degradation impurities	0.86 and 0.87	0.65 and 0.69
Ring-opened Keto-amine		Potential degradation impurities	0.91	0.80
Ring-opened Keto-formamide		Potential degradation impurities	1.14	1.21
Lactams, isomers 1 and 2		Drug product main degradation impurities	0.56 and 0.57	0.32 and 0.32
Des-glutamate		Potential degradation impurity	1.32	1.46

Table 1 (*Continued*)

Common name	Structure	Source	Drug substance method relative retention time	Drug product method relative retention time
α-Hydroxy lactams, isomers 1 and 2		Potential degradation impurities	0.39 and 0.40	0.16 and 0.17
Gamma glutamate		Drug substance synthetic route process impurity	0.88	N/A
N-methyl derivative		Drug substance synthetic route process impurity	0.79	N/A

in addition to the relevant degradation impurities, see Table 1. Both methods quantitate impurities based on percent of total area. The drug substance mobile phase consisted of ammonium formate (1.5 g/L, pH 3.5) and acetonitrile with the gradient of 5% (v) to 30% (v) acetonitrile over 45 min at 1 mL/min and at ambient column temperature. For the drug product gradient elution, separation was performed at 1 mL/min using mobile phase consisting of sodium acetate buffer (pH 5.5, 30 mM) and acetonitrile. A column temperature of 35 °C was chosen based on slight increase in selectivity at given chormatographic conditions and for further method ruggedness. The separation was achieved with the linear gradient from 3% (v) to 12.5% (v) acetonitrile over 40 min with a 5 min hold at 12.5% acetonitrile before re-equilibration to initial conditions. For both drug substance and drug product methods, HPLC autosampler temperature was controlled at 2–8 °C to minimize sample degradation.

Table 2
Columns used in column screening study.

Column type	Column
Octyl	Zorbax SB C8 Inertsil C8 Zorbax XDB C8 Zorbax RX C8 Luna C8 Supelco LC8DB Phenomenex prodigy C8
Octadecyl	Zorbax SB C18 Zorbax RX C18
Cyano	Zorbax CN
Phenyl	Zorbax SB phenyl
Varying chain length (less than or equal to C8)	YMC basic
Polymer based octadecyl	Shodex ODP

3.1.3. *Wavelength selection and impurity UV relative response factor determination*

For determination of impurities, HPLC with UV detection at 250 nm was chosen. The impurities shown in Table 1 are structurally similar and are expected to have similar UV spectral responses at this wavelength. While 250 nm is not the band with the highest response for the analytes, it is one of the maxima for pemetrexed, and is a region where all major analytes have a sufficient response. Also 250 nm provides adequate balance between analyte responses and background absorbance.

Determination of the relative response factors (RRFs) for the impurities that are not available as synthesized or isolated material with known purity can be determined using UV detection in tandem with charged aerosol detection (CAD) [9–11] or in combination with quantitative NMR [11,12]. For example, Sun et al. [9] have demonstrated the use of the HPLC-UV-CAD technology for the determination of RRFs in paclitaxel. HPLC mobile phases compatible with UV detection and the charged aerosol detector (CAD) are used and allow orthogonal means for detection. This methodology allows direct comparison of peaks without the additional complexity of needing to track peaks on different chromatographic systems where the peak order may be different. The CAD is a mass detector; therefore, the use of HPLC-UV-CAD allows for determination of response factors without having isolated impurity samples which can be difficult to obtain in high purity. In CAD the effluent from the HPLC system is nebulized under nitrogen to form small droplets followed by the removal of the volatile eluent components. The resulting sample particles collide with a stream of

positively charged nitrogen whereby charge is transferred to the analyte particles. The amount of charge detected is proportional to the amount (mass) of material in the particles. The response (area/mass) therefore can be obtained for every impurity. This technique lends itself to be an effective tool for response factor and potency determinations for analytes that are not volatile or semi-volatile.

