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Shelf Life Report for NHS Scotland

Stability Study for
Piperacillin/Tazobactam 56.9 mg/mL in Glucose 5.0% w/v in
50mL Infusion Bags

Report Reference: SCN-2020-04v2
Version: 2
Date of Issue: 17th December 2020
Supersedes: SCN-2020-04
Pages: 15 + appendices

Contents:

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1. PURPOSE OF STUDY

Stability testing of Piperacillin/Tazobactam diluted in 5.0%w/v Glucose 50mL infusion bags to give a final concentration of 56.9mg/mL was conducted to assess the chemical and physical stability. This study aimed to provide information with regards to shelf-life, storage condition and in-use period in accordance with the Yellow Cover Document for Small Molecules[1]. The stability study was carried out by Quality Control North West (QCNW) Stockport on behalf of NHS Scotland.

2. MATERIALS

Piperacillin/Tazobactam 4.5g Powder for Solution injection
Manufacturer: Bowmed Ibisqus Ltd
Batch Number: 9H75TR
Expiry: May 2022

Glucose 5.0% w/v 50mL Viaflo Infusion Bag
Manufacturer: Baxter Healthcare Ltd.
Batch Number: 19K27G62
Expiry: January 2021

Water for Injection
Manufacturer: Fresenius Kabi
Batch Number: 20NGI002
Expiry: June 2021

3. SAMPLE PREPARATION

3.1 Refrigerated Study

Samples were prepared in at QCNW Stockport on the initial day of the study under normal laboratory conditions. Using a needle and syringe, 16.5mL of water for injection was injected directly into the Piperacillin/Tazobactam vial (4.5 g) to reconstitute the contents. The vial was shaken until the powder was completely dissolved. The whole contents of the vial were then withdrawn and injected into the infusion bag via the port. No overages were removed and the final volume of the bag was approximately 79mL. The bags were inverted several times to ensure a homogenous solution was achieved. Bags were prepared with overwrap intact. A total of six bags were prepared in this way; bags 1, 2 and 3 for assay, appearance and pH analysis and an additional three bags prepared for particle count testing. A placebo bag was also prepared without the addition of the active ingredient.

3.2 In Use Study

Three additional sample bags were prepared midway through the refrigerated study under the same conditions and following the same method and technique as described in section 3.1. Bag A was analysed for assay, appearance and pH, with bags B and C prepared for particle count testing.

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

4. STORAGE AND SAMPLING PROTOCOL

4.1 Refrigerated Study

Initial time point samples were removed immediately after preparation. Bags were then stored at 5°C ± 3°C and samples removed from each of the three bags at multiple time points up to 25 days. At each time point, samples were tested for pH, appearance, Piperacillin and Tazobactam assays and monitoring of degradant peaks. At time 0, 7 and 14 days sub-visible particle matter was also tested. Approximately 5mL was removed from the bag at each time point to prepare assay samples. A further 2mL removed for pH recording.

Upon reviewing the stability of the product following the originally proposed final time point (14 days), it was agreed with the client that the study would be extended. Two additional time points of 17 and 25 days were added to the study. However, the 25 day time point showed erratic assay results for Tazobactam and was therefore removed from the stability assessment following an Out Of Trend (OOT) investigation performed in accordance with internal procedures.

4.2 In Use Study

Initial time point samples were removed immediately after preparation from bag A, as well as the initial particle count bag being analysed. Bags A and C were then stored at 5°C ± 3°C until the 17 day time point. At the 17 day time point bag A was removed from storage for assay, appearance and pH analysis. Bags A and C were transferred to and incubator for 3 hours at 25ºC/60%RH. Following storage at room temperature bag A was removed for assay, appearance and pH analysis and particle count performed on bag C.

5. METHODS

5.1 HPLC Assay

Piperacillin and Tazobactam were analysed using a validated reverse phase high performance liquid chromatography (HPLC) method, using an Agilent 1100 series HPLC system with UV detector. Chromatographic results were obtained using Agilent Technologies data handling software OpenLab CDS.

Chromatographic separation was performed at 25ºC on a reversed phase Hypersil ODS C18 column (250mm x 4mm) 5um particle size (R208) fitted with a C18 guard column. Elution was obtained using a mobile phase composition of 91% mobile phase A to 9% mobile phase B.

Mobile phase A was prepared by dissolving approximately 6.24g sodium dihydrogen phosphate and 1.92g tetrabutylammounium hydroxide-30-hydrate in 800mL HPLC grade water, adjusting the pH of solution to 5.5 with phosphoric acid and adding 200mL of HPLC grade acetonitrile.

Mobile phase B was prepared by dissolving approximately 6.24g sodium dihydrogen phosphate and 1.92g tetrabutylammounium hydroxide-30-hydrate in 350mL HPLC grade water, adjusting the pH of solution to 5.5 with phosphoric acid and adding 650mL of HPLC grade acetonitrile.

Eluent flow rate was set at 1.0mL per minute, detection wavelength set to 220 nm and an injection volume of 10µL was used. Vials for analysis were maintained at a temperature of 5ºC.

5.1.1 Standard Preparation

Standard solutions were used to quantify sample concentrations. They were prepared at each time point using Piperacillin/Tazobactam 4.0/0.5 g powder vial (Manufacturer: Bowmed Ibisqus Ltd, Batch Number: 9H75TR, Expiry: May 2022). The same vial was used throughout the study and stored at 2-8ºC between time points.

Piperacillin/Tazobactam weights of approximately 100mg (stock solution 1) and approximately 125mg (stock standard 2) were transferred into 50mL volumetric flasks and diluted to volume with diluent (NaH_2PO_4 buffer in 75% water and 25% acetonitrile) to give stock standard concentrations of approximately 1.8 and 2.1 mg/mL with regards to Piperacillin, and 0.2 and 0.3 mg/mL with regards to Tazobactam. Working standards 1 and 2 were prepared by pipetting 5.0mL of the respective stock solution into 20mL volumetric flasks and diluting to volume with diluent.

5.1.2 Sample Preparation

At each time point, infusion bags were removed from the refrigerator and left to equilibrate at room temperature. Samples were prepared in duplicate from bags 1, 2 and 3 and in singular from the placebo bag. Following sampling, bags were returned to the refrigerator as soon as possible.

