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Stability Report for NHS England

TITLE: Pemetrexed (as the disodium salt) 2mg/mL and 13.5mg/mL in 100mL Sodium Chloride 0.9%w/v Intravenous Infusion Bags (Baxter Viaflo)

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Microbiology Site Testing Chemistry

ASSURING PATIENT SAFETY

DOCUMENT CONTROL:

Version	Date	Description of Change
A	12-August-2020	New Document
B	02-September-2020	'Private and Confidential' removed from header page. 'Acknowledgements' section added on page 2. Reference for NHS yellow cover document - A Standard Protocol for Deriving and Assessment of Stability. Part 1 - Aseptic Preparations (Small Molecules) updated to current edition (Edition 5, September 2019). Reference made to USP monograph for Pemetrexed for Injection and its acceptance criteria for related substances in the discussion, in line with this further commentary on related substances levels and justification for acceptance has been given in Appendix 16. Table 11b and some associated text added in section 8.5.2.

NHS England Approval:

NHS England Approver: J M A QUALIE **(Print Name)**

(Signature)

04/09/2020 **(Date)**

TEST FACILITIES:

The work performed for this stability study was carried out at Quality Control North West (QCNW) Liverpool. QCNW Liverpool holds a certificate of GMP compliance issued by the MHRA and is accredited by UKAS to ISO17025 for those tests listed in the laboratory's Schedule of Accreditation (UKAS No: 9656), which is available on the UKAS website https://www.ukas.com/

RESPONSIBILITIES:

Quality Control North West (QCNW) Liverpool	
Luke Nelson, Analyst	Method Validation, sample preparation/analysis at test time points
Katie Milligan, Analyst	Data checks
Mark Corris, Senior Analyst (Chemistry)	Data Processing, preparation of report
Lyndsay Davies, Senior Analyst (Biochemistry)	Method Validation, sample preparation/analysis at test time points, data checks, technical approval of report
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1. PURPOSE OF STUDY AND STUDY DESIGN

The purpose of the study was to investigate the chemical and physical stability of Pemetrexed (as the disodium salt) 2mg/mL and 13.5mg/mL in 100mL Sodium Chloride 0.9%w/v Intravenous Infusion bags.

Stability was assessed for a period of up to 28 days stored at 2-8°C followed by additional time periods of 6 hours and 24 hours at 25°C (± 2ºC) / 60% Relative Humidity (RH) (±5%RH) (protected from light under both conditions) to cover the in use period for the products. The study test time points are detailed in Table 1, the testing performed and testing specification[1] is summarised in Table 2.

Table 1: Test Time Points for Stability Study LA2020001

Time points (days) / Storage Condition							
0	2 days (2-8°C)	7 days (2-8°C)	15 days (2-8°C)	23 days (2-8°C)	28 days (2-8°C)	28 days (2-8°C) + 6 hours at 25°C/60%RH	28 days (2-8°C) + 24 hours at 25°C/60%RH

NOTE: Test samples were stored protected from light during storage at both 2-8°C and 25°C/60%RH.

Table 2: Testing Specification for Stability Study LA2020001

Test	Methods	Specifications/ Limits	Time points tested
Appearance	Visual inspection. Sample appearance assessed in terms of colour, clarity and for the presence of visible particles.	No significant changes from T=0	All time points
pH*	Using in-house SOP111[2]	**Monitored for significant changes (more than 1.0 pH unit)	All time points
Assay for Pemetrexed*	In-house validated HPLC method[3]	Stability limit:- 95% of initial assay with 95% confidence	All time points
Related Substances*	In-house validated HPLC method[3]	Secondary peaks expressed as a % of the total peak area at each time point and levels monitored throughout the study duration.	All time points
Sub-Visible Particles*	In house method (SOP137)[4] following USP39<787>[5] for low volume samples, to allow single containers to be tested throughout the duration of the study.	***BP 2020 limits for single container of ≤ 100mL volume[6].	T=0, 15 day , 28 day (2-8°C) and 28 day (2-8°C) + 24 hours at 25°C/60%RH time points

*indicates that QCNW Liverpool are accredited by UKAS for this test

** pH Changes – pH was monitored for significant changes, which were taken as changes greater than 1.0 unit from Time 0.

*** BP 2020 limits for sub-visible particles for preparations supplied in containers with a nominal volume of less than or equal to 100ml[6] are;

- Average number of particles ≥ 10 micron must be less than 6000 per container, average number of particles ≥ 25 micron must be less 600 per container.

The study was performed based on in-house procedure RDP013 for Validation of Test Methods (Revision C)[7] which is written based on ICH Q2 (R1) (Validation of Analytical Procedures – Text and Methodology)[8], ICH Q1E (Evaluation of Stability Data)[9] and to a standard according to the NHS yellow cover document - A Standard Protocol for Deriving and Assessment of Stability. Part 1 - Aseptic Preparations (Small Molecules)[10]

2. MATERIALS AND EQUIPMENT

For sample manufacture:

- ALIMTA 500mg powder for concentrate for solution for infusion (containing Pemetrexed Disodium equivalent to 500mg Pemetrexed), Eli Lilly, Lot No. 197119A, Expiry Date : 09/2022
- ALIMTA 100mg powder for concentrate for solution for infusion (containing Pemetrexed Disodium equivalent to 100mg Pemetrexed), Eli Lilly, Lot No. 196884J, Expiry Date: 06/2022
- 100mL Sodium Chloride 0.9%w/v Intravenous Infusion bags, Baxter Viaflo, Code – FE1307G, Lot: 20D26G61, Expiry Date:03/2022
- Non-Vented Dispensing Pin with Luer Lock SAFSITE® Valve, B.Braun (Ref: 413501; Product Code: DP3500L)

For sampling:

- 4mL Clear glass screw top vials, Agilent Technologies, Part No: 5183-4448 with screw cap (Caps – Thermoscientific, Product Code: C4015-75A)
- 20mL Clear glass vials, Agilent Technologies, Part No: 5182-0837
- Parafilm
- Fume Cupboard, VarioLab Mobilien, Equipment Number EQ0176
- Lectrocath Line, Dia. 1.0 x 2.0mm – L. 200cm – Vol. 2.0mL; Ref: 1155.20, Code ACL 7162289, Vygon

For pH analysis:

- Mettler Toledo SevenMulti™ pH meter, Equipment Number: EQ0338
- InLab® Expert Go pH electrode, Mettler Toledo, Part Number: 51340288, Equipment Number: EQ0649 (used for adjustment of pH of aqueous portion of mobile phase for HPLC and measurement of sample pH at each time point)

- Certified pH buffer solutions used for the calibration of the pH meter were supplied by SPEX CertiPrep via Metlab Supplies Ltd

For HPLC analysis:

- Agilent Technologies High Performance Liquid Chromatography system comprising a Binary Gradient Pump (Agilent 1260 Infinity, Equipment Number: EQ0618), Degasser with solvent tray (Agilent 1260 Equipment Number: EQ0621), Variable Wavelength Detector (Agilent 1260 Infinity, Equipment Number: EQ0616), Thermostated Column Compartment (Agilent 1260 Infinity, Equipment Number: EQ0617) and Autosampler (Agilent 1260 Infinity, Equipment Number: EQ0619) with Thermostat (Agilent 1290 Infinity, Equipment Number: EQ0620).
- Agilent Technologies High Performance Liquid Chromatography system comprising a Quarternary Pump (Agilent 1260 Infinity, Equipment Number: EQ0604), Variable Wavelength Detector (Agilent 1260 Infinity, Equipment Number: EQ0602), Thermostated Column Compartment (Agilent 1260 Infinity, Equipment Number: EQ0615) and HiP Autosampler (Agilent 1260 Infinity, Equipment Number: EQ0603) with Thermostat (Agilent 1290 Infinity, Equipment Number: EQ0614).
- Chromatography data handling software - Agilent OpenLab CDS (Version 2.4)
- Accucore C18, 2.6 micron, 150 x 4.6mm HPLC column, Thermoscientific, Part No. 17126 – 154630
- Analytical Balance, Model XS205 DU/M, Mettler Toledo
- Class A Glass Volumetric Flasks and Pipettes (various sizes)
- FINNPIPETTE® F1 (100 - 1000µl), Thermoscientific, Equipment Number: EQ0487E
- FINNPIPETTE® F1 (20 - 200µl), Thermoscientific, Equipment Number: EQ0487C
- Distilled Water, prepared fresh weekly from a Laboratory Still (Fistreem™ Cyclon™), Equipment Number: EQ0077
- Glacial Acetic Acid, Fisher, Scientific, Product code: A/0400/PB17
- 5M Sodium Hydroxide, prepared in house using Sodium hydroxide, VWR, Product code- 28244.262, dissolved in distilled water
- Acetonitrile; Honeywell; Product code: 24851
- Sodium Chloride, J.T Baker, Product code: 7647-14-5
- Pemetrexed Disodium Heptahydrate, Pharmaceutical Secondary Standard, traceable to Ph.Eur, Sigma Aldrich, Product Code: PHR1596, Lot No: LRAC1932, Potency/Factor: f=0.713 in terms of Pemetrexed, Expiry Date: 31/12/2023
- Pemetrexed Impurity Mixture, European Pharmacopeia (EP) Reference Standard, supplied by Sigma Aldrich, Product Code:Y0001535, Batch No. 2.0
- 1M Hydrochloric Acid, VWR, Product Code: 7647-01-0

- 1M Sodium Hydroxide, Reagecon, Product Code: S21005
- Oven, Memmert, EQ0065 (used to heat acid degradation system suitability sample (ii) at 70°C)

For sub-visible particle count analysis

- HIAC 9703+ Particle counter, Beckman Coulter, Equipment Number: EQ0606A, (Laser Equipment No: EQ0606B)
- PharmSpec software for HIAC Liquid Particle Counters, Version 3.4.0
- Sterile Water for Irrigation, Fresenius Kabi, Product Code:31-58-589
- 20mL Clear glass vials, Agilent Technologies, Part No: 5182-0837
- Count-Cal™ Precision Standard, ThermoScientific. Cat No: CC15 Lot No. 221785 (Expiry Date: 11/2020) and 218949 (Expiry Date: 08/2020)

Equipment / areas used for storage during study:

- Refrigerator storage (2-8°C): Liebherr Refrigerator, EQ0565
- Humidity cabinet, operating at 25°C (± 2ºC) / 60%RH (±5%RH), Aralab, EQ0557
- Room temperature storage: Chemistry Laboratory Rm 3.14

The temperature of each storage area above is monitored continuously; this is done primarily using a Notion Pro temperature monitoring system, secondary to this, as a backup, areas are also monitored using Gemini Tinytag Dataloggers.

3. SAMPLE MANUFACTURE

Samples under test were manufactured aseptically by The Clatterbridge Cancer Centre Oncology Pharmacy Aseptic Unit. Samples were prepared using ALIMTA powder for concentrate for solution for infusion (containing Pemetrexed Disodium) (see section 2 – 'For sample manufacture', for further details). Batch manufacturing sheets, containing details of the sample manufacture procedure employed, are attached in Appendices 1 and 2. **(NOTE: The product preparation technique used accounts for the volume overage in the 100mL 0.9%w/v Sodium Chloride Intravenous Infusion bags (overage = 11mL), to produce final strengths of 2mg/mL and 13.5mg/mL Pemetrexed. Some units prepare a 15mg/mL strength product without accounting for the volume overage, in such cases, the actual sample strength would be 1500mg/111mL rather than 1500mg/100mL, this is equivalent to 13.5mg/mL; as a result a 15mg/mL product prepared in this way is covered by this study).** The products supplied are listed in Table 3.

Table 3: Details of samples supplied for testing during stability study

Product	Batch Number	Number of Bags
Pemetrexed 2mg/mL in 100mL Sodium Chloride 0.9%w/v Intravenous Infusion Bags (Baxter Viaflo Bags)	N/A	3
Pemetrexed 13.5mg/mL in 100mL Sodium Chloride 0.9%w/v Intravenous Infusion Bags (Baxter Viaflo Bags)	N/A	3
100mL Sodium Chloride 0.9% w/v Intravenous Infusion Bags (Baxter Viaflo Bags)	20D26G61 (Expiry Date:03/2022)	2

During sample manufacture, a non-vented dispensing pin (see section 2 for details) was inserted into the giving port of each infusion bag listed in Table 3, this was done to allow the same bags to be used for both chemical and sub visible particle analysis throughout the study.

Upon receipt the 3 x bags of each product strength were labelled as Bags A, B and C respectively. The 2 x 100mL bags of 0.9%w/v Sodium Chloride supplied were labelled as Bag A and B.

4. STORAGE CONDITIONS

Test samples were manufactured on 16/06/2020 and received by QCNW Liverpool on the same day (designated as T=0). Immediately on receipt, samples were processed before sampling from at room temperature (Chemistry Lab – Room 3.14). After sampling for T=0 testing, all samples were then transferred to a refrigerator at 2-8°C, for storage and further testing at time points up to and including T=28 days.
At T=28 days bags were sampled from for testing before transferring to a Humidity Cabinet operating at 25°C/60%RH. After 6 hours, bags were sampled from for further testing (T= 28days at 2-8°C + 6 hours at 25°C/60%RH) before returning to the Humidity Cabinet for a further 18 hours. After 24 hours stored at 25°C/60%RH, bags were sampled from for the final round of testing (T = 28 days at 2-8°C + 24 hours stored at 25°C/60%RH).

5. SAMPLING PROTOCOL

5.1 Sampling for Appearance and Chemical Testing

At each time point, test samples were removed from their storage area / cabinet and transferred to the fume cupboard situated within the Wet Chemistry Laboratory Room 3.14, for sampling. To sample, for each bag under test, a Lectrocath Line (Vygon, Dia. 1.0 x 2.0mm – L. 200cm – Vol. 2.0mL; Ref: 1155.20, Code ACL 7162289) cut down to 4.0cm length, was attached to the Dispensing Pin using the Luer Lock connection; bags were then sampled via this line. At each time point, for each bag under test, a volume of sample was initially

drained to waste before draining an additional 4ml of sample into a clean, dry, 4mL clear glass vial for appearance and chemical testing.

5.2 Sampling for Sub-Visible Particle Count Testing

At relevant time points (see Table 2), after draining a volume of sample from each bag to waste and prior to sampling for chemical tests (section 5.1), 6mL of sample was drained into a 20mL clear glass vial that had previously been rinsed 3 times with Sterile Water for Irrigation (WFI) and shaken dry, for sub-visible particle count analysis. The vial was then covered with Parafilm to protect the contents from the environment and transferred to the particle counter for analysis.

Upon sampling at each time point, the duration for which bags were outside of refrigerated storage, between T=0 and T = 28 day time points, was recorded, with the total time across the study recorded as 5 hours 5 minutes.

6. METHODS

6.1 Appearance

Sample from each bag was visually inspected within the 4mL clear glass vials used to sample into for chemical testing at each time point. Samples were viewed against a white background to assess colour and against a black background to assess clarity/signs of precipitation; Distilled Water was viewed alongside samples for comparison.

6.2 pH

The pH was measured on 3 - 4mL of sample in a suitably sized glass container, using a pH meter and a Mettler Toledo InLab® Expert Go pH electrode, at 20-25°C. This electrode is calibrated daily using pH 4, 7 and 9 certified buffer solutions within the same temperature range. At each time point, pH 7 certified buffer solution was run alongside the samples to ensure that the instrument was operating correctly at the time of use.

6.3 Assay of Pemetrexed and Monitoring of Related Substances by HPLC

Assay of Pemetrexed content and monitoring of related substances were analysed using the same in-house validated HPLC method[3]. The HPLC system(s) used are detailed in section 2. Chromatographic results were collected using Agilent OpenLab chromatography data handling software package (see section 2 for details). The chromatographic separation was performed at 30°C using an Accucore C18, 2.6 micron, 150 x 4.6mm HPLC column. Isocratic elution was performed using a mobile phase comprising 93%v/v Aqueous Phase, pH 5.3 and 7%v/v Acetonitrile. The aqueous phase was prepared as follows; 1.7mL of Glacial Acetic Acid was added to approximately 900mL Distilled Water, the pH of this solution was then adjusted to 5.3 using 5M Sodium Hydroxide before diluting to 1000mL with Distilled Water (see Deviation Report Number 114). A flow rate of 0.7mL/min was maintained at a detection wavelength of 250nm, with a sample injection volume of 20µL and an autosampler tray temperature set at 5°C. At T=0 and T=2 day time points, all solutions were injected for a run time of 15 minutes. In system suitability sample (ii) (see section 6.3.4), there are 3 x secondary peaks which elute after the Pemetrexed peak; at T=2 days it was noted that the 2 latter eluting of these 3 peaks were found to elute outside of this 15 minute run time window (Deviation Report Number 109).

To allow us to monitor for the presence of these peaks in samples, the injection run times for system suitability sample (ii) and sample preparations were extended to 30 minutes and 20 minutes respectively; this was done to allow these late eluting peaks to be monitored for any potential presence in samples (see section 8.5.3 for further details and an assessment of these late eluting peaks).

The justification for these amendments is as follows;

(i) The late eluting peaks in question are seen in system suitability sample (ii) – this solution is prepared by force degrading a solution of Pemetrexed using a combination of acid and heat (see section 6.3.4); these peaks would not be expected to elute in blank, standards and system suitability sample (i) therefore the run time for these injections was kept to 15 minutes.

(ii) The run time for system suitability sample (ii) was increased to 30 minutes to allow all of the 3 late eluting peaks to elute. This allows us to assign a retention time to each, enabling us to monitor their presence in sample injections.

(iii) Of the 3 x late eluting peaks, the first 2 peaks to elute are more prominent than the third, hence if these are not present in samples it is unlikely that the third peak would be present. The sample run time was increased to 20 minutes, to ensure that, if present, neither of the first 2 peaks of the 3 late eluting peaks in question, would co-elute with (and hence be masked by) the Pemetrexed peak in a subsequent sample injection, therefore allowing their presence to be monitored.

See section 8.5.3 for an assessment of these late eluting secondary peaks.

6.3.1 Sample preparations

Before injection onto the HPLC system, samples were diluted to a theoretical Pemetrexed concentration (assuming each sample is 100% stated strength) of 4mg/100mL. Sample dilutions were performed as follows;

2mg/mL samples: 0.5ml → 25.0ml in mobile phase

13.5mg/ml samples: 0.148ml → 50.0ml in mobile phase

Duplicate sample dilutions were prepared from each bag under test and each replicate preparation injected in single onto the HPLC (see Deviation Report Number 113).

6.3.2 Standard preparations

The Pemetrexed content in each sample at each time point was determined by assaying against standard solutions containing ~5mg/100mL Pemetrexed (Standard 1), ~4mg/100mL Pemetrexed (Standard 2) and ~3mg/100mL Pemetrexed (Standard 3) in mobile phase. These were prepared using Pemetrexed Disodium Heptahydrate, Pharmaceutical Secondary Standard, traceable to Ph.Eur, Standard 1 was injected 6 times at the beginning of the sequence, followed by injection of Standard 2 and Standard 3 as an initial demonstration of repeatability of injection and linearity. Standard 2 and 3 were then injected periodically throughout the sequence, bracketing a maximum of 6 x sample injections, to assess any potential drift in response factors throughout the run (see Deviation Report Number 110).

6.3.3 Blank injections

The following blank solutions were prepared and injected at each time point

1) 'Blank Mobile Phase'

2) 'Blank Sample' – 0.5mL of 0.9%w/v Sodium Chloride for Intravenous Infusion solution (taken from a 'Blank' bag supplied alongside test samples) diluted to 25.0mL with mobile phase (this mimics the sample diluent for 2mg/mL samples as a worse case).

These chromatograms were then assessed to allow us to distinguish system peaks from potential related substances peaks and to ensure that there was no significant carryover between injections.

6.3.4 Preparation of system suitability samples

(i) The contents of a vial of Pemetrexed Impurity Mixture (EP) Reference Standard were dissolved in 1.0mL of Distilled Water (BP2020 – Pemetrexed Disodium Heptahydrate - Related Substances Reference Solution (c) containing Impurities A and D[11]). 500µL of this solution was mixed with 500µL of Pemetrexed Standard 2 (section 6.3.2). This solution was prepared in advance of T=0 and refrigerated at 2-8°C for use at subsequent time points throughout the study.

(i) 44mg of Pemetrexed Disodium Heptahydrate Pharmaceutical Secondary Standard, traceable to Ph.Eur (equivalent to 32mg Pemetrexed) was dissolved in 200.0mL of Distilled Water. 5.0mL of this solution was added into a 20.0mL volumetric flask, 2.0mL of Acetonitrile and 1.0mL of 1M Hydrochloric Acid were then added (NOTE: The addition of Acetonitrile is to aid the solubility of the Pemetrexed at low pH). This solution was then stored at 70°C for 1 day, before cooling, neutralising with 1.0mL of 1M Sodium Hydroxide and diluting to volume with Distilled Water.