For drug substance method conditions, the actual RRFs for the impurities with respect to pemetrexed were determined at a concentration of approximately 0.2 mg/mL using UV detection in tandem with CAD since the analytes are not volatile. These analytes elute in a narrow chromatographic range, so there is minimal effect of the mobile phase composition on the CAD response. UV and CAD ratios are used together to calculate the relative response factors. Since the CAD area ratio is proportional to the mass ratio, the RRF can be calculated using the following equation:

$$\text{RRF} = \frac{\text{peak area ratio impurity}}{\text{peak area ratio pemetrexed}}$$

where peak area ratio impurity = UV peak area of impurity/CAD peak area of impurity; peak area ratio pemetrexed = UV peak area of pemetrexed/CAD peak area of pemetrexed.

Since the drug product method mobile phase is not compatible with CAD, the RRF can be determined using a combination of NMR and HPLC (UV) analyses. Quantitative NMR can serve as a universal detector for compounds containing hydrogen, so integrated areas in the ^{1}H NMR spectrum can be used to determine the exact molar ratios of species in solution. These ratios can then be used in concert with the HPLC data to calculate relative response factors for these species. As with the HPLC-UV-CAD technique, the use of NMR and HPLC-UV allows for determination of response factors even if isolated impurities with known purity values are not available. For the determination of the RRF for an impurity relative to pemetrexed, a solution that contained both analytes at (6–7 mg/mL) in acetonitrile-d3:deuterated water (1:1, v/v) was analyzed by NMR to determine the analytes molar ratio, and further diluted to 1% of the nominal pememetrexed concentration for analysis by the HPLC-UV DP method conditions. Since the NMR response is proportional to molar concentration of analytes present, the RRF can be calculated using the following equation:

$$\text{RRF} = \frac{\text{peak area ratio impurity}}{\text{peak area ratio pemetrexed}} \times \frac{\text{MW pemetrexed}}{\text{MW impurity}}$$

where peak area ratio impurity = UV peak area of impurity/NMR area of impurity; peak area ratio pemetrexed = UV peak area of pemetrexed/NMR peak area of pemetrexed; MW pemetrexed = molecular weight of pemetrexed; MW impurity = molecular weight of impurity.

The NMR area of the impurity was determined by integrating the signal near δ_H 3.48 (1 proton), the NMR area of pemetrexed was determined by integrating the signal near δ_H 6.35 (1 proton).

If the RRF values are in the 0.8–1.2 range, no response factor correction is required [13]. The RRF for all analytes, except for the lactams, were between 0.8 and 1.2 which demonstrated that no correction factors are needed for these analytes. The lactams were found to have an RRF of 0.61, therefore a correction factor of 1.64 (1/0.61) was included in the DP method for this analyte.

3.2. Analytical drug substance and drug product methods validation

3.2.1. Specificity

For the drug substance, two process impurities (the N-methyl and the gamma glutamate derivatives) and two degradation impurities (the oxidative dimers) have been identified from development work to be present in actual drug substance samples. These impurity peaks are separated from one another and from the main peak so that accurate quantitation is possible (Fig. 2). In addition, potential stress degradation impurities were also used to demonstrate the method specificity (Fig. 3).

The drug product method specificity was focused on adequate separation of degradation impurities that were typically observed in the drug product and on stability (the oxidative dimers, and the lactams). In addition, potential stress degradation impurities were also used to demonstrate the method specificity. Fig. 4 demonstrates that each impurity of interest is separated from one another and from the main peak of pemetrexed so that accurate impurities quantitation is enabled.