Samples were prepared by withdrawing approximately 5mL from the individual bags, following mixing via inversion. Sampling was conducted using a needle and syringe, piercing through the overwrap bag and into the port to withdraw the required volume of sample solution. Samples were diluted 2.0mL to 200mL with diluent.

In-use bag A, was sampled in line with the procedure used for bags 1, 2 and 3 of the refrigerated study at the in-use study time points.

5.2 pH Measurement

The pH was recorded using a calibrated pH meter. The pH meter was calibrated daily before use at pH 4, pH 7 and pH 10. pH testing of bags 1, 2, 3 and placebo were recorded at every time point. Approximately 2mL of sample was withdrawn from the infusion bag via syringe and needle and transferred to a plastic test tube. The temperature of the sample was recorded using a calibrated thermometer prior to pH readings.

In-use bag A, was analysed in line with the procedure used for bags 1, 2 and 3 of the refrigerated study at the in-use study time points.

5.3 Appearance

Appearance testing of bags 1, 2, 3 and placebo was performed at all time points with infusion bags remaining intact. The product was viewed through the infusion bag against black and white backgrounds under appropriate lighting. Samples were observed for colour, clarity and particle formation.

In-use bag A, was analysed in line with the procedure used for bags 1, 2 and 3 of the refrigerated study at the in-use study time points.

5.4 Particle Count

Particle count was performed using a PAMAS SVSS liquid particle counting system. Prior to analysis of samples an Internal Quality Control (IQC) was performed using sterile water for irrigation to ensure that the environment and equipment were appropriate for use.

Particle count was performed at three time points (Initial, 7 days and 14 days). Samples were removed from the refrigerator and inverted 20 times to ensure homogeneity. The corner of the infusion bag was then cut open to allow the sampling tube to be submerged into the solution. An initial rinse was performed prior to analysis to remove any remaining water within the tube from the IQC.

Analytical parameters were set up to perform 5 repeated measurements, with each measurement sampling 5mL of solution.

In-use bags B and C, were analysed in line with the procedure used for bags of the refrigerated study at the initial and final in use time points.

6. VALIDATION

The method was quantitatively validated by standard and sample repeatability. The method was also validated qualitatively by using forced degradation, selectivity studies. All validation performed on HPLC instrument Chrom 7 with column R208.

6.1 PRECISION

6.1.1 Standard Repeatability

Two separate Piperacillin/Tazobactam standards were prepared following the procedure stated in section 5.1.1. Standard precision was established by injecting each standard six times. The results tabulated below were obtained:

Piperacillin

The % relative standard deviation (%RSD) n=6, for each standard was found to be within a laboratory expectation of 2.0%. The response factor (RF), chromatographic peak area normalized to standard concentration, agreement between the two standards was within the laboratory expectation of 2.5%

	Concentration (mg/mL)	**%RSD** Limit = 2.0%	**RF**
Standard 1 (n=6)	1.7998	0.2	1800.05
Standard 2 (n=6)	2.1626	0.1	1784.49
		RF%RSD Limit = 2.5%	0.6%

Tazobactam

The %RSD n=6, for each standard was found to be within a laboratory expectation of 2.0%. However, agreement between standard response factors was significantly poorer than the laboratory expectation of 2.5%

	Concentration (mg/mL)	**%RSD** Limit = 2.0%	**RF**
Standard 1 (n=6)	0.2250	0.3	1034.35
Standard 2 (n=6)	0.2704	0.2	1098.11
		RF%RSD Limit = 2.5%	4.2%

Due to the nature of the standard material used (a blended mix of 0.5g Tazobactam and 4.0g Piperacillin) response factor repeatability proved challenging with regards to Tazobactam. The small composition of Tazobactam meant that only ~13mg of Tazobactam was present in each weighed standard. Any small inhomogeneity of the powder is likely to have a larger impact in the Tazobactam repeatability than Piperacillin. It is normally expected that response factor agreements are better than 2.5%. It is evident that this value was easily achieved for Piperacillin, but not for Tazobactam. This represents a limitation of the method which is unavoidable due to the unavailability of a reference Tazobactam standard.

6.1.2 Sample Repeatability

Sample repeatability was not performed on 5.0% w/v Glucose solution infusion bags, and only performed on 0.9% w/v NaCl infusion bag samples. A single infusion bag was prepared following the procedure described in section 3 to give a final concentration of Piperacillin/Tazobactam 56.9 mg/mL in 0.9% w/v sodium chloride. Six samples preparations were performed by diluting 2.0mL sample to 200mL with diluent. Sample precision was established by injecting each of the six samples in duplicate. The %RSD was within the limits of 2.0%.

Sample	**Tazobactam Concentration (mg/mL)**	**Piperacillin Concentration (mg/mL)**
1	6.2928	52.6280
2	6.3288	52.8902
3	6.3201	52.7412
4	6.3290	52.8998
5	6.3297	52.8966
6	6.3575	53.1516
%RSD Limit = 2.0%	0.2	0.3

6.2 Selectivity

A forced degradation study of Piperacillin and Tazobactam was carried out to identify related substances for the respective analytes.

A standard containing only Piperacillin and a standard containing a mixture of Piperacillin/Tazobactam were heated at 40°C for 2 hours. Heating led to the formation of several forced degradant peaks. None of the additional peaks co-eluted with either analyte peak, allowing for both analytes remaining identifiable. By comparing the Piperacillin standard to the combined Piperacillin/Tazobactam standard, known degradant products could be assigned to either analyte via a process of elimination.