See section 6.3.5 for details on the injection of these samples.

6.3.5 System suitability parameters

During method validation, there were indications that the retention time of the Pemetrexed peak is sensitive to changes in aqueous phase pH (increase by 0.1 unit), and also changes to the HPLC instrument and column employed[12]. As a result, the following system suitability criteria were selected to ensure that the system remained suitable and selective at each test time point, in the event that retention time shifts were observed during the study.

1) Replicate injections for standards must meet the following criteria at each time point;

   Standard 1: Replicate %RSD must be ≤ 2.0% (Repeatability of Injection)
   Standard 2: Replicate %RSD must be ≤ 2.5% (Drift)
   Standard 3: Replicate %RSD must be ≤ 2.5% (Drift)

2) Linearity of standards must meet the following criteria at each time point;
   Response Factor (RF) % RSD for Stds (1), (2) & (3) must be ≤ 2.5%

3) i) The retention time of the Pemetrexed peak must be 6.4 ± 1.5 minutes.
ii) The retention time of the Pemetrexed peak must drift by no more than 2% between consecutive injections.

4) The Asymmetry (10%) (EP – European Pharmacopeia calculation) for the Pemetrexed peak must be within the range 1.0 → 2.0

5) - Inject system suitability samples (i) to (ii) at both the beginning and again at the end of the HPLC sequence at each time point.

- In system suitability sample (i), there are 3 x peaks which elute. Use the retention time of Pemetrexed from standard chromatograms, to assign the Pemetrexed peak. The remaining 2 x principal peaks correspond to Pemetrexed Impurities A and D (as listed in the BP 2020 monograph for Pemetrexed Disodium Heptahydrate)[11]. Assign the first of these peaks as Impurity A and the second as Impurity D (this matches the elution order stated in the related substances test in the BP 2020 monograph for Pemetrexed Disodium Heptahydrate and in the chromatogram supplied with the Pemetrexed Impurity Mixture) – see example chromatogram attached in Appendix 3

- In system suitability sample (i) the resolution (EP) between Pemetrexed and Impurity D must be ≥ 2.0

- In system suitability sample (ii), there is a loss of Pemetrexed observed, accompanied by an increase in secondary peak areas. The principal degradant peaks are well resolved from the Pemetrexed peak by visual inspection (see example chromatogram attached in Appendix 4 in which the principal degradants elute at retention times of ~2.0 minutes, 15.3 minutes and 18.8 minutes).. Ensure that, if present in samples (which are run for 20 minutes), the 2 x latest eluting secondary peaks (eluting at 18.8 minutes and 25.6 minutes in example chromatogram attached in Appendix 4) would not co-elute with (and hence not be hidden by) the Pemetrexed peak in subsequent injections.

6.3.6 Monitoring of related substances

To monitor secondary peak areas (related substances) at each time point, the following approach was taken;

1) A minimum area equivalent to ~0.05% of the lowest Pemetrexed peak area measured in Standard 2 chromatograms was applied **(NOTE: this matches the 'reporting threshold' stated in the related substances test in the BP 2020 monograph for Pemetrexed Disodium Heptahydrate[11]; during method validation, the Limit of Quantification (LOQ) based on the Pemetrexed peak area was calculated as 0.3994µg/100mL Pemetrexed, equivalent to 0.01% of the analytical concentration of Pemetrexed (4mg/100mL)).**
2) Each sample chromatogram was monitored for the presence of secondary peaks and the area of each was measured.
3) Each individual secondary peak area was calculated as a % of the total peak area for the chromatogram.
4) The total amount of secondary peak areas were also calculated as a % of the total peak area for the chromatogram.

6.4 Sub-Visible Particle Count Analysis

Sub-visible particle count testing was performed using a HIAC 9703+ liquid particle counter and accompanying PharmSpec software. Counts were performed using method – 'User-Defined_USP39_<787>_SVI_0.5ml_Full_Count_Test_v1' (Analysis is performed based on the US Pharmacopeia (USP) monograph USP39<787>[5], which allows testing of smaller sample volumes and aliquots). A control sample of Sterile Water for Irrigation was analysed both prior to and following the analysis of samples to demonstrate that the system was free of particles. This was carried out by performing 5 x 0.5mL counts (first reading discarded), with the results averaged; the system is deemed to be acceptably free from particles if the average count for particles ≥ 10 micron does not exceed 1 particle per mL (Limit taken from USP39<787>)[5].
A Count-Cal Precision standard was also analysed, prior to and following the analysis of samples, to verify the instruments accuracy.

The sampling protocol used for test samples is described in section 5.2. After sampling and sealing with Parafilm the sample vial was left to stand for a minimum of 2 minutes to allow any air bubbles to rise to the liquid surface. The Parafilm was removed and the sampler probe was then lowered into the vial, ensuring that it was around 0.5cm from the base; 5 x 0.5mL counts were then performed. The first run primes the flow cell and these counts are therefore disregarded. The counts from the remaining 4 runs were averaged and the number of counts per container calculated and reported. To demonstrate that the 20mL glass vials used for sampling were fit for use, at each time point a control sample was run by rinsing a vial 3 times and filling to 6ml with Sterile Water for Irrigation (mimicking the sampling protocol for test samples); 5 x 0.5mL counts were then performed on this, disregarding the first 0.5mL count and calculating the average of remaining counts as per the test samples. This was done to assess the numbers of particles generated by the glass vials and the impact, if any, on sample data.

7. METHOD VALIDATION

The HPLC method used for the assay of Pemetrexed and monitoring of related substances was fully validated. Details of the validation performed and associated data is presented in a separate report (report reference LA2020001(1)[12]).

8. RESULTS

8.1 Appearance

The observations on sample appearance for each sample under test, at each time point, are presented in Tables 4a and 4b.

Table 4a: Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Observed sample appearance

Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride – observed appearance		
Time Point (days) /Storage Conditions	**Bag ID**	**Appearance**
0	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
2 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
7 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
15 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
23 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
28 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
28 (2-8°C) + 6 hours stored at 25°C/60%RH	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
28 (2-8°C) + 24 hours stored at 25°C/60%RH	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles

Table 4b: Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Observed sample appearance

Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride – observed appearance		
Time Point (days) /Storage Conditions	**Bag ID**	**Appearance**
0	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
2 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
7 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
15 (2-8°C)	**A**	Clear, colourless solution, free from visible particles
	B	Clear, colourless solution, free from visible particles
	C	Clear, colourless solution, free from visible particles
23 (2-8°C)	**A**	Clear solution, free from visible particles. Slight yellow colouration observed
	B	Clear solution, free from visible particles. Slight yellow colouration observed
	C	Clear solution, free from visible particles. Slight yellow colouration observed
28 (2-8°C)	**A**	Clear solution, free from visible particles. Slight yellow colouration observed.
	B	Clear solution, free from visible particles. Slight yellow colouration observed
	C	Clear solution, free from visible particles. Slight yellow colouration observed
28 (2-8°C) + 6 hours stored at 25°C/60%RH	**A**	Clear solution, free from visible particles. Slight yellow colouration observed
	B	Clear solution, free from visible particles. Slight yellow colouration observed
	C	Clear solution, free from visible particles. Slight yellow colouration observed
28 (2-8°C) + 24 hours stored at 25°C/60%RH	**A**	Clear solution, free from visible particles. Slight yellow colouration observed
	B	Clear solution, free from visible particles. Slight yellow colouration observed
	C	Clear solution, free from visible particles. Slight yellow colouration observed

8.2 pH

The pH data obtained is presented in Table 5.

Table 5: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH - pH data

Sample pH									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	6.75	6.83	7.15	6.84	6.76	6.94	6.80	6.75
	B	6.82	6.86	7.19	6.95	6.92	6.73	6.71	6.55
	C	6.77	6.80	7.20	6.89	6.82	6.79	6.82	6.78
13.5mg/mL	**A**	6.84	6.82	6.94	6.89	6.85	6.80	6.81	6.77
	B	6.88	6.86	6.95	6.87	6.83	6.78	6.80	6.76
	C	6.88	6.89	6.96	6.88	6.83	6.80	6.81	6.75

8.3 Assay of Pemetrexed by HPLC

The Pemetrexed assay data obtained at each time point is presented in two ways; results are expressed as mg/100mL (Table 6) and also as a % of the initial assay at T=0 (Table 7). In addition Tables 8a and 8b list the change in assayed concentration (expressed as % of initial assay) following storage for an additional 6 hours (Table 8a) and 24 hours (Table 8b) at 25°C /60%RH at the end of the study. The data in each table is the mean result from duplicate sample dilutions (n=2) for each bag under test (with the exception of those results accompanied by a (^) symbol, for these samples one of the duplicate sample dilutions failed to inject correctly on the HPLC, as a result only data from one sample dilution was used – see Deviation Report Number 113). Individual assay results, for duplicate sample dilutions, at each time point, are attached in Appendices 12 and 13. Example chromatograms from the HPLC analysis are attached in Appendices 3 to 11).

Table 6: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH - Assayed concentration of Pemetrexed (expressed as mg/100mL),

Assayed concentration of Pemetrexed expressed as mg/100mL (mean result per bag)									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	205.5	207.6	206.3	205.1	205.4	204.9	204.9	204.5
	B	198.6	199.8	198.4	197.8	197.4	196.5^	196.5	196.4^
	C	197.1	198.1	197.1	196.4	196.0	195.4	194.7	195.0^
13.5mg/mL	**A**	1361	1361	1353	1345	1323	1330	1328	1328
	B	1333	1331	1322	1305	1302	1303	1296	1294
	C	1375	1372	1363	1352	1344	1345	1337	1338

Table 7: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH - Assayed concentration of Pemetrexed (expressed as % of initial assay at T=0)

Assayed concentration of Pemetrexed expressed as % of initial assay at T=0 (mean result per bag)									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	100.0	101.0	100.4	99.8	100.0	99.7	99.7	99.5
	B	100.0	100.6	99.9	99.6	99.4	98.9^	98.9	98.9^
	C	100.0	100.5	100.0	99.6	99.4	99.1	98.8	98.9^
13.5mg/mL	**A**	100.0	100.0	99.4	98.8	97.2	97.7	97.6	97.6
	B	100.0	99.8	99.2	97.9	97.7	97.7	97.2	97.1
	C	100.0	99.8	99.1	98.3	97.7	97.8	97.2	97.3

Table 8a: Change in assayed concentration of Pemetrexed (expressed as % of initial assay at T=0) following 6 hours storage at 25°C/60%RH

Change in assayed concentration of Pemetrexed (% of initial assay at T=0) following 6 hours storage at 25°C/60%RH				
Sample concentration	**Bag ID**	**Concentration of Pemetrexed (% of initial assay at T=0) T=28d (2-8°C)**	**Concentration of Pemetrexed (% of initial assay at T=0) at T=28d (2-8°C) + 6 hours at 25°C/60%RH**	**Change in concentration of Pemetrexed (% of initial assay at T=0) following 6 hours storage at 25°C/60%RH**
2mg/mL	**A**	99.7	99.7	0.0
	B	98.9^	98.9	0.0
	C	99.1	98.8	-0.3
13.5mg/mL	**A**	97.7	97.6	-0.1
	B	97.7	97.2	-0.5
	C	97.8	97.2	-0.6

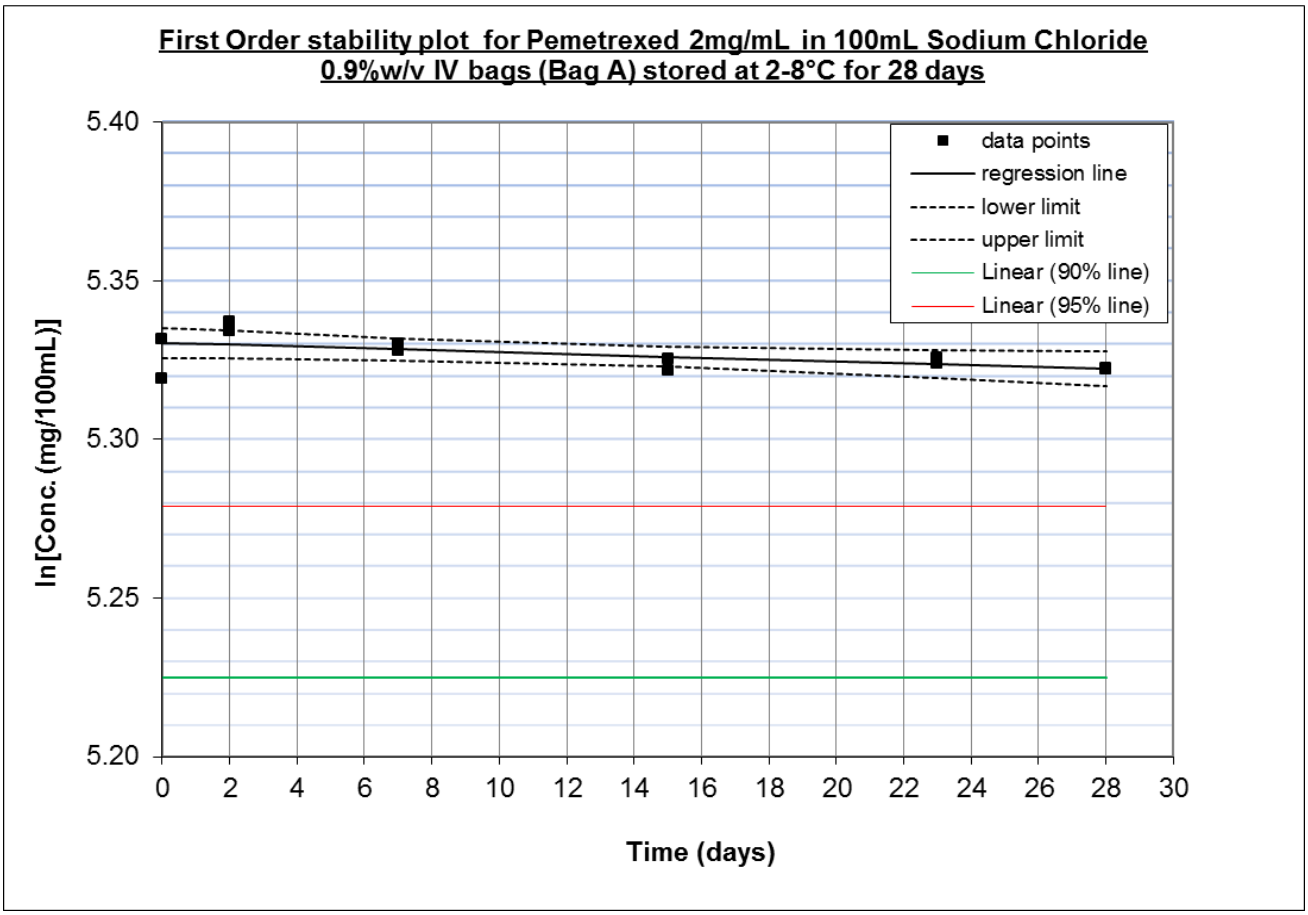
Table 8b: Change in assayed concentration of Pemetrexed (expressed as % of initial assay at T=0) following 24 hours storage at 25°C/60%RH

Change in assayed concentration of Pemetrexed (% of initial assay at T=0) following 24 hours storage at 25°C/60%RH				
Sample concentration	**Bag ID**	**Concentration of Pemetrexed (% of initial assay at T=0) T=28d (2-8°C)**	**Concentration of Pemetrexed (% of initial assay at T=0) at T=28d (2-8°C) + 24 hours at 25°C/60%RH**	**Change in concentration of Pemetrexed (% of initial assay at T=0) following 24 hours storage at 25°C/60%RH**
2mg/mL	**A**	99.7	99.5	-0.2
	B	98.9^	98.9^	-0.0
	C	99.1	98.9^	-0.2
13.5mg/mL	**A**	97.7	97.6	-0.1
	B	97.7	97.1	-0.6
	C	97.8	97.3	-0.5

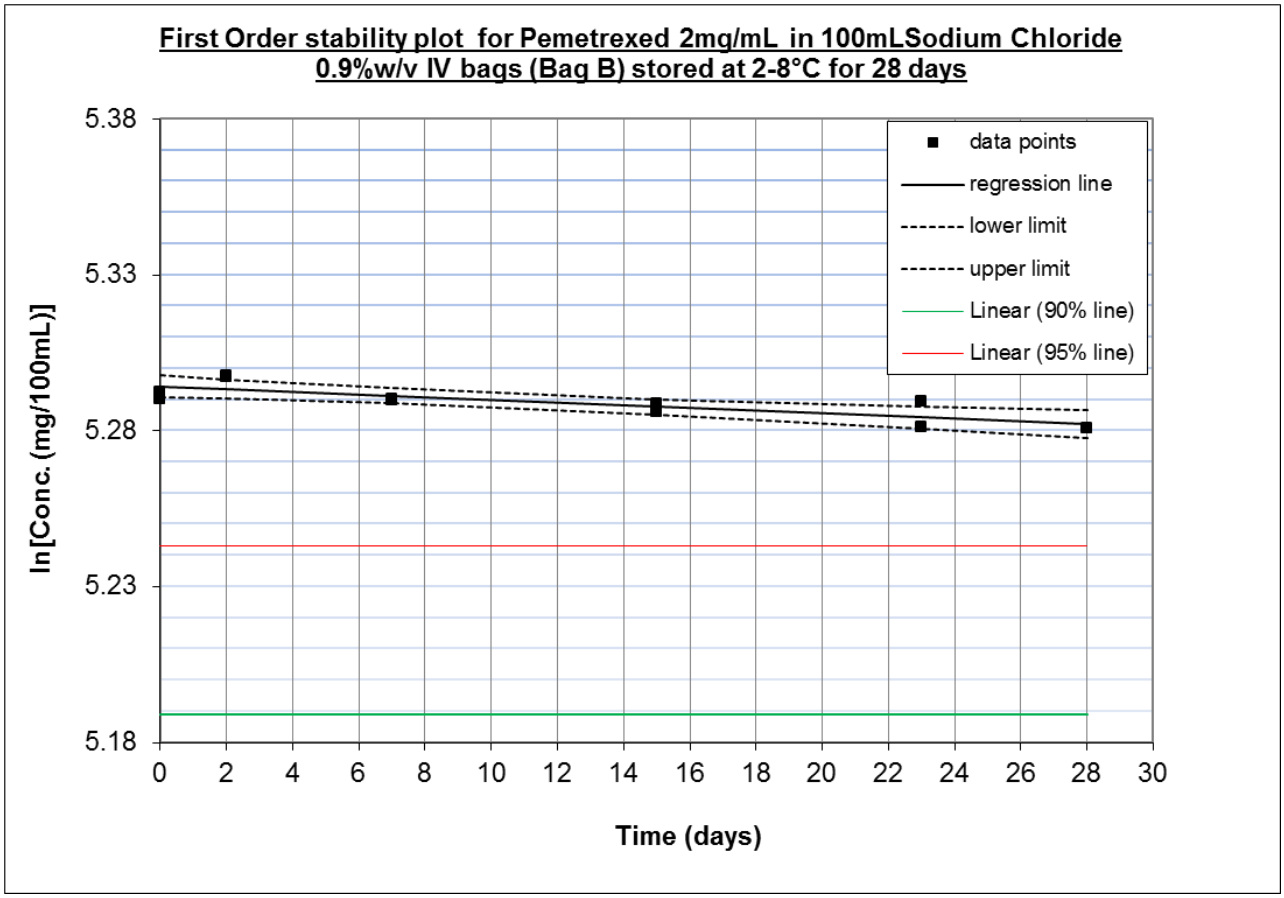
8.4 Statistical Analysis of Pemetrexed Assay Data – First Order Stability Plots

Pemetrexed assay results for samples stored for periods up to and including 28 days at 2-8°C were plotted on first order stability graphs. The slope of the regression line of Log_n (ln) [Concentration of Pemetrexed expressed as mg/100mL] versus Time (Days) represents the rate constant (k). The regression line for each plot was constructed using the duplicate results obtained for each bag tested (with the exception of those data points accompanied by a (^) symbol in Tables 6 and 7 above) at each time point. (Graphs 1 → 6)