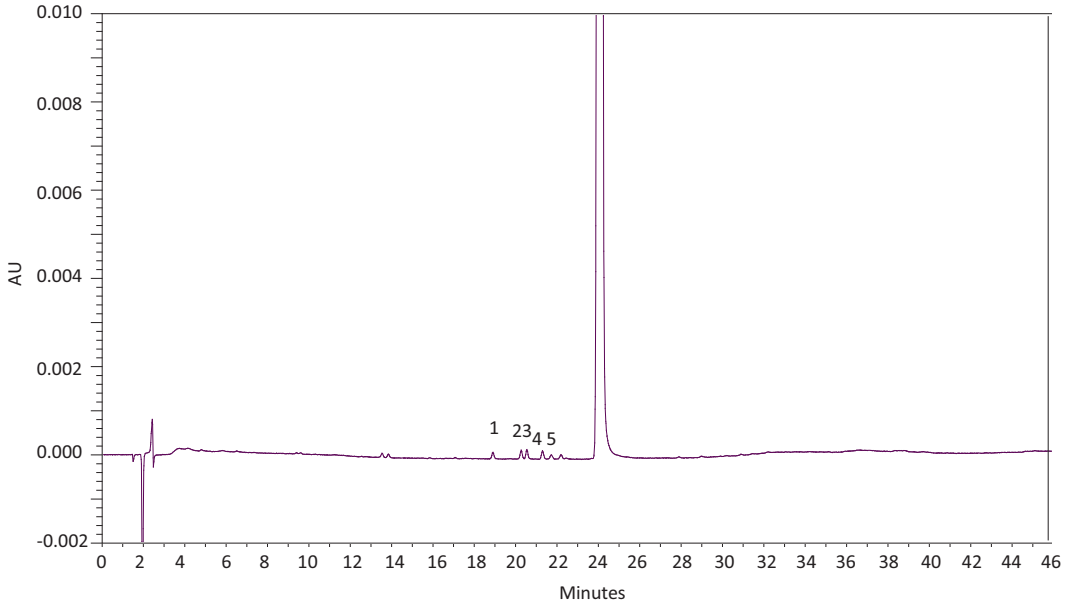


Fig. 2. Chromatogram of drug substance sample containing typical degradation and process impurities: (1) N-methyl derivative; (2, 3) oxidative dimers, isomers 1 and 2 (<0.03%); (4) gamma glutamate; (5) ring-opened keto-amine.