(See appendix 1 and 2 for chromatograms and table of degradant peaks)

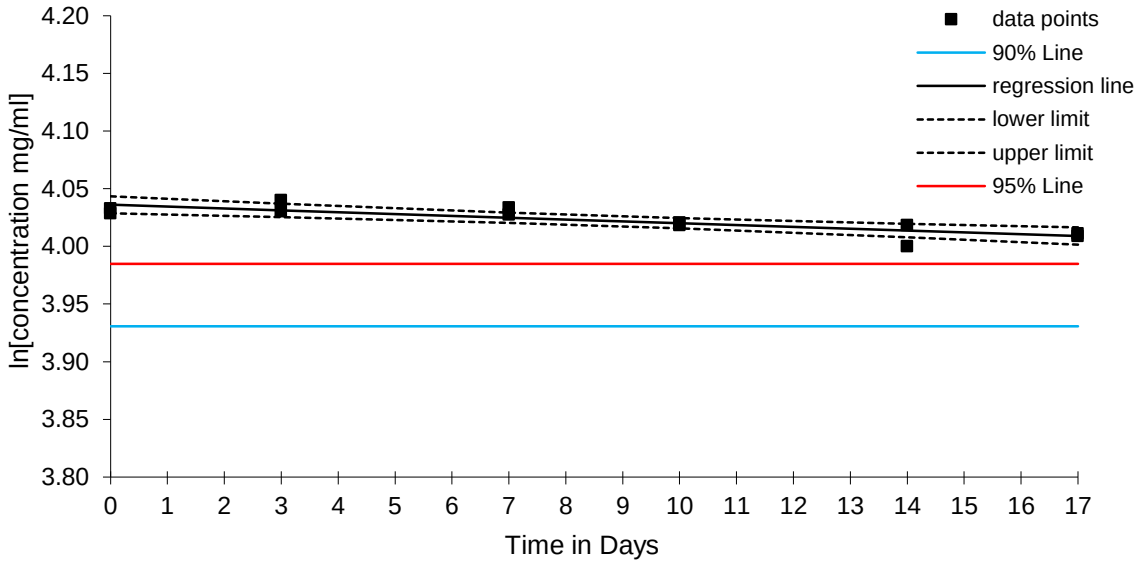
7. RESULTS – REFRIGERATED STUDY

The results for individual bags for the Piperacillin and Tazobactam assay content and pH are tabulated below. The Assay results are a mean of two samples and are expressed in terms of Piperacillin or Tazobactam concentration as a percentage of the initial assay (With the assay result obtained at time point zero set at 100.0). All subsequent data was calculated relative to the initial concentration of the respective bag.

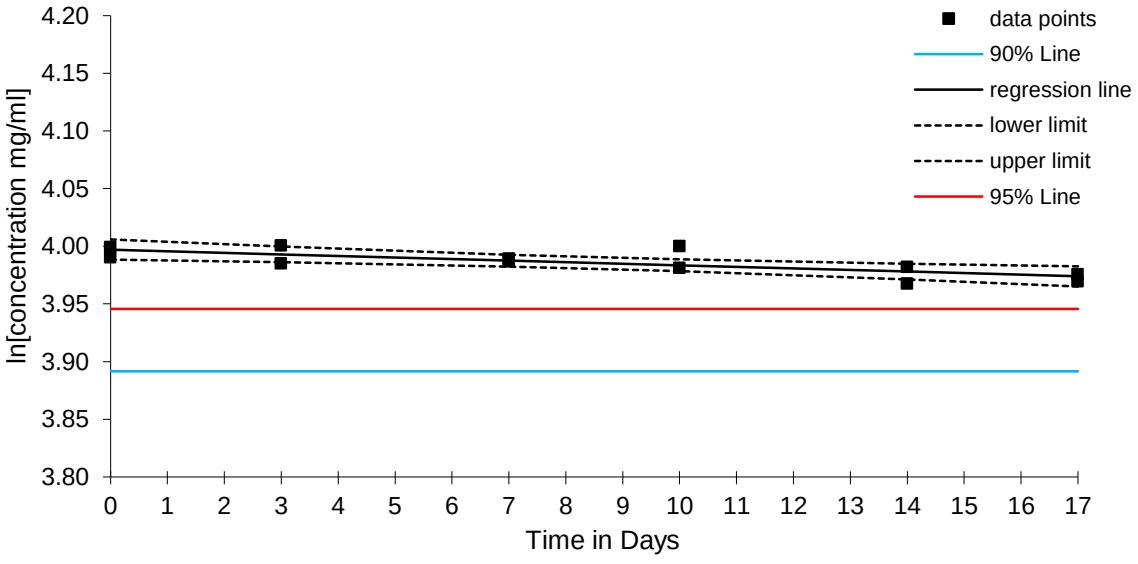
Time Point (Days)	Percentage of Initial (%) (mg/mL Piperacillin)			Percentage of Initial (%) (mg/mL Tazobactam)			pH		
	Bag 1	Bag 2	Bag 3	Bag 1	Bag 2	Bag 3	Bag 1	Bag 2	Bag 3
0	100.0 (56.30)	100.0 (54.31)	100.0 (53.90)	100.0 (6.83)	100.0 (6.62)	100.0 (6.13)	5.61	5.56	5.58
3	100.4	99.8	101.1	102.4	101.6	102.8	5.39	5.36	5.35
7	100.0	99.3	100.0	101.7	100.9	101.7	5.28	5.23	5.29
10	98.9	99.6	99.5	96.7	97.1	97.2	5.19	5.20	5.22
14	97.9	98.0	98.6	104.1	104.0	104.8	5.03	4.92	4.99
17	98.0	97.8	99.0	99.8	98.8	100.1	4.98	5.00	4.97