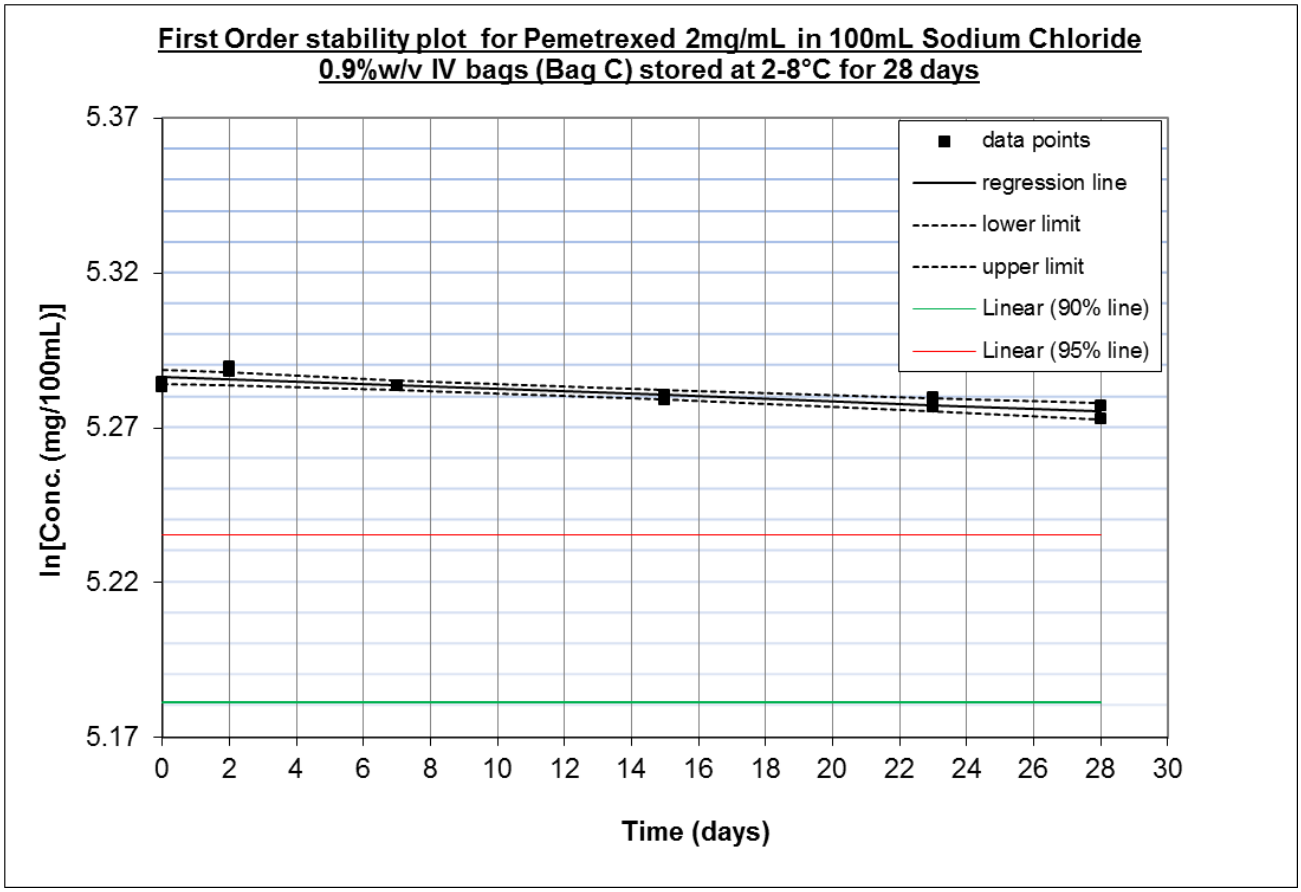
Graph 1: First order stability plot for Pemetrexed 2mg/mL in in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days – Bag A



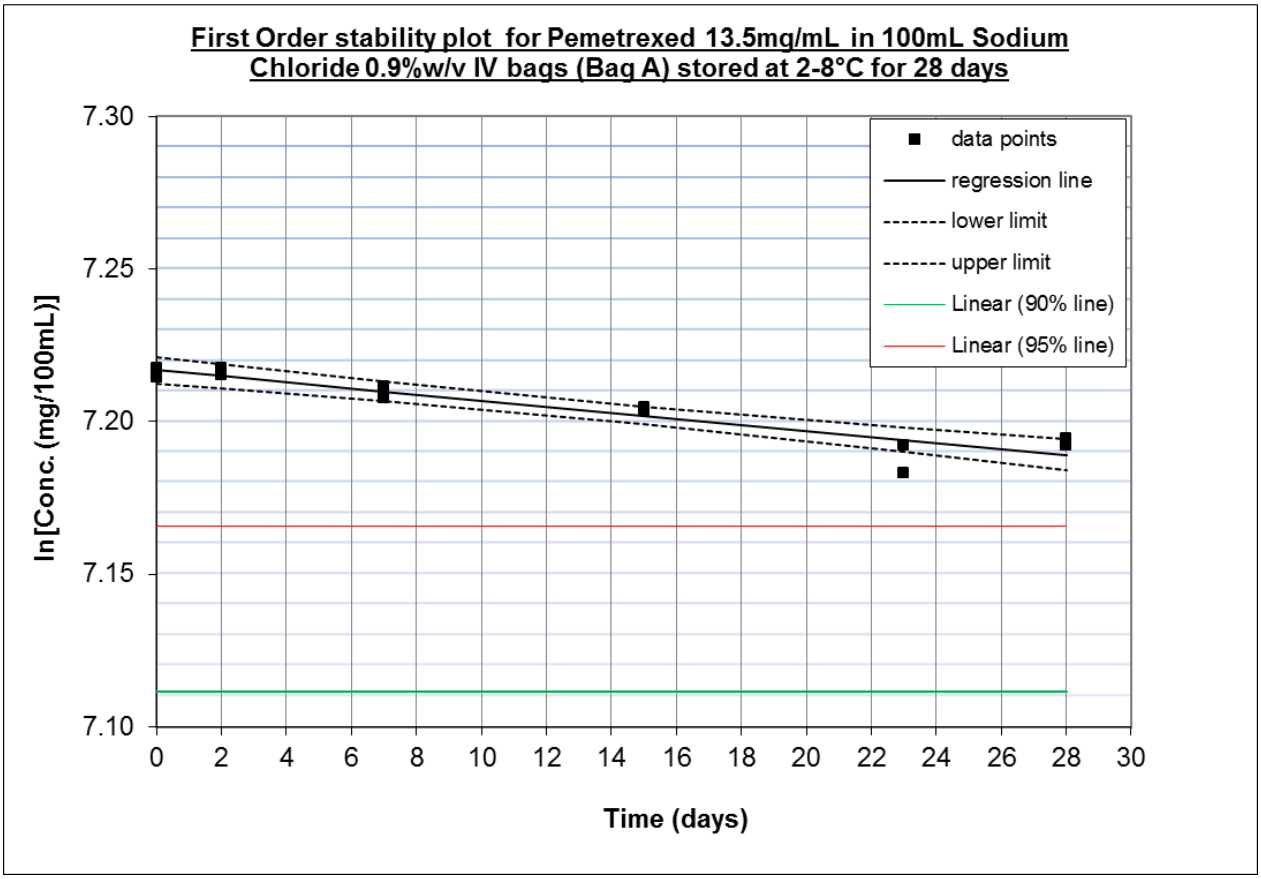
Graph 2: First order stability plot for Pemetrexed 2mg/mL in in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days – Bag B



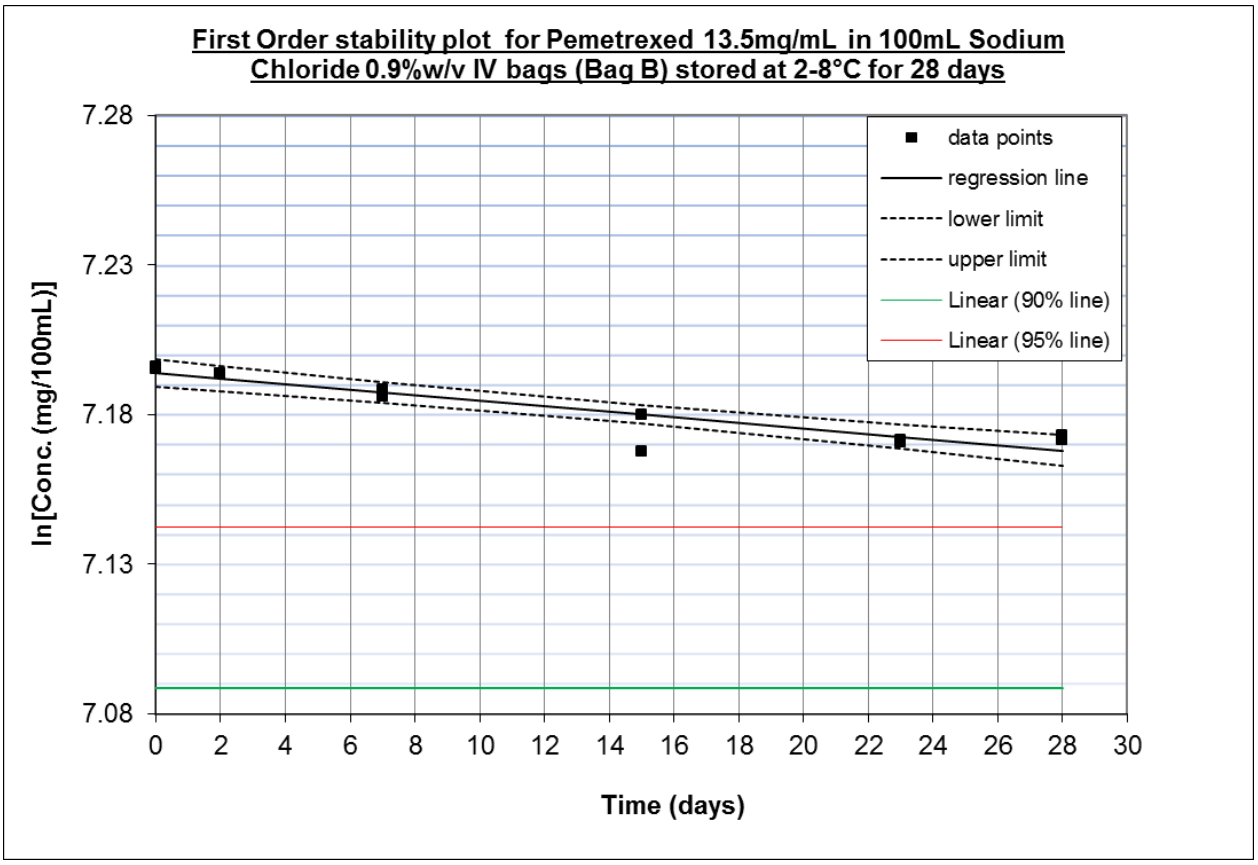
Graph 3: First order stability plot for Pemetrexed 2mg/mL in in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days – Bag C



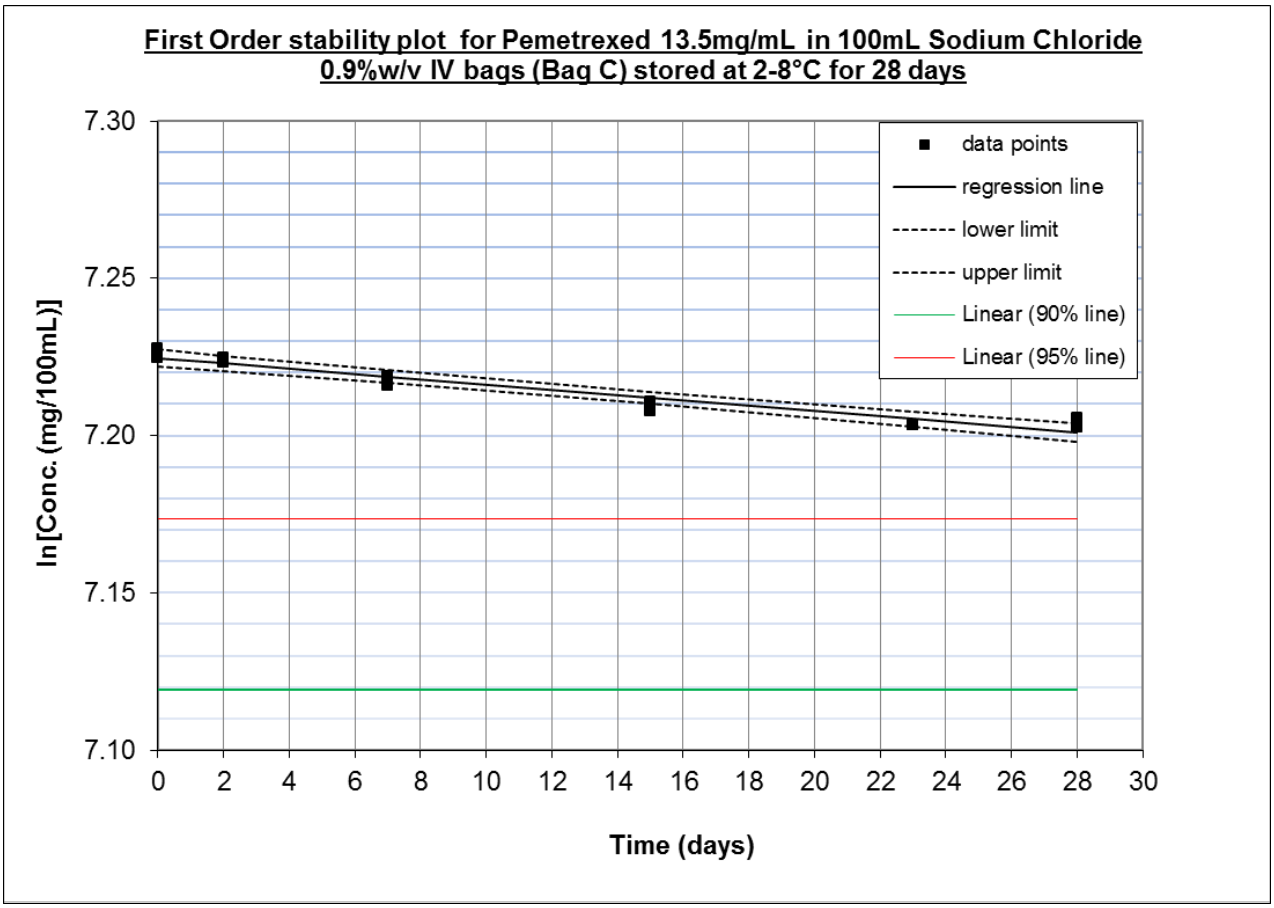
Graph 4: First order stability plot for Pemetrexed 13.5mg/mL in in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days – Bag A



Graph 5: First order stability plot for Pemetrexed 13.5mg/mL in in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days – Bag B



Graph 6: First order stability plot for Pemetrexed 13.5mg/mL in in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days – Bag C



The value for T(0.95) (Time taken for Pemetrexed assay to drop to 95% of its initial concentration at T=0 based on the lower 95% confidence lines), obtained from each plot has been presented in Table 9.

Table 9: T(0.95) values obtained from first order stability plots (based on the lower 95% confidence lines) obtained for Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride, stored for time periods up to and including 28 days, at 2-8°C

Shelf life Data from First Order Stability Plots		
Sample concentration	**Bag ID**	**T(0.95) at 2-8°C (Days)**
2mg/mL	A	88.5
	B	76.8
	C	94.0
13.5mg/mL	A	40.7
	B	42.7
	C	50.7

8.5 Pemetrexed Related Substances by HPLC

8.5.1 Total secondary peak areas

Table 10 lists the total area of secondary peaks for each sample expressed as a % of the total peak area at each time point. The data listed for each sample is the mean result from duplicate sample dilutions (with the exception of those results accompanied by a (^) symbol, for these samples one of the duplicate sample dilutions failed to inject correctly on the HPLC, as a result only data from one sample dilution was used – see Deviation Report Number 113). Secondary peaks with areas less than the applied minimum area (0.05% of the lowest Pemetrexed peak area recorded in Standard 2 chromatograms), were disregarded. Individual results, for duplicate sample dilutions, at each time point, are attached in Appendix 14. Example chromatograms from the HPLC analysis are attached in Appendices 3 to 11).

Table 10: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Related Substances: Total secondary peak areas (expressed as % of total peak area)

Related Substances: Total secondary peak areas expressed as % total peak area (mean result per bag)									
Sample concentration	**Bag ID**	**Time point (days)**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	0.06	0.06	0.16	0.35	0.62	0.63	0.63	0.70
	B	0.11	0.06	0.17	0.55	0.80	0.93^	0.94	1.01^
	C	0.07	0.07	0.17	0.54	0.82	0.92	0.87	1.02^
13.5mg/mL	**A**	0.06	0.11	0.46	0.95	1.38	1.68	1.69	1.93
	B	0.00[#]	0.11	0.60	1.00	1.45	1.72	1.77	1.99
	C	0.05	0.14	0.52	0.94	1.41	1.65	1.69	1.91

[#] No secondary peaks present with areas > minimum area (0.05% of lowest Pemetrexed peak area recorded in Standard 2 chromatograms)

8.5.2 Individual secondary peak areas

Table 11a lists the highest individual secondary peak area for each sample, expressed as a % of the total peak area. At each time point, the highest individual recorded secondary peak was seen to elute at a similar relative retention time with respect to Pemetrexed (relative retention time of ~0.4). The data listed for each sample is the mean result from duplicate sample dilutions (with the exception of those results accompanied by a (^) symbol, for these samples one of the duplicate sample dilutions failed to inject correctly on the HPLC, as a results only data from one sample dilution was used – see Deviation Report Number 113). Table 11 b lists the number of other individual secondary peaks observed with areas > 0.24% of the total area (this is the limit given in the USP 2020 monograph for Pemetrexed for Injection[17] for any other unspecified impurity); where such peaks were observed, the area of the peak(s) has been listed as a % of total peak area (in these cases, the largest peak area from duplicate sample dilutions has been reported as a worst case). Secondary peaks with areas less than the applied minimum area (0.05% of the lowest Pemetrexed peak area recorded in Standard 2 chromatograms), were disregarded. For the highest individual secondary peak data, individual results, for duplicate sample dilutions, at each time point, are attached in Appendix 15. Example chromatograms from the HPLC analysis are attached in Appendices 3 to 11).

Table 11a: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Related Substances: Highest individual secondary peak areas (expressed as % of total peak area),

Related Substances: Highest individual secondary peak areas (expressed as % total peak area (mean result per bag)									
Sample concentration	**Bag ID**	**Time point (days)**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60 %RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	0.06	0.06	0.10	0.14	0.22	0.24	0.24	0.27
	B	0.06	0.06	0.12	0.21	0.33	0.39^	0.40	0.42^
	C	0.07	0.07	0.12	0.21	0.34	0.39	0.39	0.42^
13.5mg/mL	**A**	0.06	0.11	0.23	0.42	0.64	0.78	0.79	0.89
	B	0.00#	0.11	0.26	0.44	0.67	0.80	0.82	0.92
	C	0.05	0.12	0.24	0.42	0.64	0.77	0.78	0.87

No secondary peaks present with areas > minimum area (0.05% of lowest Pemetrexed peak area recorded in Standard 2 chromatograms)

Table 11b: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Related Substances: Number of other individual secondary peaks with areas > 0.24% of total peak area.

Related Substances: Number of other individual secondary peaks with areas > 0.24% of total peak area									
Sample concentration	**Bag ID**	**Time point (days)**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60 %RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0
13.5mg/mL	**A**	0	0	0	0	1 (0.28%)	1 (0.33%)	1 (0.33%)	3 (0.36%) (0.27%) (0.26%)
	B	0	0	0	0	1 (0.29%)	1 (0.34%)	1 (0.35%)	3 (0.38%) (0.28%) (0.27%)
	C	0	0	0	0	1 (0.28%)	1 (0.33%)	1 (0.33%)	3 (0.36%) (0.27%) (0.26%)

There were no secondary peaks observed to elute at retention times corresponding to Pemetrexed Impurities A and D, with areas > minimum area (0.05% of lowest Pemetrexed peak area in Standard 2 chromatograms) throughout the duration of the study.

8.5.3 Monitoring of 3 x late eluting secondary peaks observed in system suitability sample (ii)

In system suitability sample (ii) (solution of Pemetrexed force degraded using a combination of acid and heat – see section 6.3.4 for preparation details), there are 3 x late eluting peaks (eluting after the Pemetrexed peak) observed. As detailed in section 6.3, from T=7d onwards, the injection run times for this and sample injections were extended to 30 minutes and 20 minutes respectively, to allow samples to be monitored more effectively for their potential presence. Of the 3 x late eluting peaks in question, even with the extended 20 minute run time for samples, there is still a risk that, if present, the third (latest eluting) peak could interfere with the Pemetrexed peak in the next consecutive injection. Throughout the study, the first 2 peaks were seen to elute within the extended 20 minute sample run time window (with the exception of the T=28d time point during which the second of these peaks elutes slightly later at ~21 minutes); these peaks are more prominent

than the third and therefore if these are not present in samples then the third is not likely to be present. At all time points during the study, the first 2 x late eluting peaks were not detected in any of the sample injections.