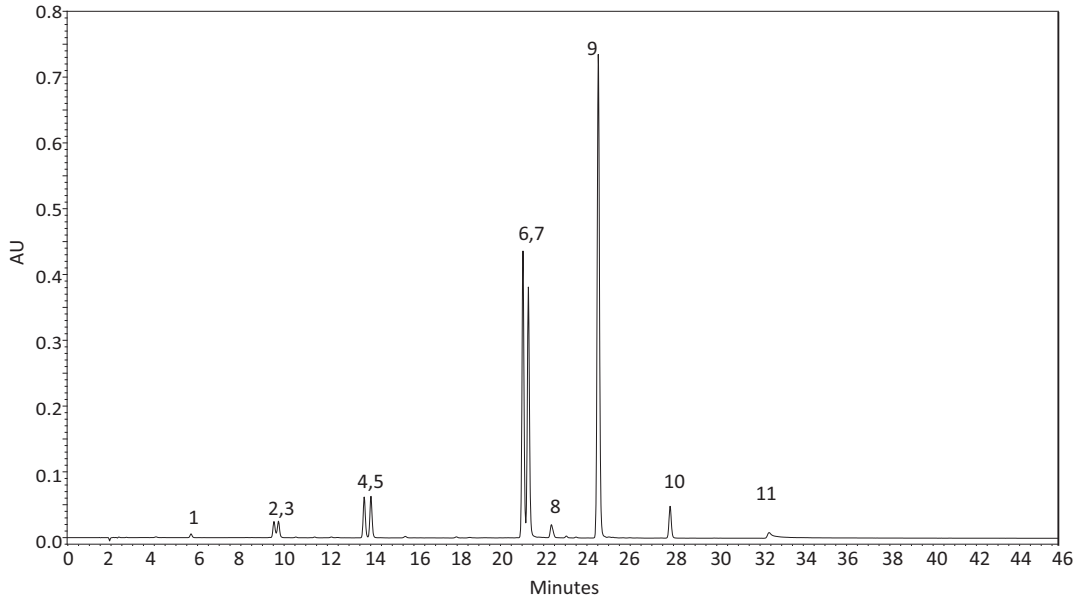


Fig. 3. Chromatogram showing specificity of the drug substance method for potential degradation impurities (1) epoxy hemiaminal; (2, 3) α-hydroxy lactams, isomers 1 and 2; (4, 5) lactams, isomers 1 and 2; (6, 7) oxidative dimers, isomers 1 and 2; (8) ring-opened keto-amine; (9) pemetrexed; (10) ring-opened keto-formamide; (11) des-glutamate.

3.2.2. Linearity precision, recovery, QL and DL

Analytical methods were validated in accordance with ICH Q2 (R1) [14]. The validation data for linearity, precision, recovery, quantitation and detection limits, and robustness/solution stability are provided in Table 3. All results demonstrate that the methods are acceptable for their intended use to accurately quantitate impurities.

3.2.3. Robustness

The robustness of a method is a measure of how the results are affected by small changes in the method parameters and provides an indication of the reliability of data during normal usage [13]. The robustness of the methods was evaluated through experimental design, and the data generated were analyzed with JMP software. The parameters varied are shown in Table 4. The responses evaluated are the individual and total impurity results. None of the factors evaluated were found to have practical significance; variation across the range of the factors evaluated does not affect the method performance or the quantitation of total and individual impurities.

3.2.4. System suitability

The generation of an appropriate system suitability sample posed a unique method development challenge for the drug substance. The process impurities are difficult to make and not readily available. It is desirable to develop conditions for a resolution

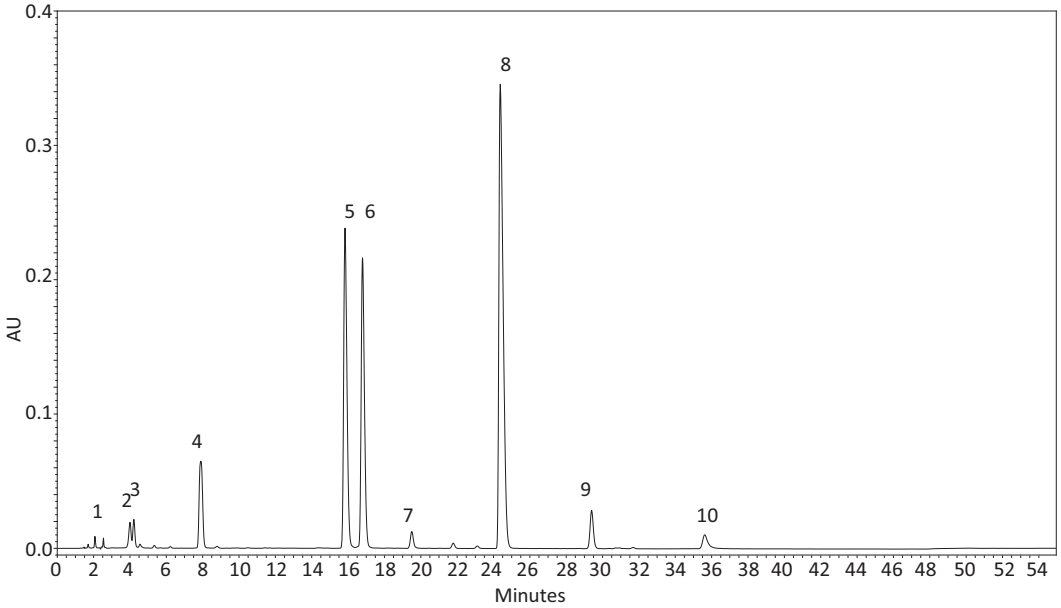


Fig. 4. Chromatogram showing specificity of the drug product method for potential impurities: (1) epoxy hemiaminal; (2, 3) α-hydroxy lactams, isomers 1 and 2; (4) lactams, isomers 1 and 2; (5,6) oxidative dimers, isomers 1 and 2; (7) ring-opened keto-amine; (8) pemetrexed; (9) ring-opened keto-formamide; (10) des-glutamate.