7.1.1 Piperacillin Stability Graphs

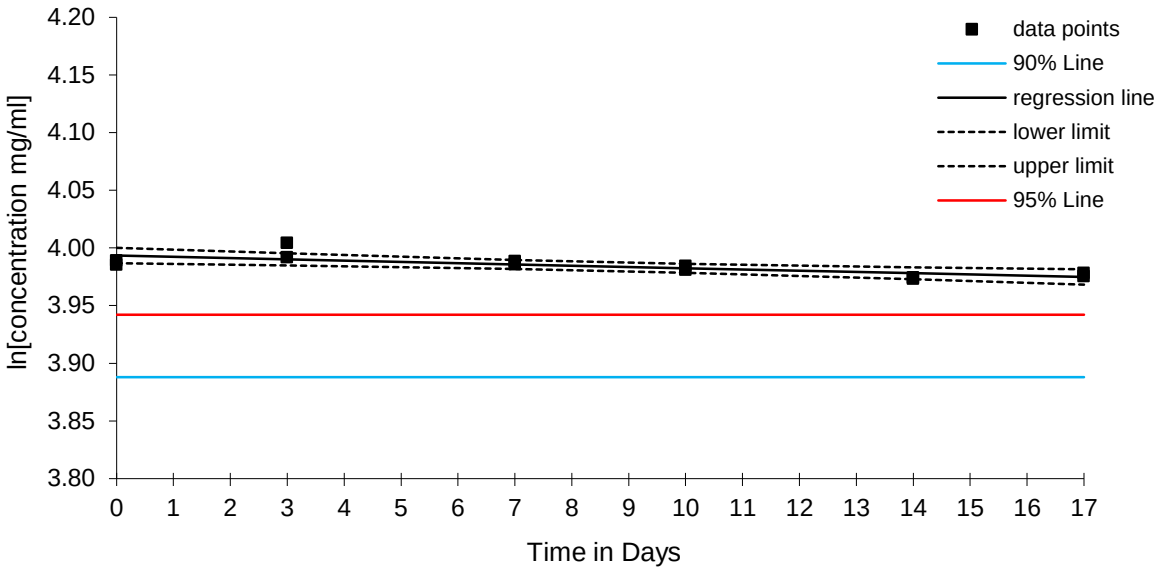
Piperacillin in 5.0 % Glucose Stability Graph - Bag 1



Piperacillin in 5.0 % Glucose Stability Graph - Bag 2



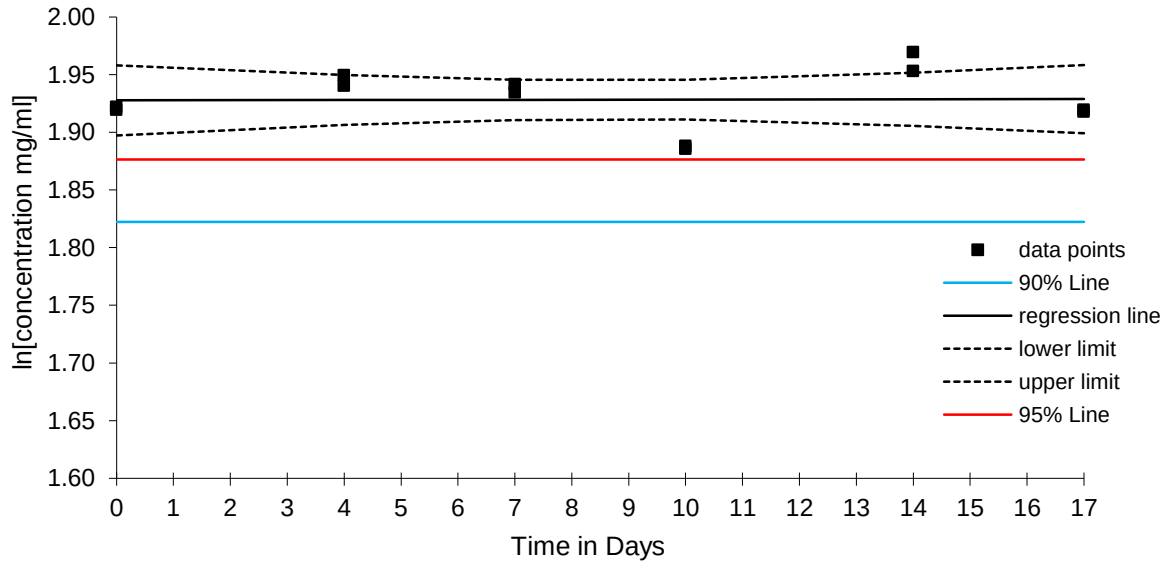
Piperacillin in 5.0 % Glucose Stability Graph - Bag_3



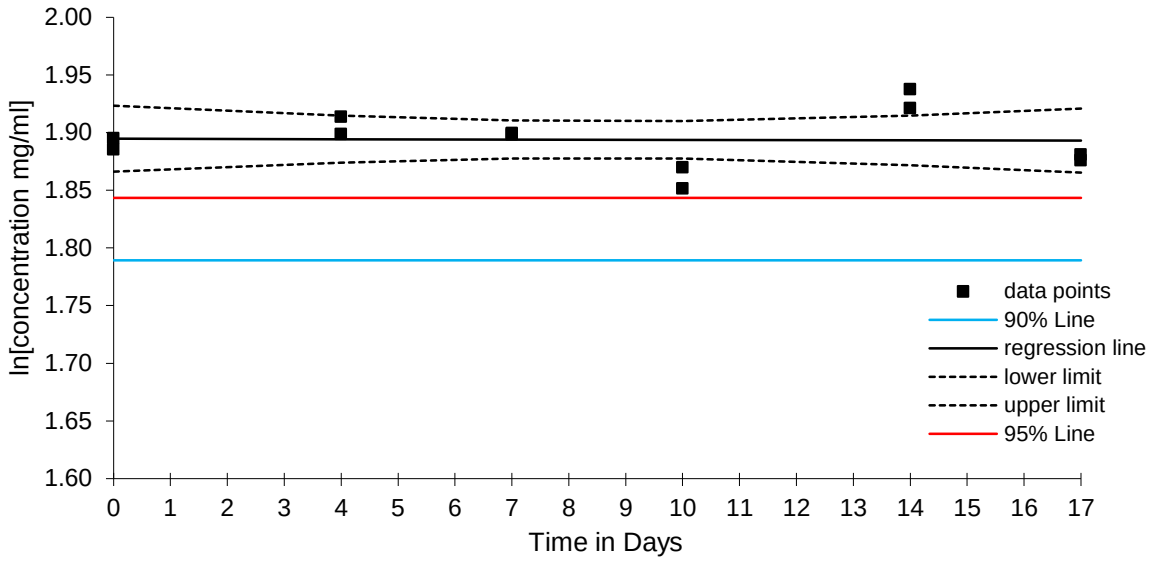
The estimated shelf life from the mean of three bags using the estimated maximum degradation rate method is 25.0 days (range 22.1 to 29.6 days)