At the end of the study the following solutions were each injected for a run time of 40 minutes to confirm that the third of the late eluting peaks was not present in sample injections (see chromatograms attached in Appendices 9 to 11);

1. System suitability sample (ii)
2. 2mg/mL sample stored for 28 days at 2-8°C and 24 hours at 25°C (End of study)
3. 13.5mg/mL sample stored for 28 days at 2-8°C and 24 hours at 25°C (End of study)

There were no secondary peaks detected in sample solutions 2 and 3 with retention times corresponding to the 3 late eluting secondary peaks seen in solution 1. This indicates that late eluting peaks would not have been unaccounted for at any stage of the study.

8.6 Sub-Visible Particle Count Analysis

The sub-visible particle count data obtained is summarised in Table 12.

Table 12: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride and Blank Samples (0.9%w/v Sodium Chloride) stored at 2-8°C for 28 days plus an additional 24 hours storage at 25°C/60%RH - Sub-Visible Particle Count Data.

Sub-Visible Particle Count Data									
Sample concentration	Bag ID	Time point (days)							
		0		15 (2-8°C)		28 (2-8°C)		28 (2-8°C) + 24hrs at 25°C/60%RH	
		Number of particles ≥10 micron (per 100mL)	Number of particles ≥ 25 micron (per 100mL)	Number of particles ≥10 micron (per 100mL)	Number of particles ≥ 25 micron (per 100mL)	Number of particles ≥10 micron (per 100mL)	Number of particles ≥ 25 micron (per 100mL)	Number of particles ≥10 micron (per 100mL)	Number of particles ≥ 25 micron (per 100mL)
2mg/mL	A	200	0	50	0	150	0	200	0
	B	50	0	50	50	100	0	0	0
	C	0	0	50	0	0	0	50	0
13.5mg/mL	A	400	0	100	0	200	0	300	0
	B	200	0	250	0	400	0	550	0
	C	150	0	100	0	100	50	100	0
0.9%w/v Sodium Chloride ('Blank')	A	0	0	0	0	100	50	0	0
	B	50	0	50	0	50	0	0	0
Limits		<6000	<600	<6000	<600	<6000	<600	<6000	<600

9. DISCUSSION

9.1. Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride

- Appearance: All 2mg/mL samples were observed to remain clear and colourless solutions, free from visible particles, throughout the study.

- The pH of 2mg/mL samples remained in the range 6.55 to 7.20 when stored for 28 days at 2-8°C followed by an additional 6 hours and 24 hours storage at 25°C/60%RH. The pH of each bag remained within ±0.43 pH units of those at T=0 throughout the duration of the study.

- The mean assayed concentrations of Pemetrexed in all 2mg/mL samples remained ≥ 98.9% of initial assay at T=0, when stored at 2-8°C for periods up to and including 28 days. From first order stability plots, the confidence intervals were narrow, with the lower confidence limit remaining well above the '95% drug

remaining' limit for each device tested, when stored for 28 days at 2-8°C. Storage at elevated temperature did not have any significant impact on assay data, with mean Pemetrexed concentrations remaining ≥ 98.9% of the initial assay at T=0, for each bag tested, following 24 hours storage at 25°C/60%RH.

- Sub visible particle counts for all 2mg/mL samples remained within the BP 2020 limits for preparations supplied in containers with a nominal volume of 100ml or less[6], when stored for 28 days at 2-8°C and also following an additional 24 hours storage at 25°C/60%RH.

9.2. Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride

- Appearance: All 13.5mg/mL samples were observed to remain clear solutions, free from visible particles, throughout the study. Samples were observed to be colourless for time points up to and including T=15 days stored at 2-8°C. From T=23 days (2-8°C) onwards a slight yellow colouration was observed. Section 6.6 ('Special precautions for disposal and other handling') within the SmPC for the ALIMTA vials used to prepare the test samples for this study, states that the appearance of the reconstituted vials (25mg/mL) results in a solution which is 'clear and ranges in colour from colourless to yellow or green-yellow without adversely affecting product quality'[13].

- The pH of 13.5mg/mL samples remained in the range 6.75 to 6.96 when stored for 28 days at 2-8°C followed by an additional 6 hours and 24 hours storage at 25°C/60%RH. The pH of each bag remained within ±0.13 pH units of those at T=0 throughout the duration of the study.

- The mean assayed concentrations of Pemetrexed in all 13.5mg/mL samples remained ≥ 97.2% of initial assay at T=0, when stored at 2-8°C for periods up to and including 28 days. From first order stability plots, the confidence intervals were narrow and despite a downward trend, the lower confidence limit remained well above the '95% drug remaining' limit for each bag tested, when stored for 28 days at 2-8°C. Storage at elevated temperature did not have any significant impact on assay data, with Pemetrexed concentration remaining ≥ 97.1% of the initial assay at T=0, for each bag tested, following 24 hours storage at 25°C/60%RH.

- Sub visible particle counts for all 13.5mg/mL samples remained within the BP 2020 limits for preparations supplied in containers with a nominal volume of 100ml or less[6], when stored for 28 days at 2-8°C and also following an additional 24 hours storage at 25°C/60%RH.

9.3 Related Substances Levels

- Secondary peak areas were seen to increase in both 2mg/mL and 13.5mg/mL samples throughout the duration of the study.

- Total secondary peak areas in all 2mg/mL samples were found to be at a maximum level of 1.02% of the total peak area and individual secondary peak areas were found to be at a maximum level of 0.42% of the total peak area, following storage for 28 days at 2-8°C, followed by 24 hours storage at 25°C/60%RH.

- Total secondary peak areas in all 13.5mg/mL samples were found to be at a maximum level of 1.99% of the total peak area and individual secondary peak areas were found to be at a maximum level of 0.92% of the total peak area, following storage for 28 days at 2-8°C followed by 24 hours storage at 25°C/60%RH.

- There were no peaks detected corresponding to Pemetrexed Impurities A and D with areas greater than the applied minimum peak area (0.05% of the Pemetrexed peak area in Standard 2 (100%)

chromatograms), in any of the samples tested, throughout the duration of the study; these levels are well below the limits outlined in the BP 2020 monograph for Pemetrexed Disodium Heptahydrate raw material[11] (≤ 0.15% of Pemetrexed peak area, for each impurity – see Appendix 16).

- There is no BP monograph for the finished product (powder for reconstitution), there is however a USP monograph for Pemetrexed for Injection (powder for reconstitution) which contains acceptance criteria for related substances levels. Further discussion on related substances levels, with consideration given to this USP monograph, is given in Appendix 16.

9.4 Additional Published Data

Additional data for Pemetrexed preparations has been presented by Stabilis[15]. The data relates to products prepared using the Accord Healthcare brand of Pemetrexed powder for concentrate for solution for infusion. Comparison of the SmPC's for both the Accord brand[14] and the ALIMTA brand[13], indicates that each brand contains Pemetrexed disodium salt as well as equivalent excipients (Mannitol, Sodium Hydroxide and Hydrochloric Acid). The data presented concludes that the Pemetrexed preparations tested in the concentration range 2.5mg/mL-.12.5mg/mL in both 5% Glucose (in Ecoflac containers) and in 0.9% Sodium Chloride (in polyolefin bags), remained physically and chemically stable for 14 days at 2-8°C and separately for 2 days at 25°C, when protected from light. It should be noted that there is no related substances data presented as part of this work, with the shelf life limiting factor appearing to be the time taken for assayed concentrations to drop to <95% of initial concentration at T=0.

10. CONCLUSION

A shelf life based on the data for appearance, pH, sub-visible particles and assay would justify a shelf life of 28 days stored at 2-8°C (protected from light). However, aseptic units are advised to consider the increase in related substances levels and apply a reduced shelf life of a maximum of 21 days stored at 2-8°C (protected from light) (further information on related substances levels is included in Appendix 16).

There were no significant changes in the stability of preparations observed, following storage at 25°C/60%RH (protected from light) for 6 hours. The SmPC for the Accord Healthcare brand of Pemetrexed powder for concentrate for solution for infusion (this brand comprises Pemetrexed disodium salt as well as equivalent excipients to the ALIMTA brand in Mannitol, Sodium Hydroxide and Hydrochloric Acid), states that, the chemical and physical in-use stability of reconstituted and infusion solutions of Pemetrexed were demonstrated for 24 hours at 25°C[14]. The infusion time stated for Pemetrexed preparations is 10 minutes[13,14].

The study design reflects the product preparation procedures described in Appendices 1 and 2; it is important to note that the microbiological stability, aseptic processes, transport processes and associated risks should be independently assured by the user.

11. DEVIATION REPORTS

(NOTE: All Deviation Reports within the laboratory are numbered successively as they are produced, the Deviation Report numbers listed are the only ones which relate to the data within this report (LA2020001(4)). In each case a summary of the nature of the deviation has been detailed here, full copies of each Deviation listed are supplied alongside this report).

Deviation Report Number 109:

Nature of deviation:
On review of the data at the T=2d time point, it was noted that there are 2 x late eluting secondary peaks in system suitability sample (ii); these elute outside of the 15 minute run time window and therefore are carried over into the next consecutive injection (associated Analytical Method Number: LA2020001-1AM – Revision A). On re-visiting the T=0 data, the same observation was made.

Deviation Report Number 110:

Nature of deviation:
At T=7d, the final standard (Standard 3 Injection 2) in the HPLC sequence failed to inject correctly. The peak area of the Pemetrexed peak in this injection was recorded as 9.980 compared with an area of 2287.772 recorded in Standard 3 Injection 1.

Deviation Report Number 113:

Nature of deviation:
The following solutions failed to inject properly at the indicated time points;

T=15d (2-8°C):
- Standard 3 (75%) Injection 2.

T=28d (2-8°C)
- 2mg/mL Sample 2B (Bag B)

T=28d (2-8°C) + 24 hours at 25°C/60%RH
- 2mg/mL Sample 2B (Bag B)
- 2mg/mL Sample 3B (Bag C)

Deviation Report Number 114:

Nature of deviation:
At T=23d (2-8°C) and T=28d (2-8°C) + 24 hours at 25°C/60%RH, preview injections showed the observed retention time of the Pemetrexed peak to be later than that observed at previous time points; in some cases the observed retention time was found to be outside of the acceptance window stated within associated analytical method LA2020001-1AM-Revision B of 6.4 ± 1.5 minutes. In order to reduce the retention time of the Pemetrexed peak (ensuring that it is within the acceptance window and in closer agreement with the retention time observed at other time points), the amount of Acetonitrile in the mobile phase was increased from 7%v/v (93%v/v Aqueous Phase) as detailed in LA2020001-1AM – Revision B, as follows;

T= 23d (2-8°C)
Mobile Phase Composition: 92%v/v Aqueous Phase: 8%v/v Acetonitrile

T=28d (2-8°C) + 24 hours at 25°C/60%RH
Mobile Phase Composition: 92.5%v/v Aqueous Phase: 7.5%v/v Acetonitrile

Deviation Report Number 115:

Nature of deviation:
Changes from Study Protocol:

1. The signed Study Protocol had sample concentrations listed as Pemetrexed 3.5mg/mL and 12mg/mL. The study concentrations were changed to 2mg/mL and 13.5mg/mL.

2. Study time points were listed as;

Time points and Storage Conditions					
0	2 days at 2-8°C	7 days at 2-8°C	14 days at 2-8°C	28 days at 2-8°C	28 days at 2-8°C + 24 hours stored at 25°C/60%RH

Study time points were amended to;

Time points (days) / Storage Condition							
0	2 days (2-8°C)	7 days (2-8°C)	15 days (2-8°C)	23 days (2-8°C)	28 days (2-8°C)	28 days (2-8°C) + 6 hours at 25°C/60%RH	28 days (2-8°C) + 24 hours at 25°C/60%RH

12. REFERENCES

[1] Quality Control North West (Liverpool) Research and Development (R&D) Specification, LA2020001 – Spec1, Pemetrexed (as the disodium salt) 2mg/ml and 13.5mg/ml in 100mL 0.9%w/v Sodium Chloride Intravenous Infusion, Revision A.

[2] Quality Control North West (Liverpool) Standard Operating Procedure (SOP) 111, Use of the Mettler Toledo SevenMulti™ in pH Mode, Revision E.

[3] Quality Control North West (Liverpool) Research and Development (R&D) Analytical Method Number: LA2020001, Pemetrexed (as the disodium salt) 2mg/mL and 13.5mg/mL in 100mL 0.9%w/v Sodium Chloride Intravenous Infusion – Assay of Pemetrexed and monitoring of related substances, Revision A.

[4] Quality Control North West (Liverpool), Standard Operating Procedure (SOP) 137, Operation of HIAC 9703+ Particle Counter, Revision D.

[5] USP39 <787> Sub visible Particulate Matter in Therapeutic Protein Injections, Light Obscuration Particle Count Test.

[6] British Pharmacopeia (BP) 2020, Volume V, Appendix XIIIA. Particulate Contamination: Sub-visible Particles, Method 1, Light Obscuration Particle Count Test.

[7] Quality Control North West (Liverpool) Research and Development Procedure (RDP) 013, Validation of Routine QC Methods / Analytical Tests, Revision C.

[8] ICH (International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) Guideline Q2 (R1) (Validation of Analytical Procedures – Text and Methodology), Step 5, August 2003.

[9] ICH (International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) Guideline Q1E (Evaluation of Stability Data), Step 5, August 2003.

[10] A Standard Protocol for Deriving and Assessment of Stability. Part 1 - Aseptic Preparations (Small Molecules), Edition 5, September 2019, NHS Pharmaceutical Quality Assurance Committee.

[11] British Pharmacopeia (BP) 2020 (as published), monograph for Pemetrexed Disodium Heptahydrate, Related Substances.

[12] Quality Control North West (Liverpool) Method Validation Report Reference: LA2020001(1), Validation of a stability indicating HPLC method for the assay of Pemetrexed and analysis of related substances (validated for use for stability study reference number LA2020001) – Version A.

[13] Summary of Product Characteristics (SmPC) for ALIMTA powder for concentrate for solution for infusion, Date of revision of text: 17 April 2020

[14] https://www.ema.europa.eu/en/documents/product-information/pemetrexed-accord-epar-product-information_en.pdf

[15] Stabilis Newsletter (INFOSTAB) No. 41, June 2018, Short Report : Extended Stability Studies on Bortezomib Injection and Infusions of Cisplatin and Pemetrexed (all Accord Healthcare), Toral Patel and Graham Sewell, Faculty of Health and Human Sciences, University of Plymouth, Plymouth PL68BH, UK.

[16] US Pharmacopeia (USP) 2020, monograph for Pemetrexed Disodium, USP-NF, Document ID: 1_GUID-74EEB2EB-AE83-4A3B-BDAE-40BDB0493B7B_6_en-US.

[17] US Pharmacopeia (USP) 2020, monograph for Pemetrexed for Injection, USP-NF, Document ID: 1_GUID-FC3E7388-251E-4561-99CE-2D2CE27F6FF3_5_en-US.

[18] Pemetrexed (as pemetrexed disodium) 2mg/mL and 13.5mg/mL in 100mL Sodium Chloride 0.9%w/v Intravenous Infusion Bags (Baxter Viaflo) LA2020001(4) - Justification of related substances levels reported – (unspecified impurities) which exceed ICH Q3(B) levels, Assessment carried out by Mark Santillo.

[19] ICH (International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) Guideline Q3B(R2) (Impurities in New Drug Products), Step 5, June 2006.

13. APPENDICES

Appendix 1: Batch Production sheets for Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM200 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

PRODUCT INFORMATION					
Pemetrexed 200mg in 100ml Sodium Chloride 0.9% (FOR QCNW TESTING PURPOSES ONLY)					
Strength	2mg/ml	**Final Container**	100ml Baxter Viaflow	**Batch size**	3 x 100ml
Expiry	N/A	**Storage**	Store between 2-8°C Protect from light	**Stability Ref**	N/A

BATCH SPECIFIC DETAILS					
Batch number (YYMMXXX)		Date Worksheet Prepared		Worksheet Prepared by	
Expiry date	N/A	Date of Dispensing		Worksheet Checked by	

LABELS	
Quantity of labels produced should include TWO sample labels. Of the batch of labels produced, the first and last labels must be attached to the batch sheet. If an error is made during label production and more labels are to be produced, TWO more samples must be produced. TWO labels per product must be produced if light protective outer bag is required.	
Master Product Label	**Sample Label**
	Attach Sample Label (First label printed)
	Attach Sample Label (Last label printed)

Label Code	PEM200STABILITY	No. Labels Required (+ 2 for batch sheet)	8

Page **1** of **6**

Appendix 1 (cont.): Batch Production sheets for Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM200 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

No. Labels Produced		No. attached to Batch sheet	
Labels produced by		Date produced	
Labels checked by		Date checked	

AUTHORISATION TO PROCEED			
Signature of Senior Quality Team member		Date approved	

INGREDIENTS AND CONSUMABLES

Assemble all starting materials as listed below and enter the batch numbers and expiry dates for each item. Sign for assembling each item. Each item must then be checked by a second person.

Ingredients	Strength	Quantity	Volume	Batch Number	Expiry	Manufacturer	Assembled by	Checked By
Pemetrexed vial	500mg	1	-					
Pemetrexed vial	100mg	1	-					
Sodium Chloride 0.9%	-	3	100ml					
Sodium Chloride 0.9%	-	1	250ml					

Consumables	Strength	Quantity	Volume	Batch Number	Expiry	Manufacturer	Assembled by	Checked by
Syringe	-	1	5ml					
Syringe	-	3	10ml					
Syringe	-	4	20ml					
Green needle	-	2	-					
White needle	-	8	-					
Dispensing pin	-	1	-					
End cap	-	3	-					
Alcohol wipes	-	1 pack	-					
Non-vented Dispensing Pins (Supplied by QCNW)	-	-	-			B Braun		

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Appendix 1 (cont.): Batch Production sheets for Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM200 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

TRANSFER SANITISATION					
Transfer materials in accordance with the SOP: Transfer Disinfection					
Sporicidal stage Performed by		Transfer into isolator room performed by		Transfer into isolator performed by	

FACILITY STATUS		
Operational status of the unit has been confirmed as satisfactory	Checked by (operator)	

	PRODUCTION METHOD	IN-PROCESS CHECK BY
1	Transfer all items required into the isolator body as per the Transfer Disinfection SOP.	
2	Swab the additive port of each 100ml NaCl 0.9% bag. Remove 19ml of NaCl from the bag.	
3	Repeat step (2) a further 2 times so that 19ml has been removed from each 100ml bag. Place syringes containing withdrawals into the bag to be sent out to the accuracy checker.	
4	Swab the rubber bung of each pemetrexed vial with an alcohol wipe and add a green needle to each vial (to vent).	
5	Swab the port of the 250ml NaCl 0.9% bag with an alcohol wipe and insert a dispensing pin.	
6	Withdraw 4.2ml from the 250ml NaCl 0.9% bag into a 5ml syringe. Add a white needle to the syringe and reconstitute the **100mg vial**. Gently swirl until the powder has completely dissolved.	
7	Withdraw 20ml from the NaCl 0.9% bag into a 20ml syringe. Add a white needle to the syringe and reconstitute the **500mg vial**. Gently swirl until the powder has completely dissolved. Both vials will contain a 25mg/ml solution.	
8	Using a new 10ml syringe and white needle, and leaving the green needles in place, withdraw 8ml from a constituted vial. Add this 8ml (200mg) to a 100ml NaCl 0.9% bag. Once the addition has been made, place an end cap on the bag immediately.	
9	Repeat step (8) a further 2 times, until all 3 bags have had 200mg (8ml) added.	
10	Add a non-vented dispensing pin to the giving port of each bag	
11	Wipe bag with an alcohol wipe. Remove bags from the isolator for labelling and transfer for testing. Place empty vials and syringes containing removal volumes into the rubbish bag for the accuracy checker. Ensure all other syringes and needles have been placed into the sharps bin.	