Table 3
Validation data for drug substance and drug product impurities methods.

Drug substance			Drug product		
Linearity					
Analyte	Range, %	r^2	Analyte	Range, %	r^2
Pemetrexed (low)	0.03–3.0	1.000	Pemetrexed (low)	0.02–0.5	1.000
Pemetrexed (high)	0.04–141	1.000	Pemetrexed (high)	63–146	1.000
Oxidative dimer isomer 1	0.01–0.64	0.999	Oxidative dimers, isomers 1 and 2	0.02–0.5	
Oxidative dimer isomer 2	0.01–0.64	0.999	Lactams, isomers 1 and 2	0.02–0.5	0.999
			Ring-opened keto-amine	0.02–0.5	0.999
			α-Hydroxy lactams, isomers 1 and 2	0.02–0.5	0.998
			Ring-opened keto-formamide	0.02–0.5	1.000
Accuracy					
Analyte	Range, %	Recovery, %	Analyte	Range, %	Recovery, %
Oxidative dimer isomer 1	0.01–0.45	86.1–105.6	Oxidative dimers, isomer 1 and isomer 2	0.02–0.5	81.0–104.9
Oxidative dimer isomer 2	0.01–0.45	86.7–101.8	Lactams, isomers 1 and 2	0.02–0.5	73.3–99.2
			Ring-opened keto-amine	0.01–0.4	68.2–118.4
			α-Hydroxy lactams, isomers 1 and 2	0.01–0.2	101.3–127.0
Repeatability					
Analyte	Average area, %	Standard deviation area, %	Analyte	Average area, %	Standard deviation area, %
Pemetrexed	99.3	0.022	Total impurities	0.15	0.005
Total impurities	0.65	0.022	Oxidative dimer isomer 1	0.015	0.0015
N-methyl derivative	0.06	0.004	Oxidative dimer isomer 2	0.03	0.02
Gamma glutamate	0.05	0.007	Lactams, isomers 1 and 2	0.03	0.002
			Ring-opened keto-amine	0.01	0.001
			α-Hydroxy lactam isomer 1	0.006	0.0007
			α-Hydroxy lactam isomer 2	0.006	0.0006
Intermediate precision					
3 instruments, 3 analysts, 5 columns, over 1 year			2 instruments, 2 analysts, 12 independent HPLC runs with 3 replicate per run		
Response (n = 37)	Mean, %	Standard deviation area, %	Response (n = 36)	Average area, %	Standard deviation area, %
Total impurities	0.74	0.03%	Total impurities	0.15	0.009
Largest impurity	0.26	Oxidative dimer isomer 1	Oxidative dimer isomer 1	0.015	0.006
			Oxidative dimer isomer 2	0.03	0.01
			Lactams, isomers 1 and 2	0.03	0.003
Detection (LOD) and quantitation (LOQ) limits					
LOD % of nominal sample concentration		0.01	LOD % of nominal sample concentration		0.01
LOQ % of nominal sample concentration		0.02	LOQ % of nominal sample concentration		0.02

Table 4
Robustness data for drug substance and drug product impurity methods.