7.1.2 Tazobactam Stability Graphs

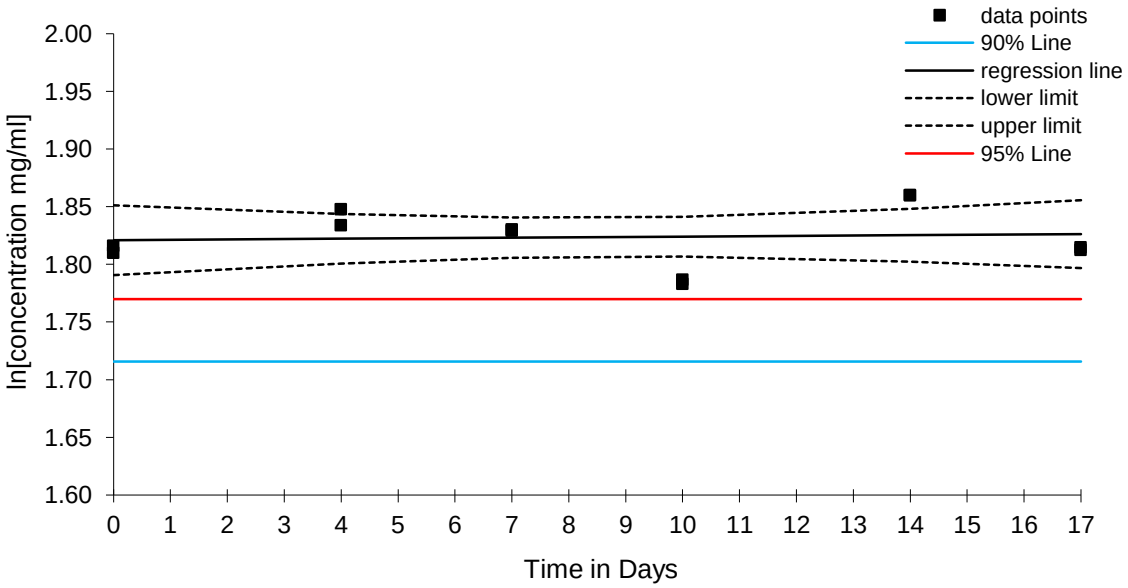
Tazobactam in 5.0 % Glucose Stability Graph - Bag_1



Tazobactam in 5.0 % Glucose Stability Graph - Bag 2



Tazobactam in 5.0 % Glucose Stability Graph - Bag 3



The estimated shelf life from the mean of three bags using the estimated maximum degradation rate method is 18.6 days (range 18.0 to 19.7 days)

7.1.3 Response factor (RF) agreements during the study

Time Point (Days)	RF RSD (%)	
	Tazobactam	Piperacillin
0	2.0	1.1
3	1.3	0.1
7	7.5	1.1
10	10.8	2.0
14	3.4	0.5
17	7.1	0.7

Due to the nature of the standard material (a blended mix of 0.5g Tazobactam and 4.0g of Piperacillin), RF repeatability proved challenging with regards to Tazobactam. The small composition of Tazobactam meant that only ~13mg of Tazobactam was present in each weighed sample. Any small inhomogeneity of the powder is likely to have a larger impact in the Tazobactam repeatability than Piperacillin. Consequently, the assay/time profiles show greater scatter and uncertainty for Tazobactam

7.2 Particle Count

Results were calculated per ml and converted to per container in line with the British Pharmacopeia requirement to report results for samples of 100ml or less as 'per container'.

Limits (per container): 10 µ = less than 6000 25 µ = less than 600

Time Point (Days)	Counts per mL		Counts per Container (79mL)	
	10 Micron	25 Micron	10 Micron	25 Micron
Initial	25	1	1940	52
7	23	1	1754	48
14	10	1	739	8

7.3 Appearance

The appearance of solutions in all bags remained clear, colourless and free from visible particulate matter up to day 10 of the study.

At day 14, one bag contained a single fibrous particle. At day 17, all bags contained visible particles. These particulates did not appear to be precipitate related to the active ingredients but are likely to be small particles of plastic from continuous sampling of the bags.

7.4 Degradation Products

Several related substance peaks were identified in the chromatograms from each bag from the initial time point. Four peaks were identified as likely degradation products from the selectivity studies conducted during method validation. These were labelled as Unknowns A-D. (*See appendix 1 and 2)*

Peaks labelled as Unknowns A, B, C2 and D were found to be likely piperacillin degradants. Unknown C1 was found to be a likely Tazobactam degradant. However, unknowns C1 and C2 co-eluted for the main part of the refrigerated study which made tracking of each difficult. During the analysis of the in-use bags (17 days and 17 days + 3 hours at room temperature) resolution was re-established and assessment of individual peak areas performed (Section 8.4)

The following table shows the change in peak area of likely Piperacillin degradants at day 7 and day 17 for bag 1. Results are the average of duplicate preparations.

Time Point	Peak Area	Peak Area (%)	Increase from Initial (%)
Unknown A : Relative to Piperacillin			
Initial	2.45	0.06	
7 days	6.38	0.16	+0.1
17 days	13.69	0.35	+0.3
Unknown B : Relative to Piperacillin			
Initial	3.92	0.10	
7 days	23.22	0.57	+0.5
17 days	52.91	1.34	+1.2
Unknown D : Relative to Piperacillin			
Initial	6.82	0.17	
7 days	10.93	0.27	+0.1
17 days	21.48	0.54	+0.4

(See appendix 3 and 4 for identification of degradant peaks at 0 and 17 days)

8. RESULTS – IN USE STUDY

Three additional infusion bags, A, B and C were prepared. Bag A was sampled at time zero and then 17 days after refrigerated storage. The bag was then placed at 25°C/60%RH for 3 hours to replicate an in-use bag at room temperature during infusion. Bag B was analysed for initial particle count and therefore not stored. Bag C was analysed for 17 days + 3 hours particle count.

8.1 In-Use Study - Assay and pH

Time Point	Piperacillin (mg/mL)	Tazobactam (mg/mL)	pH
17 days	54.45	6.48	4.96
17 days (+3 hours)	54.48	6.49	4.97

After 17 days refrigeration and a further 3 hours storage at room temperature (25ºC/60%RH), there was no significant change in assay or pH

8.2 In-Use Study - Particle Count

Time Point	Counts per mL		Counts per container (79mL)	
	10 Micron	**25 Micron**	**10 Micron**	**25 Micron**
17 days (+3 hours)	20	1	1580	32

Particle count data for bags stored for 17 days refrigerated and a further 3 hours storage at room temperature (25ºC/60%RH) demonstrated continued compliance with pharmacopeial limits for parenteral products.