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Appendix 1 (cont.): Batch Production sheets for Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM200 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

PREPARATION CHECKS			
Isolator used		Dispensed by	
Line clearance by		Line clearance check by	
Isolator cleaned before preparation by		Isolator cleaned after preparation by	
Date manufactured		Time manufactured	

PRODUCT INSPECTION			
Starting Material Reconciliation check	**Checked by**	**Product Inspection**	**Inspected by**
Check 1 x 100mg vial and 1 x 500mg vial present		Check final product for leakages	
Check 3 x 100ml Sodium Chloride 0.9% bags present		Check bags for visible particles	
No evidence of damage or defects for components used		Confirm product appearance satisfactory	
Confirm excess removed from bag		Confirm dispensing pins are in place	

Total number for labelling		Checked by	
Total number failed			
Reasons for failures			

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Appendix 1 (cont.): Batch Production sheets for Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM200 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

PRODUCT AND LABEL RECONCILIATION			
Label each product according to local procedure. Label each bag/syringe, seal in clear plastic bag, and overwrap in light protective bag if required. Each light protective bag must also be labelled.			
Product Reconciliation		**Label Reconciliation**	
Number expected		Number produced	
Number dispensed		Number attached to products	
Number passed		Number excess	
Number failed		Number destroyed	
Product reconciliation completed by		Destroyed by	
		Label reconciliation completed by	
Reasons for failures (product/labels destroyed)			

AUTHORISED DEVIATIONS FROM SOP AND NON-STANDARD EVENTS			
Description of event		**Outcome**	
Documented by		Date	
Approved by Authorised Pharmacist		Date	

Appendix 1 (cont.): Batch Production sheets for Pemetrexed 2mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM200 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

PRODUCT APPROVAL					
Worksheet checks	**Y**	**N**	**Component Reconciliation Checks**	**Y**	**N**
Batch number on worksheet is unique and matches batch records			All ingredients and consumables used are in date		
All worksheet checks are present			Components do not expire before finished product		
Deviations are authorised			All ingredients and consumables have been recorded completely on the worksheet		
Alterations are initialled or covered by an authorised deviation, as appropriate			**Final Product Checks**	**Y**	**N**
Facility status has been confirmed as satisfactory			Final product has been prepared in 100ml bags		
Label/Packing check	**Y**	**N**	Visible particles are absent		
Product labels match the master label on the worksheet			The solution is clear		
			Free from visible defects and leakage		
Labels display the correct expiry date					
Labels have a batch number that matches the hand written one on the worksheet					
Labels adhere to the syringe barrel or infusion bag, and do not obscure the syringe scale, if applicable					
Label reconciliation tallies and are recorded on the worksheet					
All products are sealed neatly inside clear plastic bags and are in light protective bags as required, both have labels attached.					

Product approval by		Date	
Total number quarantined	N/A	Number sent for QC testing	

Appendix 2: Batch Production sheets for Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM1325 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

PRODUCT INFORMATION					
Pemetrexed 1325mg in 100ml Sodium Chloride 0.9% (FOR QCNW TESTING PURPOSES ONLY)					
Strength	13.5mg/ml	**Final Container**	100ml Baxter Viaflow	**Batch size**	3 x 100ml
Expiry	N/A	**Storage**	Store between 2-8°C Protect from light	**Stability Ref**	N/A

BATCH SPECIFIC DETAILS					
Batch number (YYMMXXX)		Date Worksheet Prepared		Worksheet Prepared by	
Expiry date	N/A	Date of Dispensing		Worksheet Checked by	

LABELS	
Quantity of labels produced should include TWO sample labels. Of the batch of labels produced, the first and last labels must be attached to the batch sheet. If an error is made during label production and more labels are to be produced, TWO more samples must be produced. TWO labels per product must be produced if light protective outer bag is required.	
Master Product Label	**Sample Label**
	Attach Sample Label (First label printed)
	Attach Sample Label (Last label printed)

Label Code	PEM1325STABILITY	No. Labels Required (+ 2 for batch sheet)	8

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Appendix 2 (cont.): Batch Production sheets for Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM1325 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

No. Labels Produced		No. attached to Batch sheet	
Labels produced by		Date produced	
Labels checked by		Date checked	

AUTHORISATION TO PROCEED			
Signature of Senior Quality Team member		Date approved	

INGREDIENTS AND CONSUMABLES

Assemble all starting materials as listed below and enter the batch numbers and expiry dates for each item. Sign for assembling each item. Each item must then be checked by a second person.

Ingredients	Strength	Quantity	Volume	Batch Number	Expiry	Manufacturer	Assembled by	Checked By
Pemetrexed vial	500mg	8	-					
Sodium Chloride 0.9%	-	3	100ml					
Sodium Chloride 0.9%	-	1	250ml					

Consumables	Strength	Quantity	Volume	Batch Number	Expiry	Manufacturer	Assembled by	Checked by
Syringe	-	11	20ml					
Syringe	-	6	30ml					
Syringe	-	3	50ml					
Green needle	-	8	-					
White needle	-	20	-					
Dispensing pin	-	1	-					
End cap	-	3	-					
Alcohol wipes	-	1 pack	-					
Non-vented Dispensing Pins (Supplied by QCNW)	-	-	-			B Braun		

Appendix 2 (cont.): Batch Production sheets for Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM1325 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

TRANSFER SANITISATION					
Transfer materials in accordance with the SOP: Transfer Disinfection					
Sporicidal stage Performed by		Transfer into isolator room performed by		Transfer into isolator performed by	

FACILITY STATUS		
Operational status of the unit has been confirmed as satisfactory	Checked by (operator)	

	PRODUCTION METHOD	IN-PROCESS CHECK BY
1	Transfer all items required into the isolator body as per the Transfer Disinfection SOP.	
2	Swab the additive port of each 100ml NaCl 0.9% bag. Remove 66ml of NaCl from the bag using 50ml and 20ml syringes.	
3	Repeat step (2) a further 2 times so that 66ml has been removed from each 100ml bag. Place syringes containing withdrawals into the bag to be sent out to the accuracy checker.	
4	Swab the rubber bung of each pemetrexed vial with an alcohol wipe and add a green needle to each vial (to vent).	
5	Swab the port of the 250ml NaCl 0.9% bag with an alcohol wipe and insert a dispensing pin.	
6	Withdraw 20ml from the 250ml NaCl 0.9% bag into a 20ml syringe. Add a white needle to the syringe and reconstitute a 500mg vial. Gently swirl until the powder has completely dissolved.	
7	Repeat step (6) until all 8 vials have been reconstituted with 20ml of NaCl 0.9%. Vials will contain a 25mg/ml solution.	
8	Using 2 x new 30ml syringes and white needles, leave the green needles in place, withdraw 53ml from the constituted vials. Add this 53ml (1325mg) to a 100ml NaCl 0.9% bag. Once the addition has been made, place an end cap on the bag immediately.	
9	Repeat step (8) a further 2 times, until all 3 bags have had 1325mg (53ml) added.	
10	Add a non-vented dispensing pin to the giving port of each bag	
11	Wipe bag with an alcohol wipe. Remove bags from the isolator for labelling and transfer for testing. Place empty vials and syringes containing removal volumes into the rubbish bag for the accuracy checker. Ensure all other syringes and needles have been placed into the sharps bin.	

PREPARATION CHECKS			
Isolator used		Dispensed by	
Line clearance by		Line clearance check by	
Isolator cleaned before preparation by		Isolator cleaned after preparation by	
Date manufactured		Time manufactured	

Appendix 2 (cont.): Batch Production sheets for Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM1325 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

PRODUCT INSPECTION			
Starting Material Reconciliation check	**Checked by**	**Product Inspection**	**Inspected by**
Check 8 x 500mg vials present		Check final product for leakages	
Check 3 x 100ml Sodium Chloride 0.9% bags present		Check bags for visible particles	
No evidence of damage or defects for components used		Confirm product appearance satisfactory	
Confirm excess removed from bag		Confirm dispensing pins are in place	

Total number for labelling		Checked by	
Total number failed			
Reasons for failures			

PRODUCT AND LABEL RECONCILIATION			
Label each product according to local procedure. Label each bag/syringe, seal in clear plastic bag, and overwrap in light protective bag if required. Each light protective bag must also be labelled.			
Product Reconciliation		**Label Reconciliation**	
Number expected		Number produced	
Number dispensed		Number attached to products	
Number passed		Number excess	
Number failed		Number destroyed	
Product reconciliation completed by		Destroyed by	
		Label reconciliation completed by	
Reasons for failures (product/labels destroyed)			

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Appendix 2 (cont.): Batch Production sheets for Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride test samples

The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM1325 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

AUTHORISED DEVIATIONS FROM SOP AND NON-STANDARD EVENTS			
Description of event		**Outcome**	
Documented by		Date	
Approved by Authorised Pharmacist		Date	

PRODUCT APPROVAL					
Worksheet checks	**Y**	**N**	**Component Reconciliation Checks**	**Y**	**N**
Batch number on worksheet is unique and matches batch records			All ingredients and consumables used are in date		
All worksheet checks are present			Components do not expire before finished product		
Deviations are authorised			All ingredients and consumables have been recorded completely on the worksheet		
Alterations are initialled or covered by an authorised deviation, as appropriate			**Final Product Checks**	**Y**	**N**
Facility status has been confirmed as satisfactory			Final product has been prepared in 100ml bags		
Label/Packing check	**Y**	**N**	Visible particles are absent		
Product labels match the master label on the worksheet			The solution is clear		
			Free from visible defects and leakage		
Labels display the correct expiry date					
Labels have a batch number that matches the hand written one on the worksheet					
Labels adhere to the syringe barrel or infusion bag, and do not obscure the syringe scale, if applicable					
Label reconciliation tallies and are recorded on the worksheet					
All products are sealed neatly inside clear plastic bags and are in light protective bags as required, both have labels attached.					

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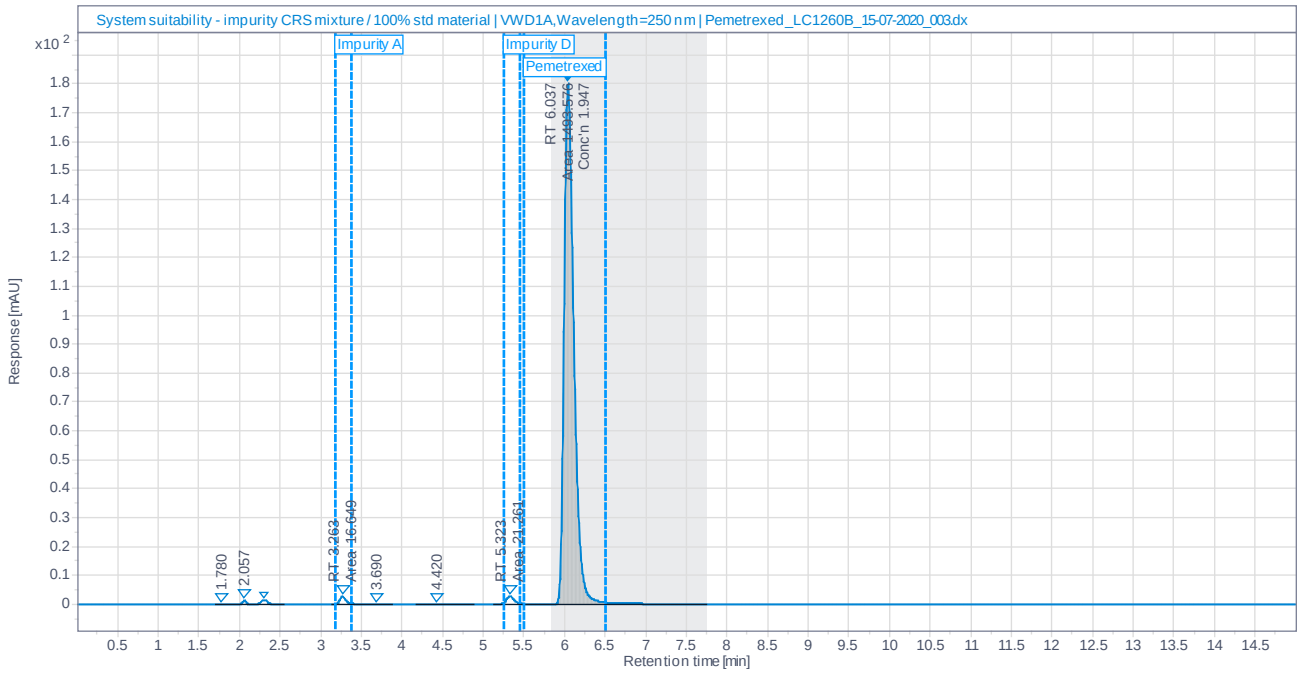
Appendix 2 (cont.): Batch Production sheets for Pemetrexed 13.5mg/mL in 0.9%w/v Sodium Chloride test samples

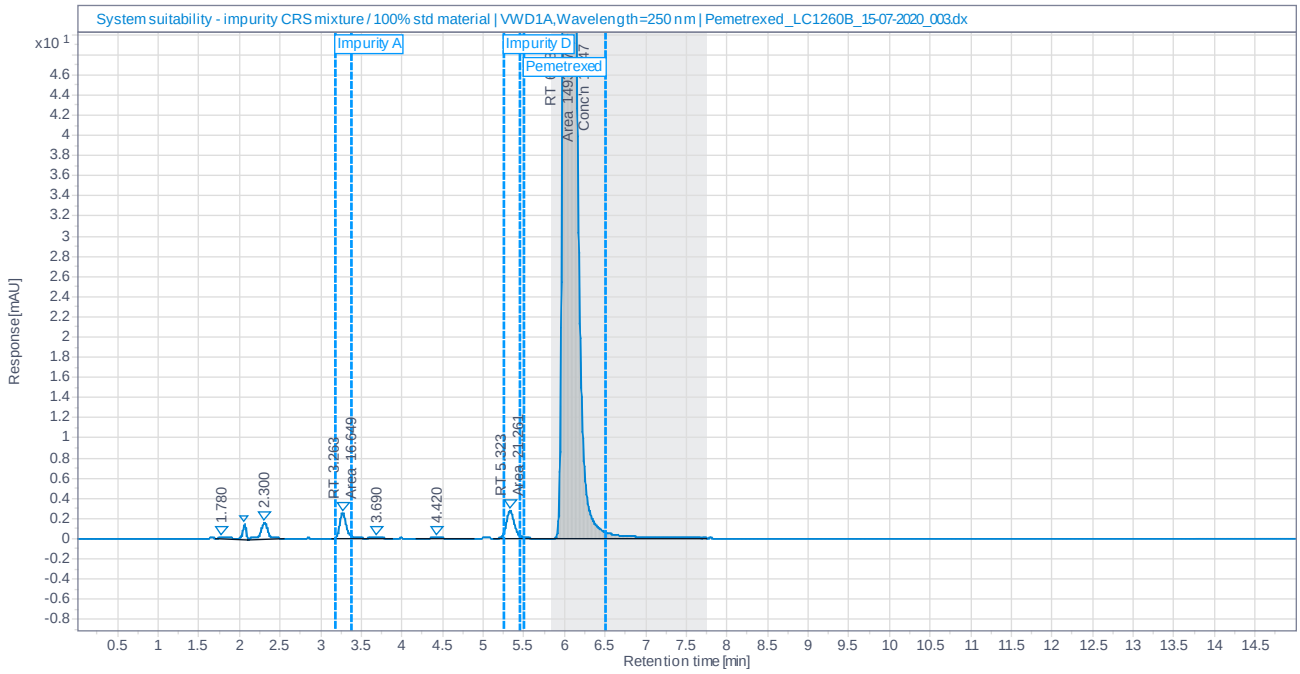
The Clatterbridge Cancer Centre Oncology Pharmacy					
Batch Sheet Reference:	PEM1325 STABILITY	**Prepared by:**	Laura Jayne Allen	**Date Approved:**	15/06/2020
Version Number:	1.0	**Approved by:**		**Review Date:**	15/06/2022

Product approval by		Date	
Total number quarantined	N/A	Number sent for QC testing	

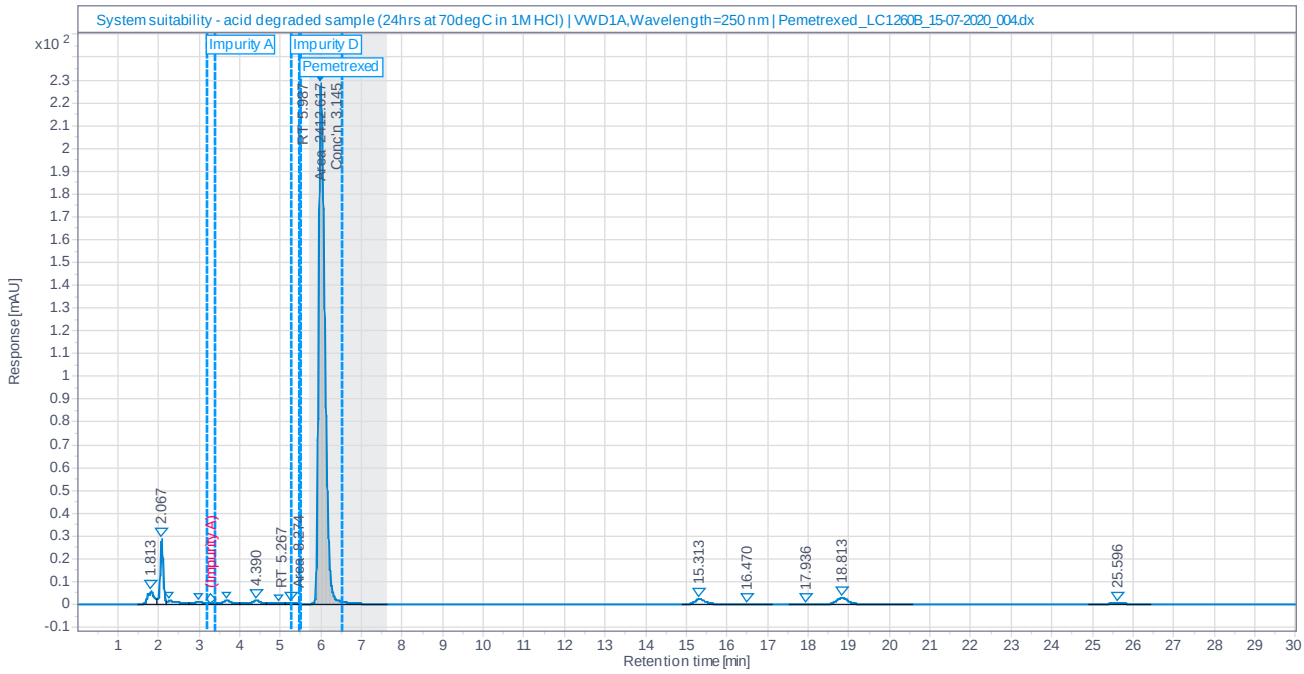
Appendix 3a: HPLC analysis – Chromatogram for system suitability sample (i) (normal view)



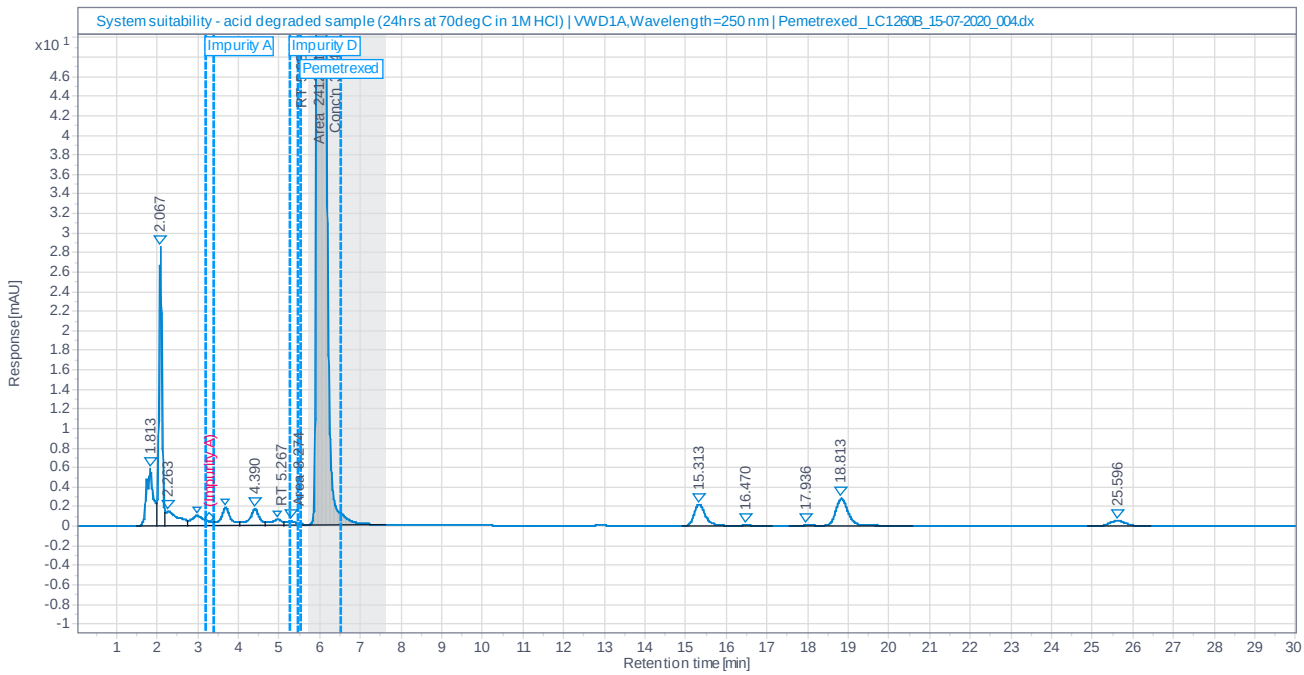
Appendix 3b: HPLC analysis – Chromatogram for system suitability sample (i) (enhanced view)