Robustness study design			
Drug substance		Drug product	
Parameter	Midpoint/range	Parameter	Midpoint/range
Wavelength, nm	250 ± 5	Wavelength, nm	250 ± 5
Column temperature, °C	26 ± 4	Column temperature, °C	35 ± 5
Ammonium formate concentration, g/L	1.5 ± 0.5	Sodium acetate buffer concentration, mM	30 ± 5
Ammonium formate buffer, pH	3.5 ± 0.2	Sodium acetate buffer pH	5.5 ± 0.1
Initial organic concentration in mobile phase, %ACN	5.0 ± 1.0	Initial organic concentration in mobile phase, %ACN	3 ± 0.5
Flow rate, mL/min	1.0 ± 0.2	Final organic concentration in mobile phase, %ACN	12.5 ± 1
Robustness results			
Response	Mean, area (%)	Standard deviation, area (%)	Response	Mean, area (%)	Standard deviation, area (%)
Total impurities (Sample 1)	0.60	0.036	Total impurities	0.15	0.009
Largest impurity (Sample 1)	0.30	0.008	Oxidative dimer isomer 1	0.015	0.006
Total impurities (Sample 2)	0.78	0.056	Oxidative dimer isomer 2	0.03	0.01
Largest impurity (Sample 2)	0.27	0.006	lactams, isomers 1 and 2	0.03	0.003

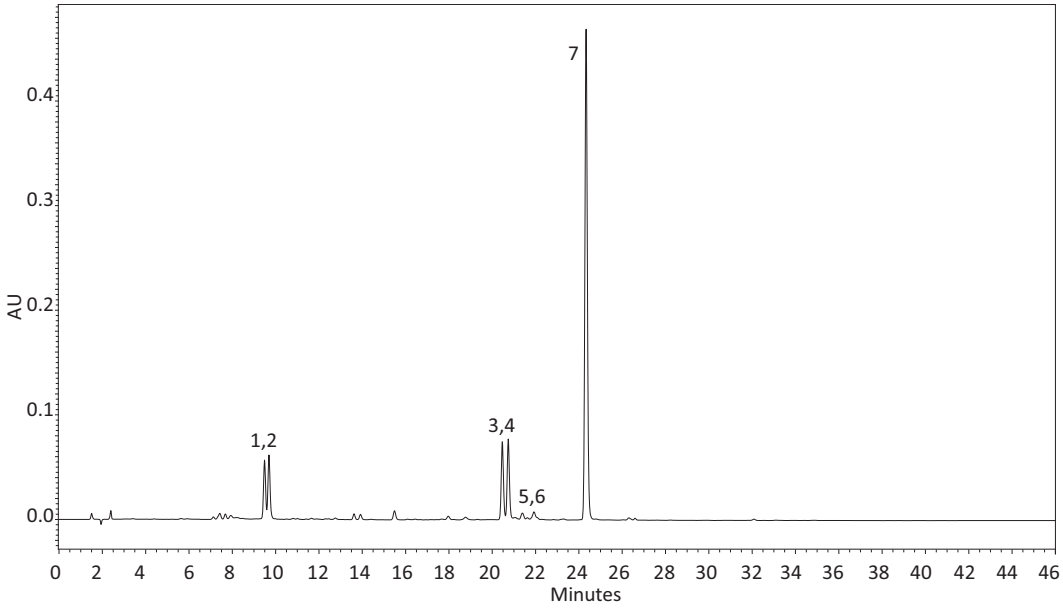


Fig. 5. Chromatogram of drug substance resolution solution: (1, 2) α-hydroxy lactams, isomers 1 and 2; (3,4) oxidative dimers, isomers 1 and 2; (5) gamma glutamate; (6) ring-opened keto-amine; (7) pemetrexed.

solution that can be routinely utilized to assure adequate chromatography. In the robustness study for drug substance and drug product methods, the resolution of the oxidative dimers was monitored as well as the separation of other peak pairs. Within the design space of the various chromatographic parameters described above, the oxidative dimers were indeed the critical pair and were indicative of the overall selectivity of the method. When these isomers had resolution ≥0.7, then the other impurities were all resolved. Since the oxidative dimers are the main degradation compounds and represent the main critical pair, their in situ preparation was investigated as an alternate to having a supply of each impurity.