8.3 In-Use Study - Appearance

The appearance of solutions in all bags remained clear, colourless and free from visible particulate matter up to day 17 of the study.

8.4 In-Use Study - Degradant Products

The following table shows the change in peak area of likely Piperacillin degradants at day 17 refrigerated storage and for a further 3 hours storage at room temperature from bag A. Results are the average of duplicate preparations

Peak areas relative to the Tazobactam peak for unknown C1 are also tabulated although no progression of this peak was observed with an unexplained reduction in peak area.

Time Point	Peak Area	Peak Area (%)	Increase from Initial (%)
Unknown A – Relative to Piperacillin			
Initial	5.01	0.12	
17 days	11.41	0.28	+ 0.2
17 days (+ 3 hours)	12.77	0.32	+0.2
Unknown B – Relative to Piperacillin			
Initial	6.29	0.15	
17 days	47.35	1.18	+1.0
17 days (+ 3 hours)	49.97	1.24	+1.1

Unknown C1 – Relative to Tazobactam			
Initial	15.87	5.48	
17 days	13.79	4.76	-0.7
17 days (+ 3 hours)	13.83	4.77	-0.9

Unknown C2 – Relative to Piperacillin			
Initial	15.95	0.39	
17 days	29.72	0.74	+0.4
17 days (+3 hours)	29.00	0.72	+0.3
Unknown D – Relative to Piperacillin			
Initial	6.41	0.16	
17 days	20.87	0.52	+0.4
17 days (+3 hours)	22.44	0.56	+0.4

The results show that the likely Piperacillin related substances determined from the HPLC chromatograms of bag A from the In-Use study are in broad agreement with those determined for bag 1 in the refrigerated study. The principal changes occur from the development of Unknown C and detectable changes in Unknown A, B and D. Storage for 3 hours at room temperature does not appear to cause any detectable changes in related substances.

The results suggest there is no significant formation of Tazobactam degradants for the duration of the study either under refrigerated or refrigerated plus room temperature storage

9. DISCUSSION

- The appearance of all three bags remained clear and colourless throughout the duration of the study. Some small particulates were obtained, however, these are thought to related to repeated piercing of the infusion port rather than deterioration of the product.

- Characteristic reduction in solution pH was observed, final pH values of 5.0 were obtained from a starting pH of 5.6.

- The HPLC method was validated to allow quantification of the two principal components Piperacillin and Tazobactam without interference from related compounds, including those resulting from degradation.

  A blended powder mix of Piperacillin and Tazobactam was used out of necessity for the HPLC analytical standard. Results showed that the repeatability for Tazobactam assays were higher than normally expected by HPLC analysis; this translated to more scatter in the regression plot for Tazobactam.

  However, a shelf life assessment was still allowed to be made based on a 95% confidence interval of the data. Based on assay data from Piperacillin and Tazobactam a shelf life of 17 days (refrigerated) + 3 hours (room temperature) is supported.

- The HPLC method was also used to track those related substances assumed to be degradation products, namely unknown A-D. Relatively small quantities of Piperacillin degradants were observed and quantified by their relative peak area to Piperacillin. However due to the absence of reference degradants, these could not be formally identified
- Particle count data for all infusion bags tested in the study demonstrated compliance with pharmacopoeial limits for parenteral products. No time related trends were observed.

10. CONCLUSION

In conclusion, Piperacillin/Tazobactam 56.9 mg/mL in Glucose 5.0%w/v in 50mL Baxter Viaflo bags exhibited chemical and physical stability under refrigerated storage conditions (2-8ºC) for up to 17 days. Chemical and Physical stability was also shown at 17 days 2-8ºC + 3 hours at 25ºC/60%RH. A risk based approach to shelf life allocation in relation to related substances/ degradation products is advised.

11. CHANGE CONTROL

Document Reference	Date	Description of Change
SCN-2020-04	21/05/2020	Initial Version
SCN-2020-04v2	17/12/2020	Removal of 'Private and Confidential' at request of client

12. APPENDICES

APPENDIX 1 – Standard Selectivity (Piperacillin only)

Chromatogram of Piperacillin forced degradation (2 hours at 40 °C)