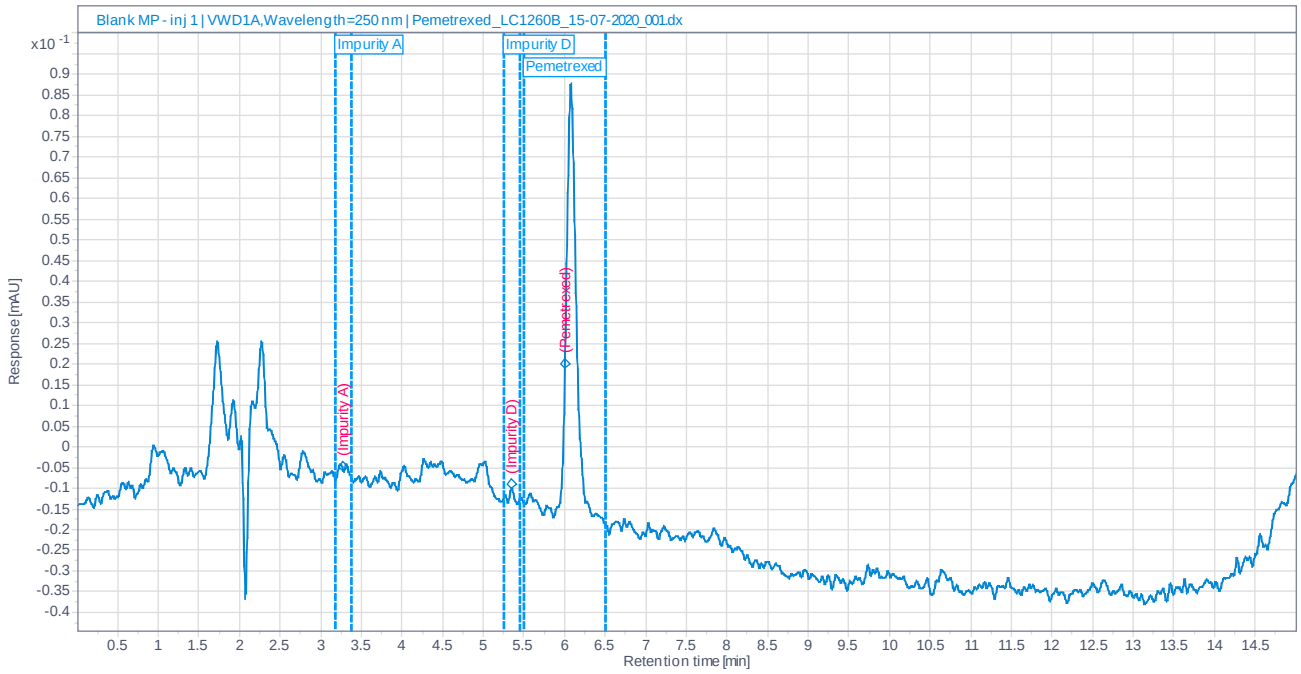
Appendix 4a: HPLC analysis – Chromatogram for system suitability sample (ii) (normal view)



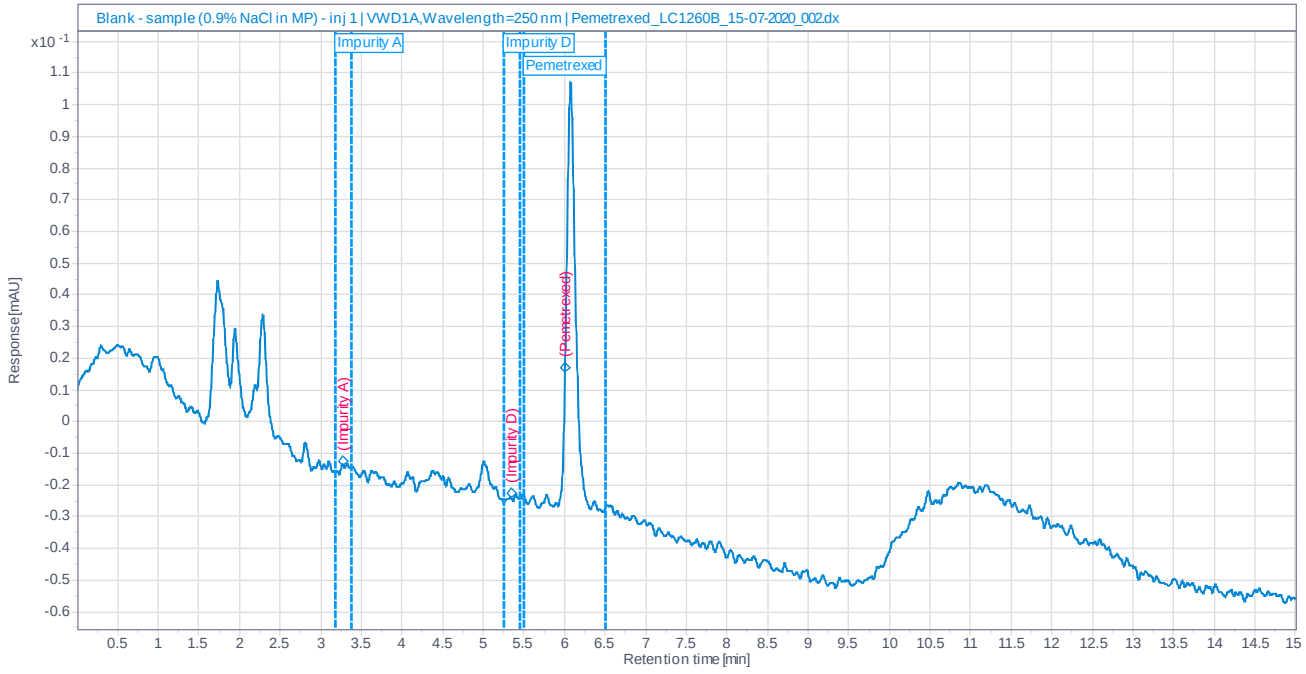
Appendix 4b: HPLC analysis – Chromatogram for system suitability sample (ii) (enhanced view)



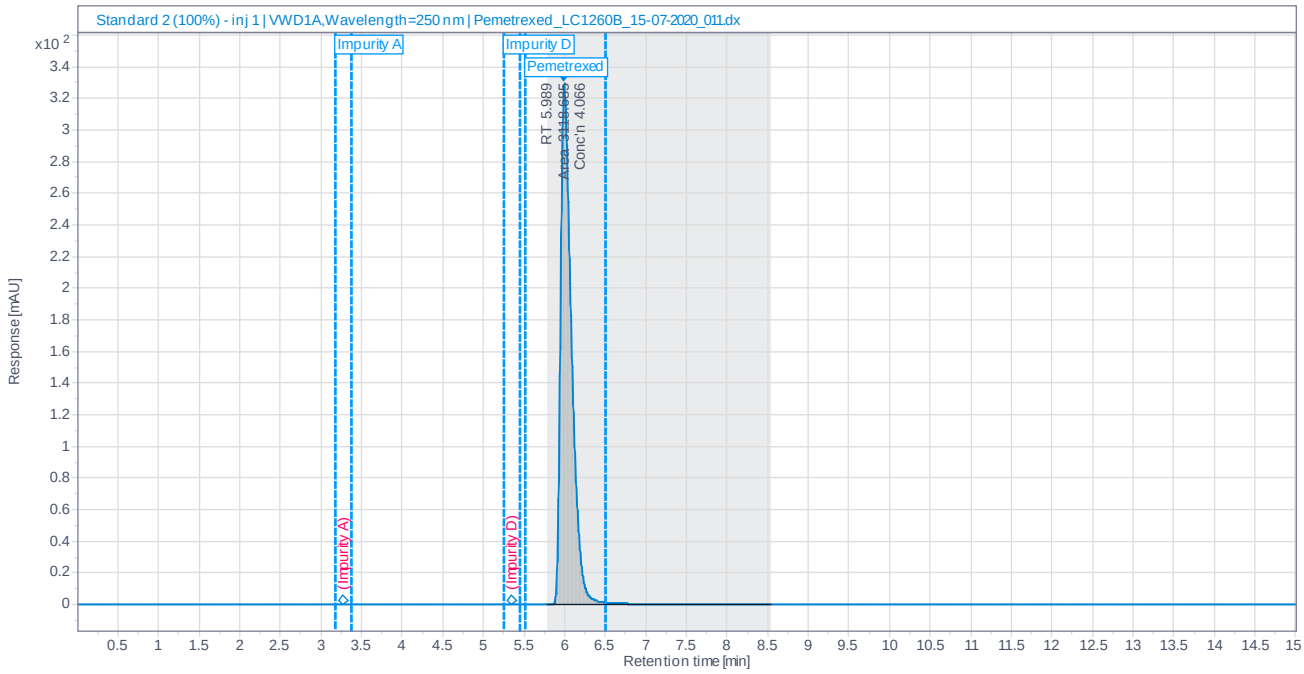
Appendix 5a: HPLC analysis – Chromatogram for 'Blank Mobile Phase'



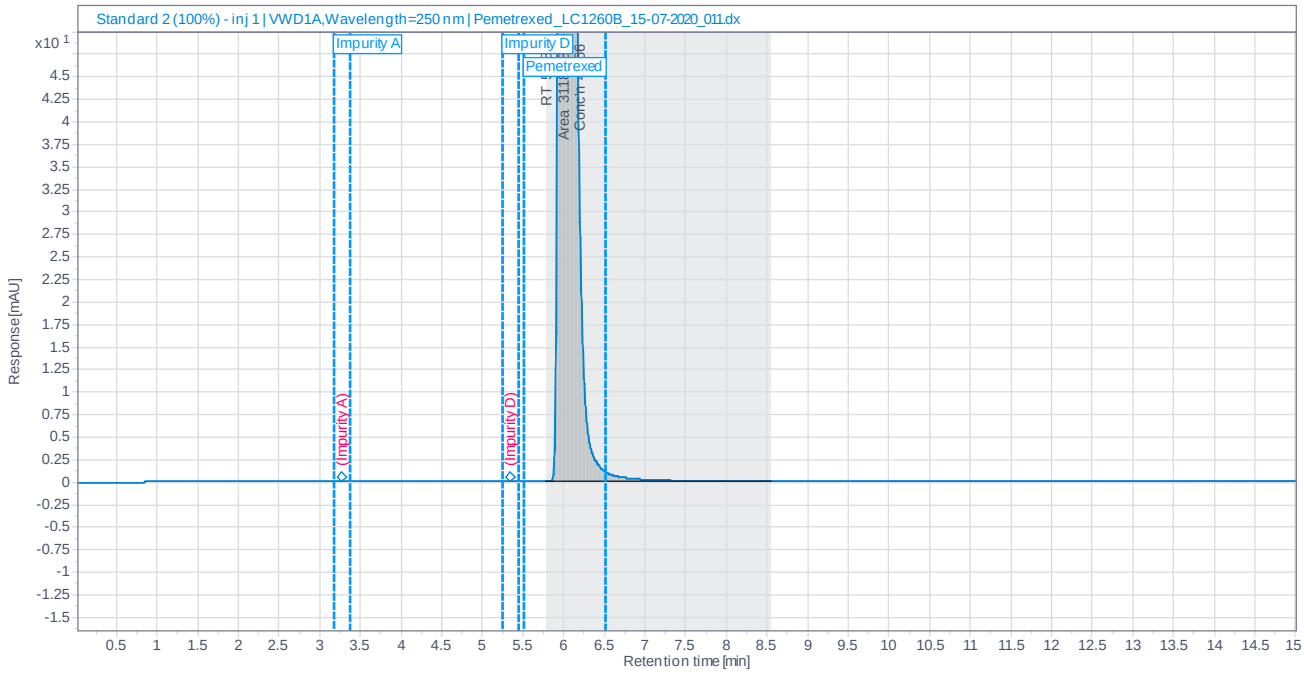
Appendix 5b: HPLC analysis – Chromatogram for 'Blank Sample'



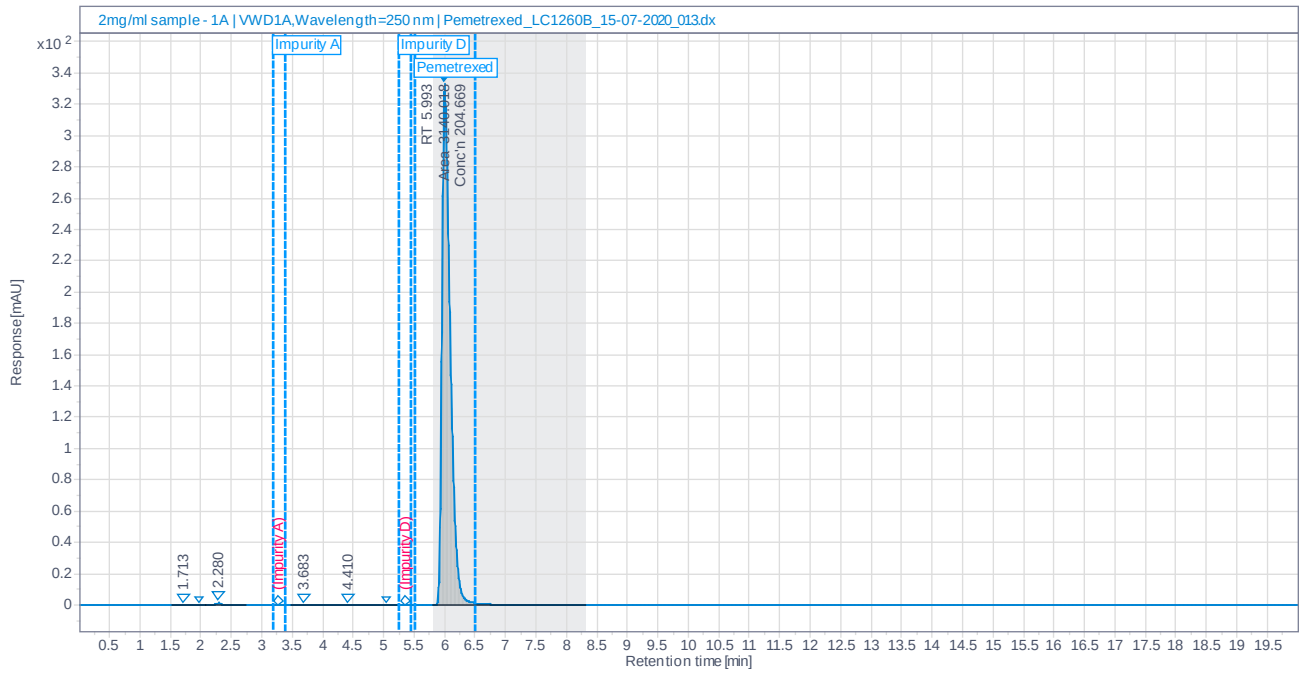
Appendix 6a: HPLC analysis – Chromatogram for Standard 2 (normal view)



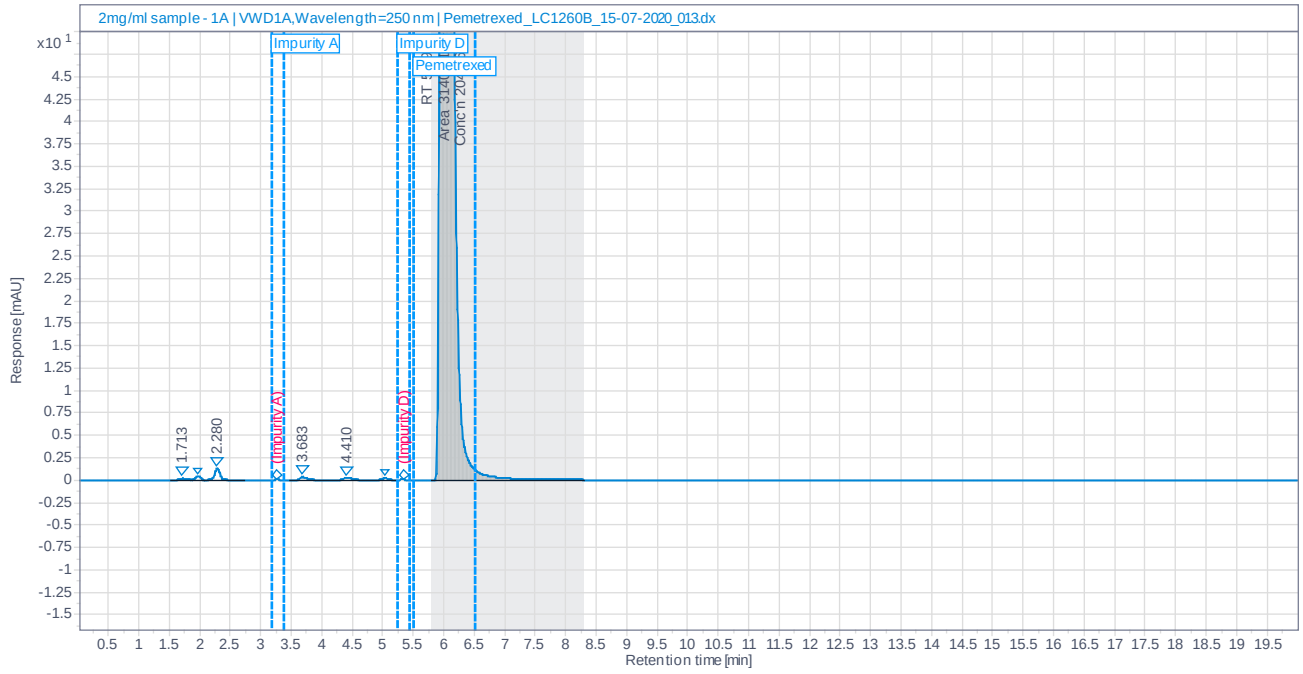
Appendix 6b: HPLC analysis – Chromatogram for Standard 2 (enhanced view)



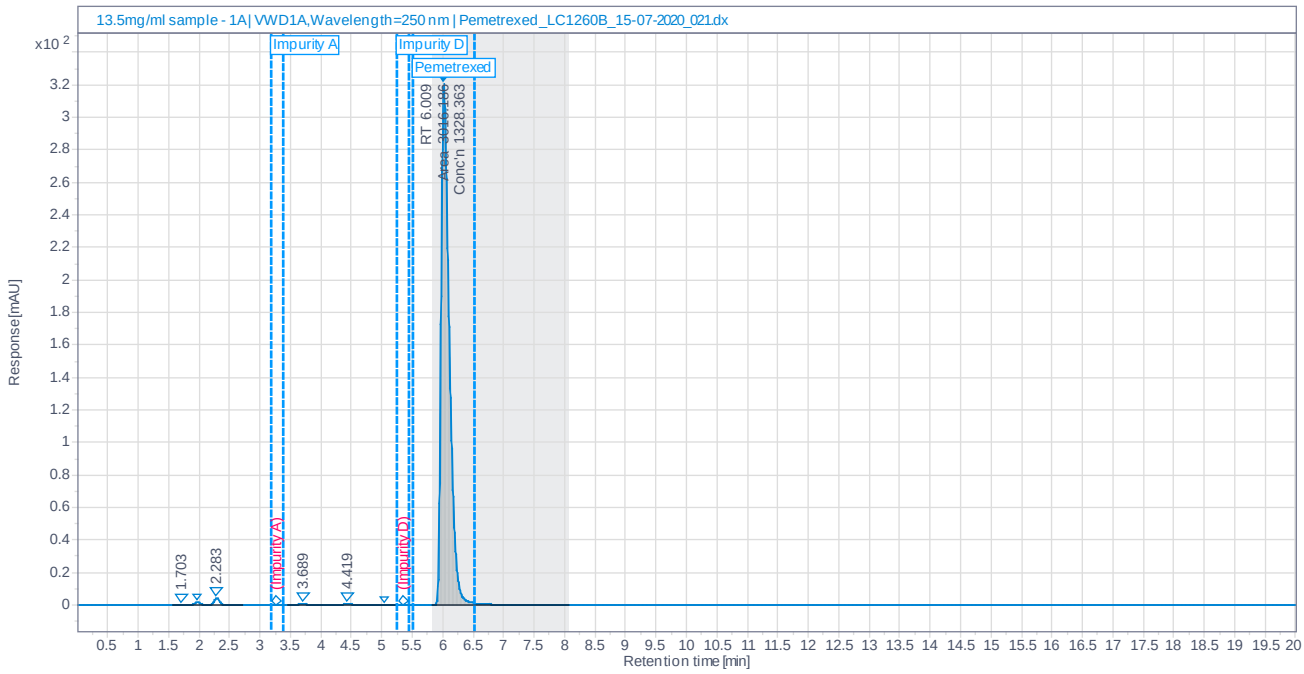
Appendix 7a: HPLC analysis – Chromatogram for 2mg/mL sample (T=28d + 24 hours at 25°C/60%RH) (normal view)