For the DS method a sample solution of pemetrexed disodium was degraded in 0.1 M NaOH to generate the oxidative dimers (see Section 2.3). The conditions to generate the resolution solution are harsh and generate additional impurities that typically are not seen for samples, namely the α-hydroxyl lactams and a small amount of ring-opened keto-amine. These impurities along with the oxidative dimers can provide a confident identification of the degradation peaks (Fig. 5). In addition, the main process impurities are also well resolved and the system suitability solution provides a fingerprint for peak identification; the N-methyl derivative elutes just prior to the oxidative dimers, and the gamma glutamate derivative elutes just after the oxidative dimers but prior to ring-opened keto-amine (Fig. 5).

For the DP method a sample solution of pemetrexed disodium was degraded with oxidizing agent such as Vazo 52 or hydrogen peroxide to generate the critical resolution pair of oxidative dimers and the lactams to provide a reliable detection of these peaks (see Section 2.3). Similar chromatographic profiles are obtained using either solution preparation (Fig. 6).

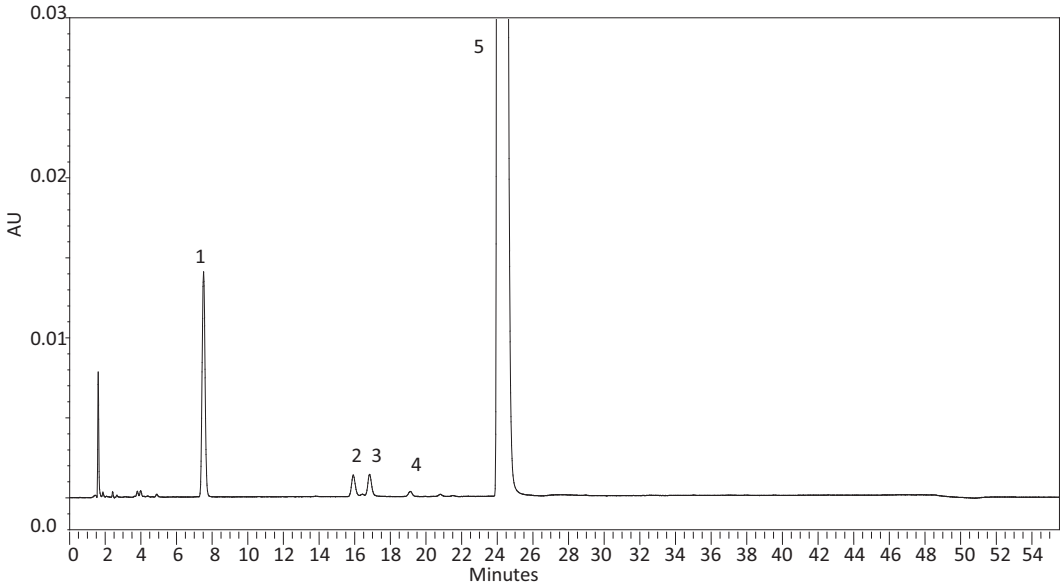


Fig. 6. Chromatogram of drug product resolution solution: (1) lactams, isomers 1 and 2; (2, 3) oxidative dimers, isomers 1 and 2; (4) ring-opened keto-amine (5) pemetrexed.

Method robustness included an evaluation of solution stability. Drug substance sample solutions were stable for 3 days at 5 °C. Drug product samples were found to be stable for at least for 24 h at 5 °C. The DS and DP system suitability solutions demonstrated acceptable stability for 21 days at refrigerated conditions (2–8 °C).

4. Conclusions

HPLC-UV methods have been developed for the determination of impurities in the pemetrexed disodium drug substance and drug product. UV relative response factors (RRFs) and impurities identification were performed using UV detection in tandem with charged aerosol detection (CAD) or in combination with quantitative NMR detection. The suitability solutions for drug substance and drug product methods were prepared in situ with dual purpose. The system suitability solutions generate a critical resolution pair and assure that potential impurities are both separated from the main peak and from each other. In addition, the system suitability samples generate main degradation impurities and are used to establish a fingerprint of impurities from which impurities in the chromatographic region can be determined. The methods have been validated and demonstrated to be robust over typical operating parameter ranges.

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