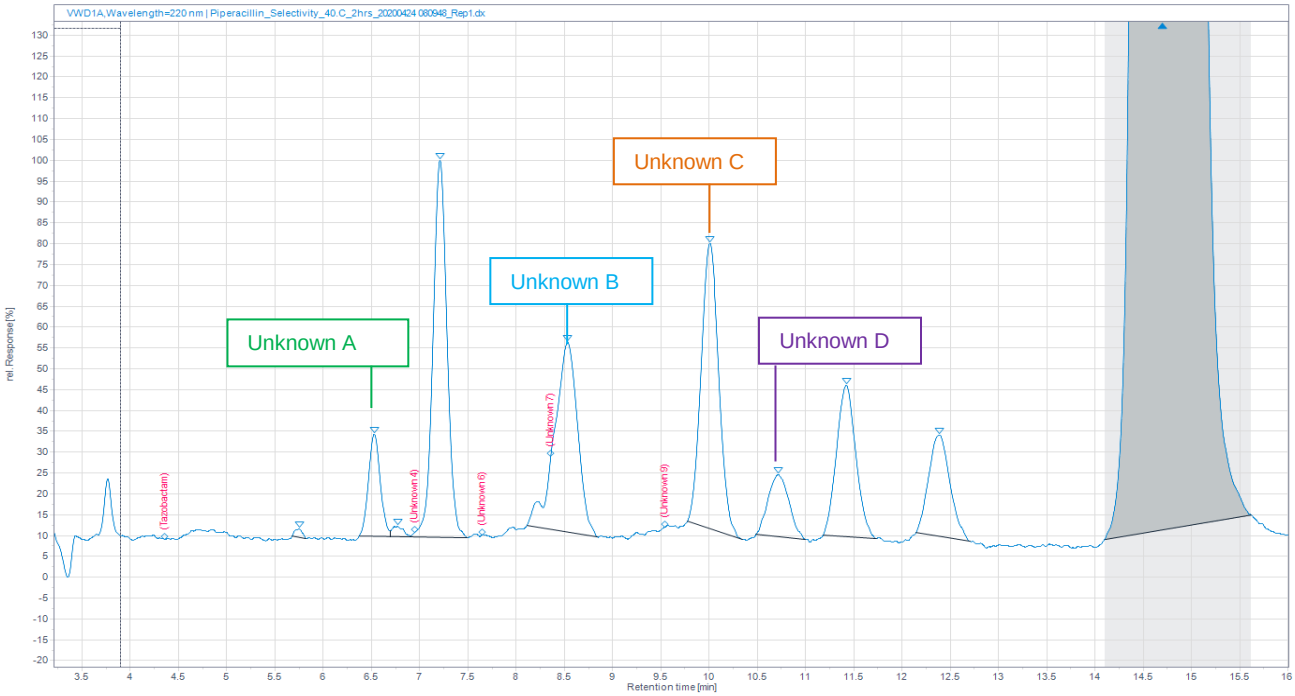


Table of degradants observed in Piperacillin forced degradation (2 hours at 40 °C)

Peak	Retention Time (Minutes)	Area	Relative Retention Time (Minutes)	% Area
Piperacillin	14.70	3904.97	1.00	100
Unknown 1	5.75	0.22	0.39	0.01
Unknown 2 **(Referred to as Unknown A)**	**6.53**	**4.16**	**0.44**	**0.11**
Unknown 3	6.77	0.41	0.46	0.01
Unknown 4	N/A	N/A	N/A	N/A
Unknown 5	7.21	18.18	0.49	0.47
Unknown 6	N/A	N/A	N/A	N/A
Unknown 7	N/A	N/A	N/A	N/A
Unknown 8 **(Referred to as Unknown B)**	**8.53**	**15.22**	**0.58**	**0.39**
Unknown 9	N/A	N/A	N/A	N/A
Unknown 10 **(Referred to as Unknown C2)**	**10.01**	**17.61**	**0.68**	**0.45**

Unknown 11 **(Referred to as Unknown D)**	**10.72**	**4.46**	**0.73**	**0.11**
Unknown 12	11.43	10.06	0.78	0.26
Unknown 13	12.39	7.42	0.84	0.19

APPENDIX 2– Standard Selectivity (Piperacillin and Tazobactam mix)

Chromatogram of Piperacillin and Tazobactam forced degradation (2 hours at 40 °C)