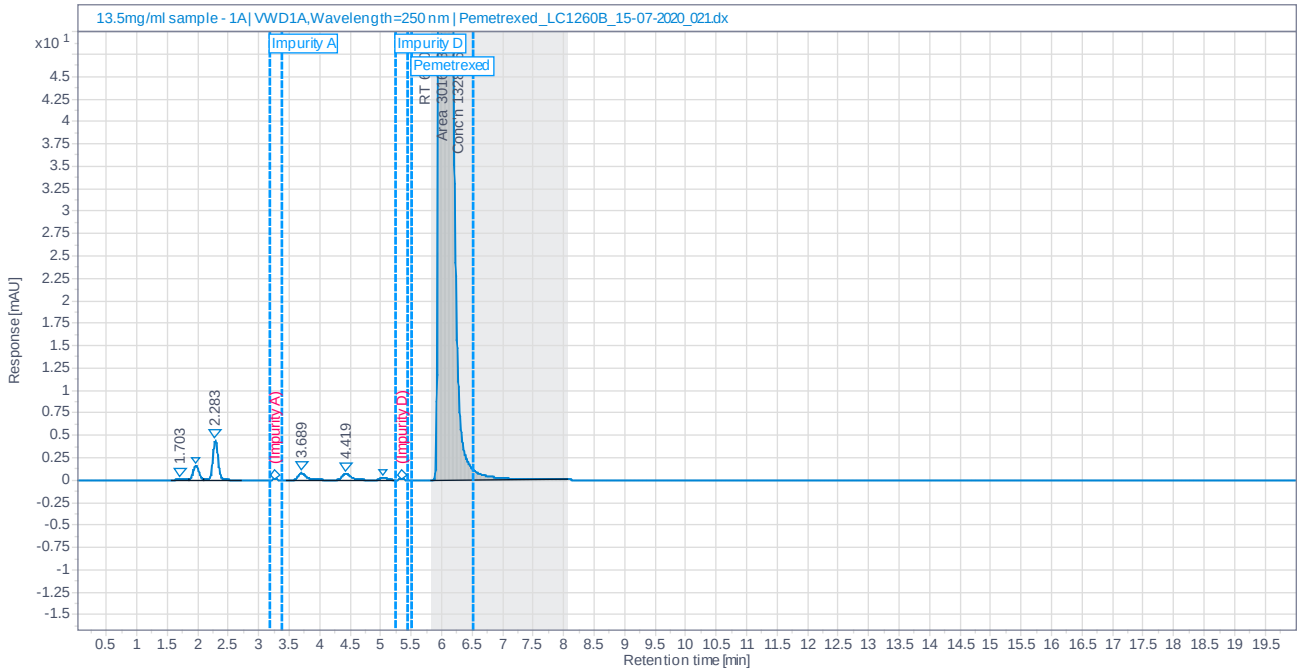
Appendix 7b: HPLC analysis – Chromatogram for 2mg/mL sample (T=28d + 24 hours at 25°C/60%RH (enhanced view)



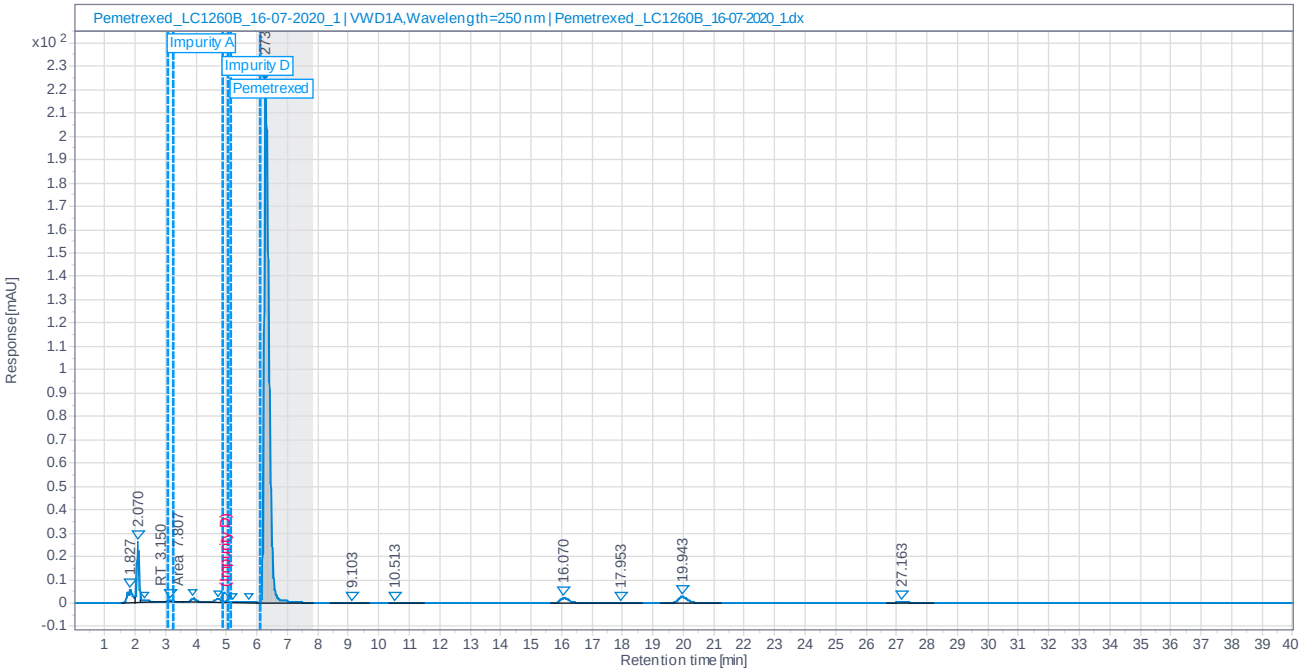
Appendix 8a: HPLC analysis – Chromatogram for 13.5mg/mL sample (T=28d + 24 hours at 25°C/60%RH) (normal view)



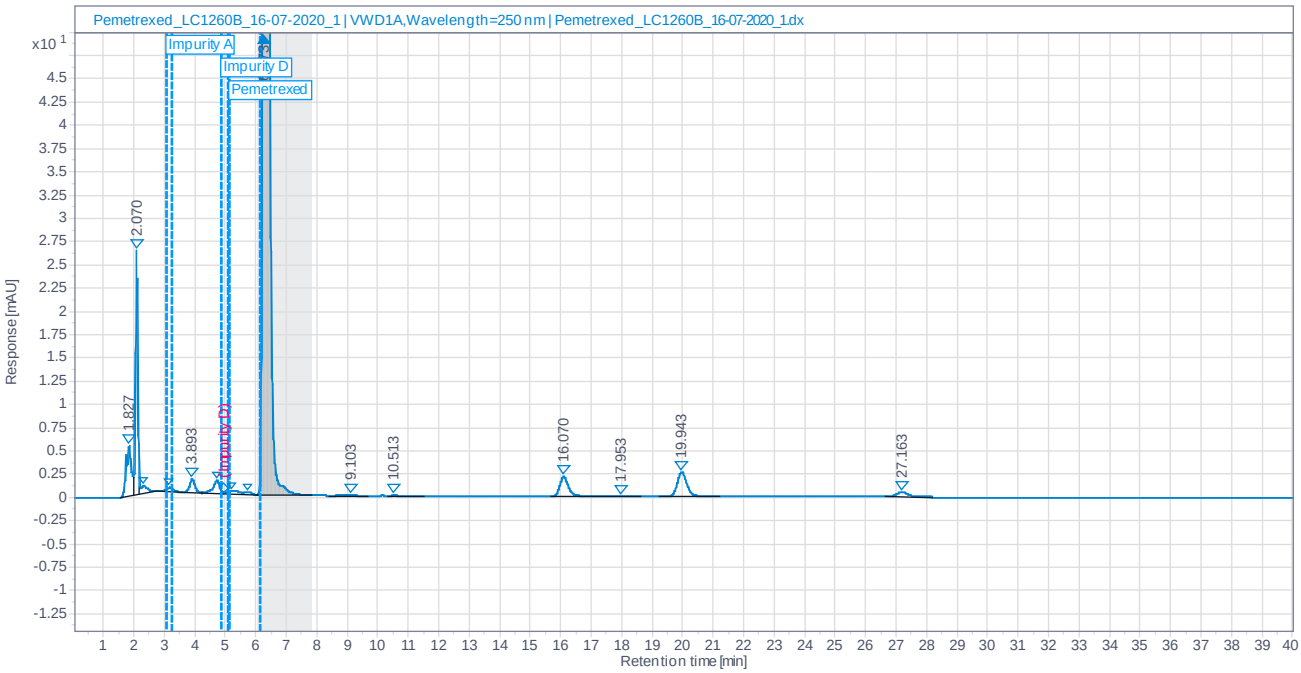
Appendix 8b: HPLC analysis – Chromatogram for 13.5mg/mL sample (T=28d + 24 hours at 25°C/60%RH) (enhanced view)



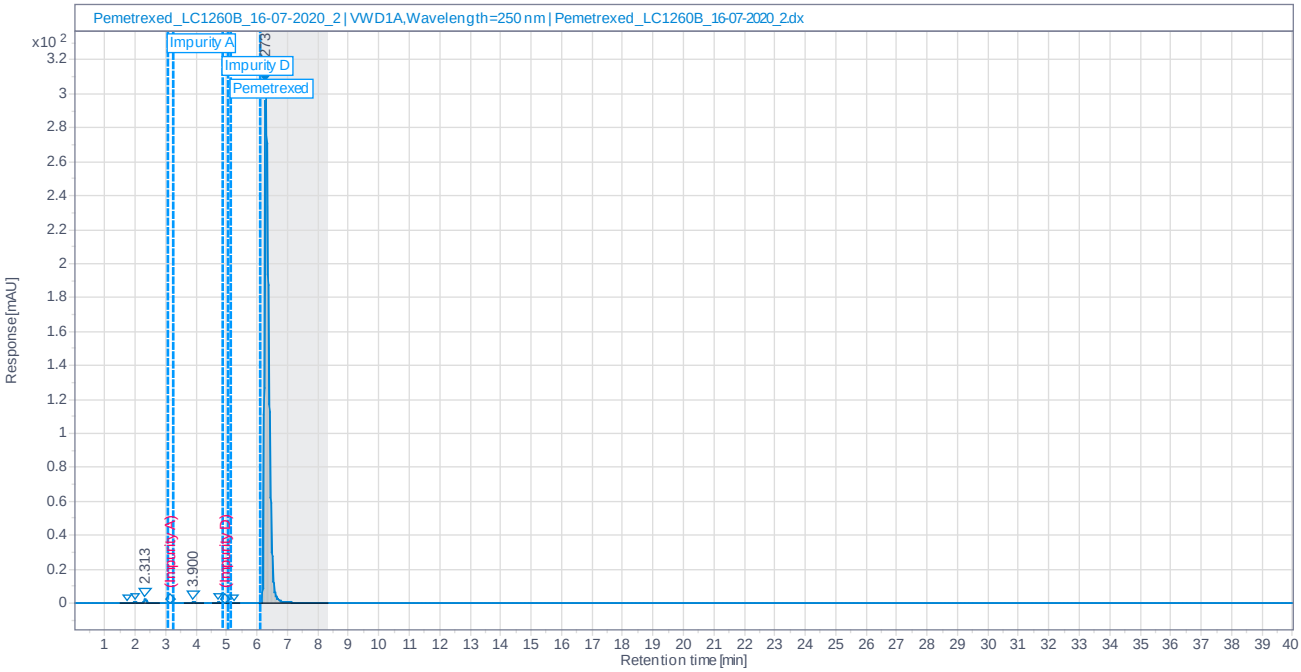
Appendix 9a: HPLC analysis – Chromatogram for system suitability sample (ii) run for 40 minutes at end of study (normal view)



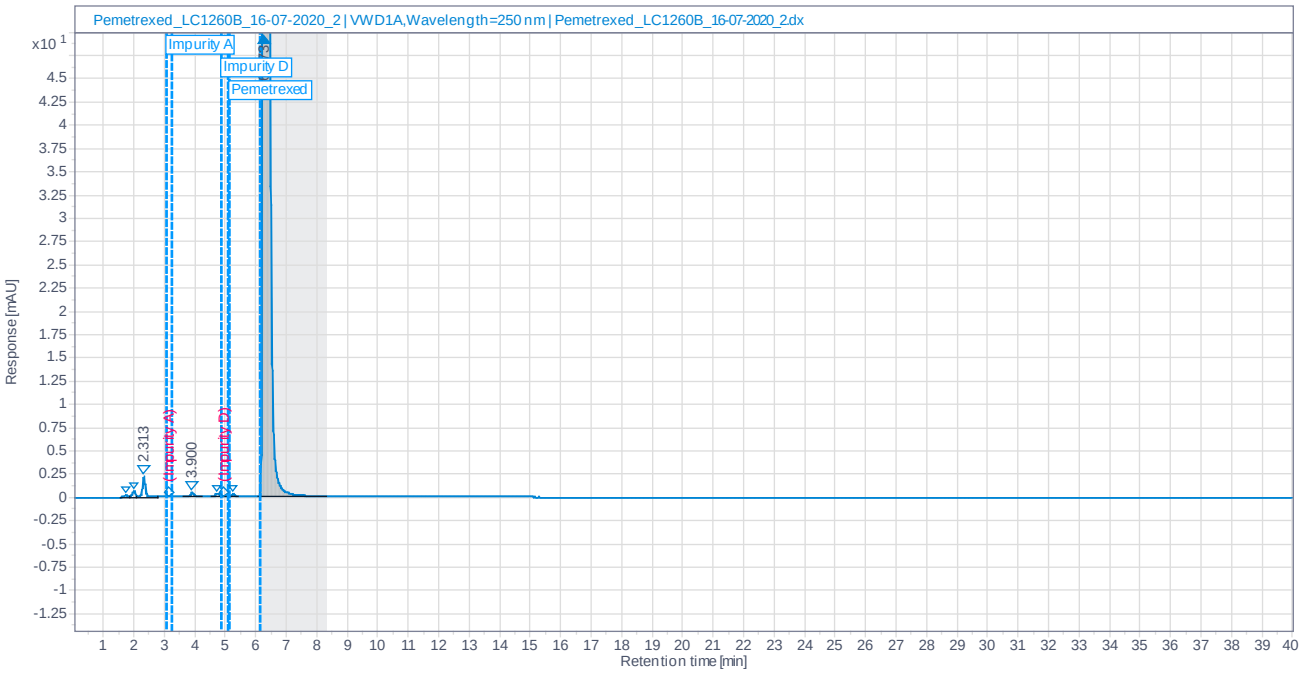
Appendix 9b: HPLC analysis – Chromatogram for system suitability sample (ii) run for 40 minutes at end of study (enhanced view)



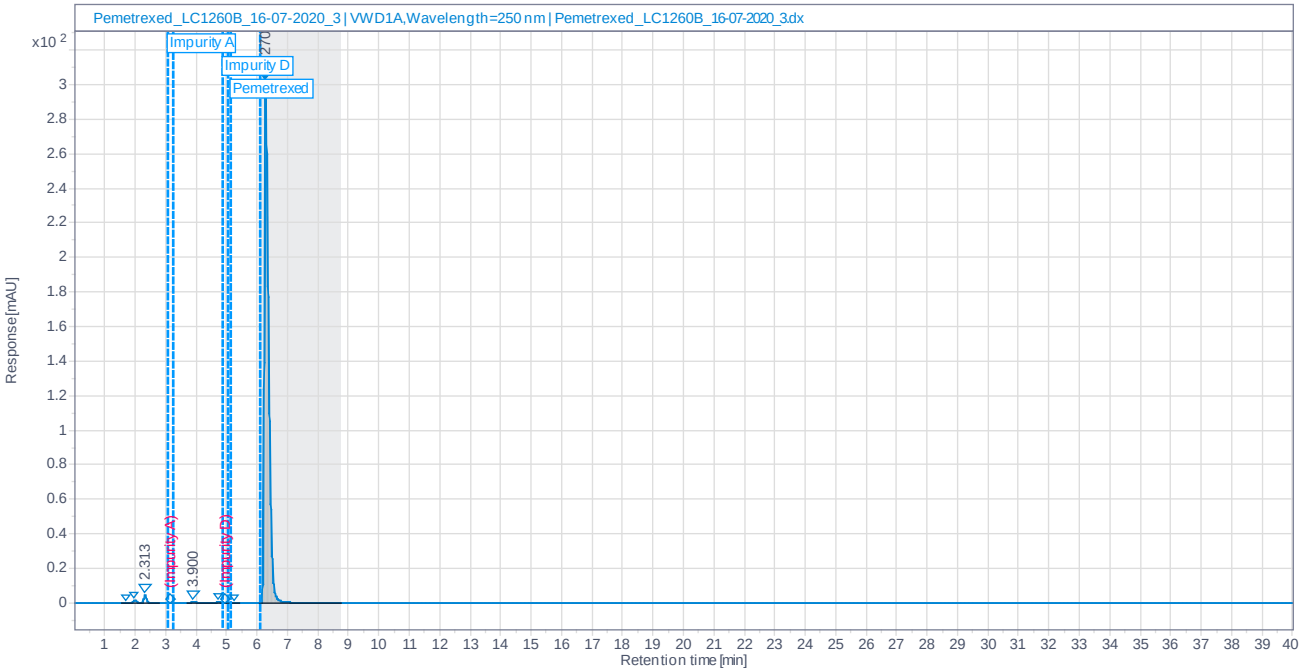
Appendix 10a: HPLC analysis – Chromatogram for 2mg/mL sample run for 40 minutes at end of study (normal view)



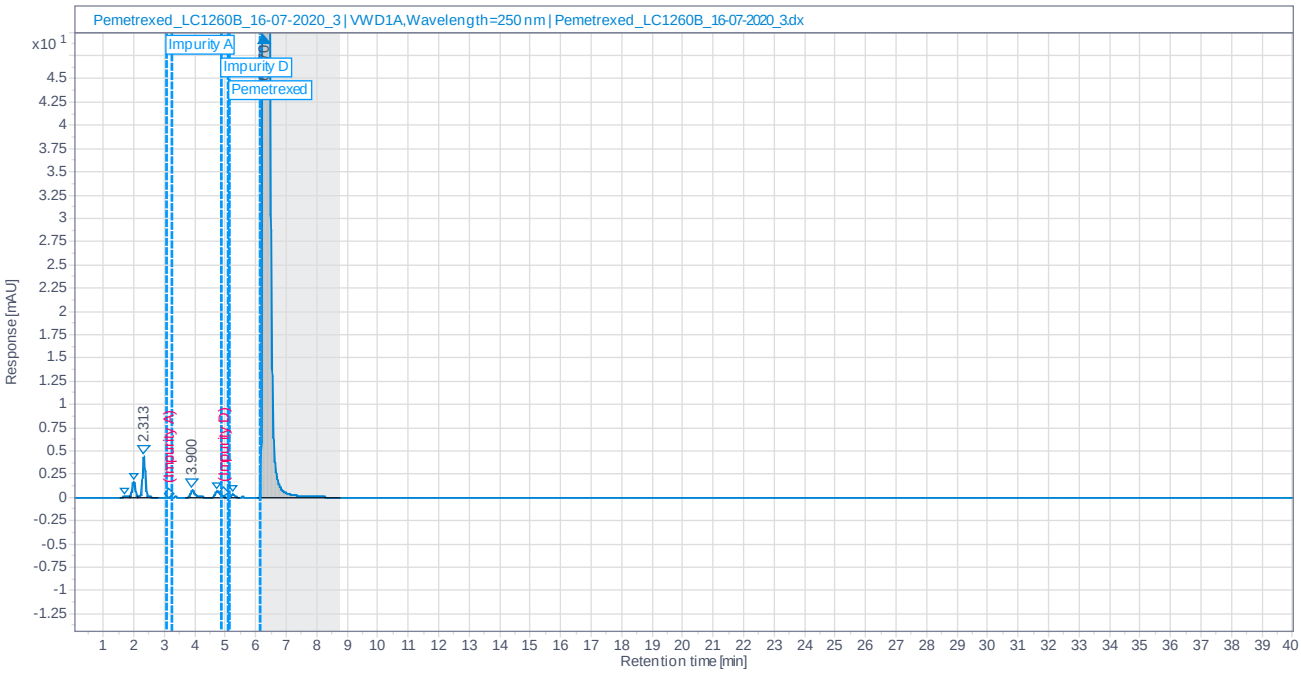
Appendix 10b: HPLC analysis – Chromatogram for 2mg/mL sample run for 40 minutes at end of study (enhanced view)



Appendix 11a: HPLC analysis – Chromatogram for 13.5mg/mL sample run for 40 minutes at end of study (normal view)



Appendix 11b: HPLC analysis – Chromatogram for 13.5mg/mL sample run for 40 minutes at end of study (enhanced view)



Appendix 12: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH - Assayed concentration of Pemetrexed (expressed as mg/100mL) - individual data from duplicate sample dilutions

Assayed concentration of Pemetrexed expressed as mg/100mL (individual data from duplicate sample dilutions from each bag)									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	(1) 204.2 (2) 206.8 $\bar{x}$: 205.5	(1) 207.9 (2) 207.3 $\bar{x}$: 207.6	(1) 206.5 (2) 206.0 $\bar{x}$: 206.3	(1) 205.5 (2) 204.7 $\bar{x}$: 205.1	(1) 205.6 (2) 205.2 $\bar{x}$: 205.4	(1) 204.9 (2) 204.8 $\bar{x}$: 204.9	(1) 205.3 (2) 204.5 $\bar{x}$: 204.9	(1) 204.7 (2) 204.4 $\bar{x}$:204.5
	B	(1) 198.8 (2) 198.4 $\bar{x}$: 198.6	(1)199.9 (2)199.8 $\bar{x}$: 199.8	(1) 198.4 (2) 198.3 $\bar{x}$: 198.4	(1) 198.1 (2) 197.6 $\bar{x}$: 197.8	(1) 196.6 (2) 198.2 $\bar{x}$: 197.4	(1) 196.5 (2) N/A ^ $\bar{x}$: 196.5	(1) 196.4 (2) 196.6 $\bar{x}$: 196.5	(1) 196.4 (2) N/A^ $\bar{x}$: 196.4
	C	(1) 197.3 (2) 197.0 $\bar{x}$: 197.1	(1)198.3 (2)198.0 $\bar{x}$: 198.1	(1) 197.1 (2) 197.1 $\bar{x}$: 197.1	(1) 196.5 (2) 196.2 $\bar{x}$: 196.4	(1) 196.3 (2) 195.7 $\bar{x}$: 196.0	(1) 195.8 (2) 195.0 $\bar{x}$: 195.4	(1) 194.9 (2) 194.5 $\bar{x}$: 194.7	(1) 195.0 (2) N/A^ $\bar{x}$: 195.0
13.5mg/mL	**A**	(1) 1363 (2) 1359 $\bar{x}$: 1361	(1) 1360 (2) 1363 $\bar{x}$: 1361	(1) 1355 (2) 1350 $\bar{x}$: 1353	(1) 1346 (2) 1344 $\bar{x}$: 1345	(1) 1317 (2) 1329 $\bar{x}$: 1323	(1) 1329 (2) 1332 $\bar{x}$: 1330	(1) 1331 (2) 1325 $\bar{x}$: 1328	(1) 1328 (2) 1328 $\bar{x}$:1328
	B	(1) 1333 (2) 1334 $\bar{x}$:1333	(1) 1331 (2) 1331 $\bar{x}$: 1331	(1) 1324 (2) 1321 $\bar{x}$: 1322	(1) 1313 (2) 1297 $\bar{x}$: 1305	(1) 1301 (2) 1302 $\bar{x}$: 1302	(1) 1304 (2) 1302 $\bar{x}$: 1303	(1) 1296 (2) 1296 $\bar{x}$: 1296	(1) 1290 (2) 1298 $\bar{x}$: 1294
	C	(1) 1377 (2) 1373 $\bar{x}$: 1375	(1) 1373 (2) 1371 $\bar{x}$: 1372	(1) 1365 (2) 1361 $\bar{x}$: 1363	(1) 1350 (2) 1354 $\bar{x}$: 1352	(1) 1344 (2) 1344 $\bar{x}$: 1344	(1) 1347 (2) 1343 $\bar{x}$: 1345	(1) 1338 (2) 1335 $\bar{x}$: 1337	(1) 1336 (2) 1339 $\bar{x}$: 1338

^ Injection error resulted in sample replicate failing to inject correctly (see Deviation report number 113)

Appendix 13: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH - Assayed concentration of Pemetrexed (expressed as % of initial assay at T=0) – individual data from duplicate sample dilutions

Assayed concentration of Pemetrexed expressed as % of initial assay at T=0 (individual data from duplicate sample dilutions from each bag)									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	(1) 99.4 (2) 100.6 $\bar{x}$: 100.0	(1) 101.2 (2) 100.9 $\bar{x}$: 101.0	(1) 100.5 (2) 100.2 $\bar{x}$: 100.4	(1) 100.0 (2) 99.6 $\bar{x}$: 99.8	(1) 100.0 (2) 99.9 $\bar{x}$: 100.0	(1) 99.7 (2) 99.7 $\bar{x}$: 99.7	(1) 99.9 (2) 99.5 $\bar{x}$: 99.7	(1) 99.6 (2) 99.5 $\bar{x}$: 99.5
	B	(1) 100.1 (2) 99.9 $\bar{x}$: 100.0	(1) 100.7 (2) 100.6 $\bar{x}$: 100.6	(1) 99.9 (2) 99.8 $\bar{x}$: 99.9	(1) 99.7 (2) 99.5 $\bar{x}$: 99.6	(1) 99.0 (2) 99.8 $\bar{x}$: 99.4	(1) 98.9 (2) N/A ^ $\bar{x}$:98.9	(1) 98.9 (2) 99.0 $\bar{x}$: 98.9	(1) 98.9 (2) N/A^ $\bar{x}$: 98.9
	C	(1) 100.1 (2) 100.0 $\bar{x}$: 100.0	(1) 100.6 (2) 100.5 $\bar{x}$: 100.5	(1) 100.0 (2) 100.0 $\bar{x}$: 100.0	(1) 99.7 (2) 99.5 $\bar{x}$: 99.6	(1) 99.6 (2) 99.3 $\bar{x}$: 99.4	(1) 99.3 (2) 98.9 $\bar{x}$: 99.1	(1) 98.9 (2) 98.7 $\bar{x}$: 98.8	(1) 98.9 (2) N/A^ $\bar{x}$: 98.9
13.5mg/mL	**A**	(1) 100.2 (2) 99.9 $\bar{x}$: 100.0	(1) 99.9 (2) 100.1 $\bar{x}$: 100.0	(1) 99.6 (2) 99.2 $\bar{x}$: 99.4	(1) 98.9 (2) 98.8 $\bar{x}$: 98.8	(1) 96.8 (2) 97.6 $\bar{x}$: 97.2	(1) 97.6 (2) 97.9 $\bar{x}$: 97.7	(1) 97.8 (2) 97.4 $\bar{x}$: 97.6	(1) 97.6 (2) 97.6 $\bar{x}$: 97.6
	B	(1) 100.0 (2) 100.1 $\bar{x}$:100.0	(1) 99.8 (2) 99.8 $\bar{x}$: 99.8	(1) 99.3 (2) 99.1 $\bar{x}$: 99.2	(1) 98.5 (2) 97.3 $\bar{x}$: 97.9	(1) 97.6 (2) 97.7 $\bar{x}$: 97.7	(1) 97.8 (2) 97.7 $\bar{x}$: 97.7	(1) 97.2 (2) 97.2 $\bar{x}$: 97.2	(1) 96.8 (2) 97.4 $\bar{x}$: 97.1
	C	(1) 100.1 (2) 99.9 $\bar{x}$: 100.0	(1) 99.9 (2) 99.7 $\bar{x}$: 99.8	(1) 99.3 (2) 99.0 $\bar{x}$: 99.1	(1) 98.2 (2) 98.5 $\bar{x}$: 98.3	(1) 97.7 (2) 97.7 $\bar{x}$: 97.7	(1) 98.0 (2) 97.7 $\bar{x}$:97.8	(1) 97.3 (2) 97.1 $\bar{x}$: 97.2	(1) 97.2 (2) 97.4 $\bar{x}$: 97.3

^ Injection error resulted in sample replicate failing to inject correctly (see Deviation report number 113)

Appendix 14: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Related Substances: Total secondary peak areas (expressed as % of total peak area) – individual data from duplicate sample dilutions

Related Substances: Total secondary peak areas expressed as % total peak area (individual data from duplicate sample dilutions from each bag)									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	(1) 0.00[#] (2) 0.06 $\bar{x}$: 0.06[##]	(1) 0.06 (2) 0.06 $\bar{x}$: 0.06	(1) 0.17 (2) 0.15 $\bar{x}$: 0.16	(1) 0.38 (2) 0.31 $\bar{x}$: 0.35	(1) 0.60 (2) 0.64 $\bar{x}$: 0.62	(1) 0.63 (2) 0.62 $\bar{x}$: 0.63	(1) 0.63 (2) 0.63 $\bar{x}$: 0.63	(1) 0.71 (2) 0.69 $\bar{x}$:0.70
	B	(1) 0.11 (2) 0.00[#] $\bar{x}$: 0.11[##]	(1) 0.06 (2) 0.06 $\bar{x}$: 0.06	(1) 0.17 (2) 0.17 $\bar{x}$: 0.17	(1) 0.55 (2) 0.54 $\bar{x}$:0.55	(1) 0.78 (2) 0.81 $\bar{x}$: 0.80	(1) 0.93 (2) N/A ^ $\bar{x}$: 0.93	(1) 0.93 (2) 0.95 $\bar{x}$: 0.94	(1) 1.01 (2) N/A^ $\bar{x}$: 1.01
	C	(1) 0.08 (2) 0.05 $\bar{x}$: 0.07	(1) 0.07 (2) 0.07 $\bar{x}$: 0.07	(1) 0.17 (2) 0.17 $\bar{x}$: 0.17	(1) 0.54 (2) 0.54 $\bar{x}$: 0.54	(1) 0.79 (2) 0.85 $\bar{x}$: 0.82	(1) 0.91 (2) 0.93 $\bar{x}$: 0.92	(1) 0.87 (2) 0.86 $\bar{x}$: 0.87	(1) 1.02 (2) N/A^ $\bar{x}$: 1.02
13.5mg/mL	**A**	(1) 0.06 (2) 0.05 $\bar{x}$: 0.06	(1) 0.11 (2) 0.11 $\bar{x}$: 0.11	(1) 0.46 (2) 0.46 $\bar{x}$: 0.46	(1) 0.94 (2) 0.96 $\bar{x}$: 0.95	(1) 1.36 (2) 1.40 $\bar{x}$: 1.38	(1) 1.70 (2) 1.66 $\bar{x}$: 1.68	(1) 1.69 (2) 1.68 $\bar{x}$: 1.69	(1) 1.92 (2) 1.93 $\bar{x}$:1.93
	B	(1) 0.00[#] (2) 0.00[#] $\bar{x}$: 0.00[#]	(1) 0.10 (2) 0.11 $\bar{x}$: 0.11	(1) 0.60 (2) 0.59 $\bar{x}$: 0.60	(1) 1.00 (2) 0.99 $\bar{x}$: 1.00	(1) 1.44 (2) 1.46 $\bar{x}$: 1.45	(1) 1.71 (2) 1.73 $\bar{x}$: 1.72	(1) 1.77 (2) 1.76 $\bar{x}$: 1.77	(1) 1.99 (2) 1.99 $\bar{x}$: 1.99
	C	(1) 0.05 (2) 0.05 $\bar{x}$: 0.05	(1) 0.12 (2) 0.16 $\bar{x}$: 0.14	(1) 0.52 (2) 0.51 $\bar{x}$: 0.52	(1) 0.94 (2) 0.94 $\bar{x}$: 0.94	(1) 1.35 (2) 1.47 $\bar{x}$: 1.41	(1) 1.65 (2) 1.64 $\bar{x}$: 1.65	(1) 1.68 (2) 1.69 $\bar{x}$: 1.69	(1) 1.91 (2) 1.90 $\bar{x}$: 1.91

[#] No secondary peaks present with areas > minimum area (0.05% of lowest Pemetrexed peak area recorded in Standard 2 chromatograms)

[##] Where one of the duplicate sample results returned a result of 0.00%, the mean result reported is the result returned for the other dilution for that sample

^ Injection error resulted in sample replicate failing to inject correctly (see Deviation report number 113)

Appendix 15: Pemetrexed 2mg/mL and 13.5mg/mL in 0.9%w/v Sodium Chloride stored at 2-8°C for 28 days plus an additional 6 hours and 24 hours storage at 25°C/60%RH – Related Substances: Highest individual secondary peak areas (expressed as % of total peak area) – individual data from duplicate sample dilutions

Related Substances: Highest individual secondary peak areas expressed as % total peak area (individual data from duplicate sample dilutions from each bag)									
Sample concentration	**Bag ID**	**Time point (days) / Storage Conditions**							
		0	**2 (2-8°C)**	**7 (2-8°C)**	**15 (2-8°C)**	**23 (2-8°C)**	**28 (2-8°C)**	**28 (2-8°C) + 6hrs at 25°C/60% RH**	**28 (2-8°C) + 24hrs at 25°C/60% RH**
2mg/mL	**A**	(1) 0.00[#] (2) 0.06 $\bar{x}$: 0.06[##]	(1) 0.06 (2) 0.06 $\bar{x}$: 0.06	(1) 0.10 (2) 0.10 $\bar{x}$: 0.10	(1) 0.14 (2) 0.14 $\bar{x}$: 0.14	(1) 0.21 (2) 0.22 $\bar{x}$: 0.22	(1) 0.24 (2) 0.23 $\bar{x}$: 0.24	(1) 0.24 (2) 0.24 $\bar{x}$: 0.24	(1) 0.27 (2) 0.26 $\bar{x}$:0.27
	B	(1) 0.06 (2) 0.00[#] $\bar{x}$: 0.06[##]	(1) 0.06 (2) 0.06 $\bar{x}$: 0.06	(1) 0.12 (2) 0.12 $\bar{x}$: 0.12	(1) 0.21 (2) 0.21 $\bar{x}$:0.21	(1) 0.33 (2) 0.33 $\bar{x}$: 0.33	(1) 0.39 (2) N/A ^ $\bar{x}$: 0.39	(1) 0.39 (2) 0.40 $\bar{x}$: 0.40	(1) 0.42 (2) N/A^ $\bar{x}$: 0.42
	C	(1) 0.08 (2) 0.05 $\bar{x}$: 0.07	(1) 0.07 (2) 0.07 $\bar{x}$: 0.07	(1) 0.12 (2) 0.12 $\bar{x}$: 0.12	(1) 0.21 (2) 0.21 $\bar{x}$: 0.21	(1) 0.33 (2) 0.34 $\bar{x}$: 0.34	(1) 0.38 (2) 0.39 $\bar{x}$: 0.39	(1) 0.39 (2) 0.39 $\bar{x}$: 0.39	(1) 0.42 (2) N/A^ $\bar{x}$: 0.42
13.5mg/mL	**A**	(1) 0.06 (2) 0.05 $\bar{x}$: 0.06	(1) 0.11 (2) 0.11 $\bar{x}$: 0.11	(1) 0.23 (2) 0.23 $\bar{x}$: 0.23	(1) 0.42 (2) 0.42 $\bar{x}$: 0.42	(1) 0.63 (2) 0.64 $\bar{x}$: 0.64	(1) 0.78 (2) 0.77 $\bar{x}$: 0.78	(1) 0.79 (2) 0.79 $\bar{x}$: 0.79	(1) 0.89 (2) 0.88 $\bar{x}$: 0.89
	B	(1) 0.00[#] (2) 0.00[#] $\bar{x}$: 0.00[#]	(1) 0.10 (2) 0.11 $\bar{x}$: 0.11	(1) 0.25 (2) 0.26 $\bar{x}$: 0.26	(1) 0.42 (2) 0.45 $\bar{x}$: 0.44	(1) 0.66 (2) 0.67 $\bar{x}$: 0.67	(1) 0.80 (2) 0.80 $\bar{x}$: 0.80	(1) 0.82 (2) 0.82 $\bar{x}$: 0.82	(1) 0.91 (2) 0.92 $\bar{x}$: 0.92
	C	(1) 0.05 (2) 0.05 $\bar{x}$: 0.05	(1) 0.12 (2) 0.11 $\bar{x}$: 0.12	(1) 0.24 (2) 0.24 $\bar{x}$: 0.24	(1) 0.42 (2) 0.42 $\bar{x}$: 0.42	(1) 0.62 (2) 0.65 $\bar{x}$: 0.64	(1) 0.76 (2) 0.77 $\bar{x}$: 0.77	(1) 0.78 (2) 0.78 $\bar{x}$: 0.78	(1) 0.87 (2) 0.87 $\bar{x}$: 0.87

[#] No secondary peaks present with areas > minimum area (0.05% of lowest Pemetrexed peak area recorded in Standard 2 chromatograms)

[##] Where one of the duplicate sample results returned a result of 0.00%, the mean result reported is the result returned for the other dilution for that sample

^ Injection error resulted in sample replicate failing to inject correctly (see Deviation report number 113)

Appendix 16: Further Information with regard to related substances levels

There is no BP monograph for Pemetrexed finished product (Pemetrexed for Injection). The BP 2020 monograph for Pemetrexed Disodium Heptahydrate raw material[11] states the following acceptance criteria;

- Pemetrexed Impurities A and D: ≤ 0.15% of Pemetrexed peak area
- Other unspecified impurities: ≤ 0.10% of Pemetrexed peak area
- Total: ≤ 0.6% of Pemetrexed peak area

There is a USP monograph for both Pemetrexed Disodium raw material[16] and for Pemetrexed for Injection[17] (powder for reconstitution) which contain the following acceptance criteria for related substances levels;

USP 2020 related substances limits for Pemetrexed Disodium raw material

- N-Methyl Pemetrexed: ≤ 0.15%
- Pemetrexed Glutamide: ≤ 0.15%
- Any individual unspecified impurity: ≤ 0.10%
- Total Impurities: ≤ 0.60%

USP 2020 related substances limits for Pemetrexed for Injection

- Ketopemetrexed: ≤ 0.60% (relative retention time with respect to Pemetrexed = 0.31; relative response factor = 0.61)
- Any individual unspecified impurity: ≤ 0.24% (relative response factor = 1.0)
- Total Impurities: ≤ 1.30%

In the samples under test within this report, at each time point, the highest individual secondary peak was seen to elute at a relative retention time of ~0.4 with respect to Pemetrexed, this is similar to that stated for the principal degradant, Ketopemetrexed, in the USP monograph for Pemetrexed for Injection. Despite differences in chromatographic conditions used within this study compared against those listed in the USP monograph, the data for the highest individual secondary peak has been assessed against the limits given for Ketopemetrexed.

2mg/mL samples remain compliant with the USP related substances limits for Pemetrexed for Injection for the recommended shelf life of 21 days stored at 2-8°C.

For the 13.5mg/mL samples, when accounting for the relative response factor for Ketopemetrexed, the highest individual secondary peak area is just above the acceptance limit of 0.60% and total peak areas would be close to the acceptance limit of 1.3%, following 15 days storage at 2-8°C (Highest individual secondary peak area across all 13.5mg/mL samples = 0.72%; Highest total secondary peak areas across all 13.5mg/mL samples = 1.28% when accounting for relative response factor for Ketopemetrexed). There were no other secondary peaks with areas > 0.24% of the total peak area, following 15 days storage at 2-8°C.

Appendix 16 (cont.): Further Information with regard to related substances levels

During 6 hours storage at 25°C/60%RH, we observed the following increases in related substances levels:

2mg/mL:
Largest individual peak increase by 0.01%.
Total increases by 0.01%

13.5mg/mL:
Largest individual peak increases by 0.02%.
No significant change in other secondary peaks with areas > 0.24% of total peak area.
Total increase by 0.05%

During 24 hours storage at 25°C/60%RH, we observed the following increases in related substances levels:

2mg/mL:
Largest individual peak increase by 0.03%.
Total increases by 0.10%

13.5mg/mL:
Largest individual peak increases by 0.12%.
Number of other secondary peaks with areas > 0.24% of total area increased from 1 to 3.
Total increase by 0.27%.

There is no significant change in the related substances levels following 6 hours storage at 25°C/60 %RH. The SmPC for the Accord Healthcare brand of Pemetrexed powder for concentrate for solution for infusion (this brand comprises Pemetrexed disodium salt as well as equivalent excipients to the ALIMTA brand in Mannitol, Sodium Hydroxide and Hydrochloric Acid), states that, the chemical and physical in-use stability of reconstituted and infusion solutions of Pemetrexed were demonstrated for 24 hours at 25°C[14]. The infusion time stated for Pemetrexed preparations is 10 minutes[13,14].

A further review of the related substances data, has been performed by Mark Santillo (Regional Quality Assurance Pharmacist (SW England) Chair of NHS Pharmaceutical Research and Development Group[18]. This review offers justification for acceptance of related substances levels which exceed those stated in the USP monograph for Pemetrexed for Injection and also those in ICH Q3(B)[19] .

A shelf life based on the data for appearance, pH, sub-visible particles and assay would justify a shelf life of 28 days stored at 2-8°C (protected from light). However, aseptic units are advised to consider the increase in related substance levels and apply a reduced shelf life of a maximum of 21 days stored at 2-8°C (protected from light).