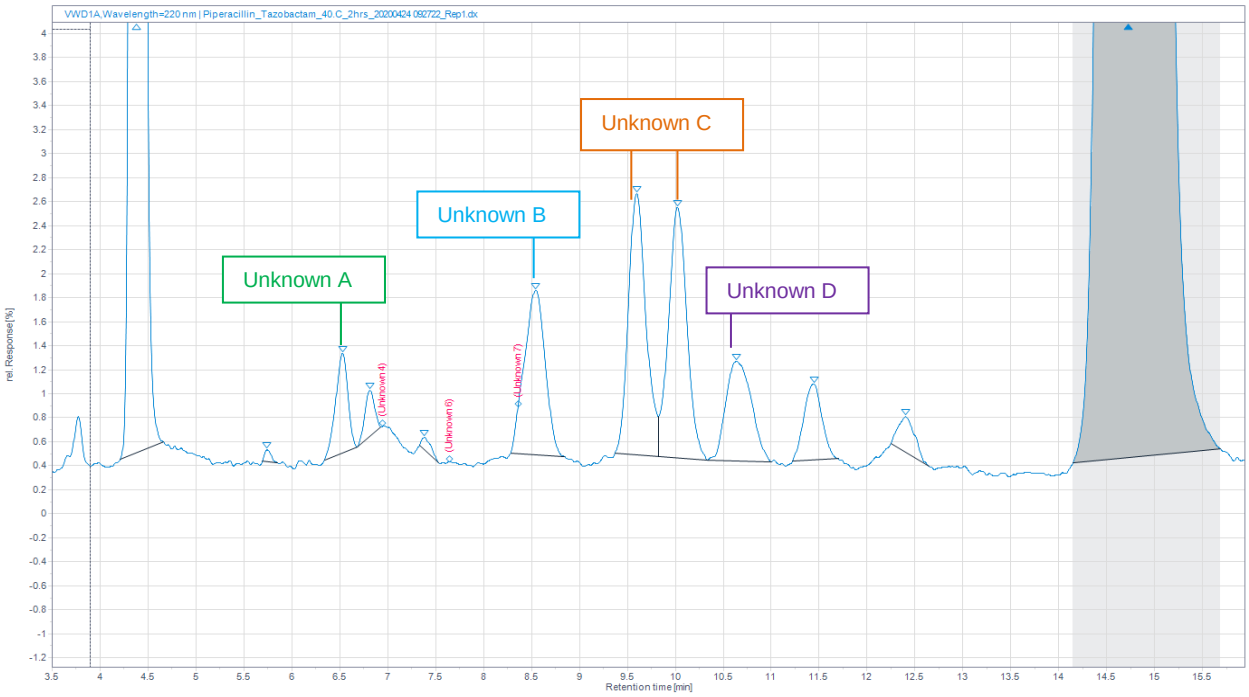


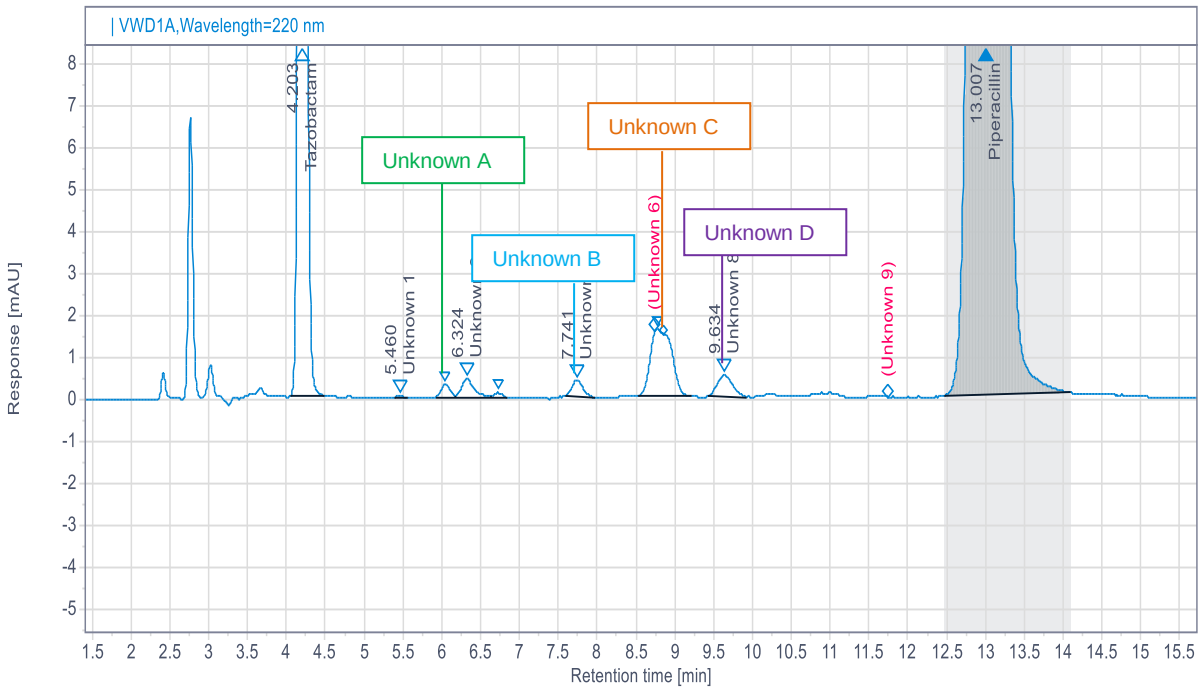
Table of degradants observed in Piperacillin/Tazobactam forced degradation (2 hours at 40 °C)

Peak	Retention Time (Minutes)	Area	Relative Retention Time (Minutes)	% Area	Related to Analyte
Piperacillin	14.73	3279.24	1.00	100.00	N/A
Tazobactam	4.38	259.71	0.30	7.92	N/A
Unknown 1	5.74	0.21	0.39	0.01	Pip
Unknown 2 **(Referred to as Unknown A)**	**6.53**	**3.29**	**0.44**	**0.10**	**Pip**
Unknown 3	6.81	1.18	0.46	0.04	Pip
Unknown 4	N/A	N/A	N/A	N/A	N/A
Unknown 5	7.38	0.36	0.50	0.01	Pip
Unknown 6	N/A	N/A	N/A	N/A	N/A
Unknown 7	N/A	N/A	N/A	N/A	N/A
Unknown 8 **(Referred to as Unknown B)**	**8.54**	**9.18**	**0.58**	**0.28**	**Pip**
Unknown 9 **(Referred to as Unknown C1)**	**9.60**	**12.38**	**0.65**	**0.38**	**Taz**
Unknown 10 **(Referred to as Unknown C1)**	**10.02**	**12.43**	**0.68**	**0.38**	**Pip**

Unknown 11 **(Referred to as Unknown D)**	**10.64**	**6.85**	**0.72**	**0.201**	**Pip**
Unknown 12	11.44	3.69	0.78	0.11	Pip
Unknown 13	12.40	1.61	0.84	0.05	Pip

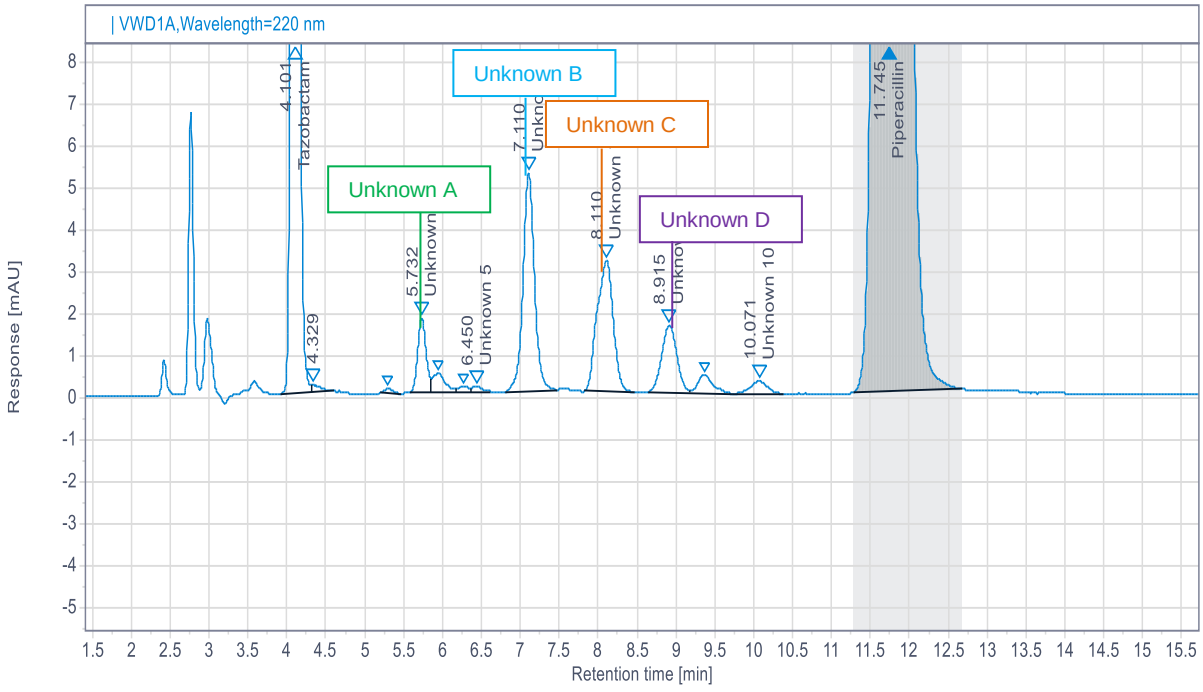
APPENDIX 3 – Initial Chromatogram

Chromatogram of Related Substances at Initial Time Point (Bag 1)



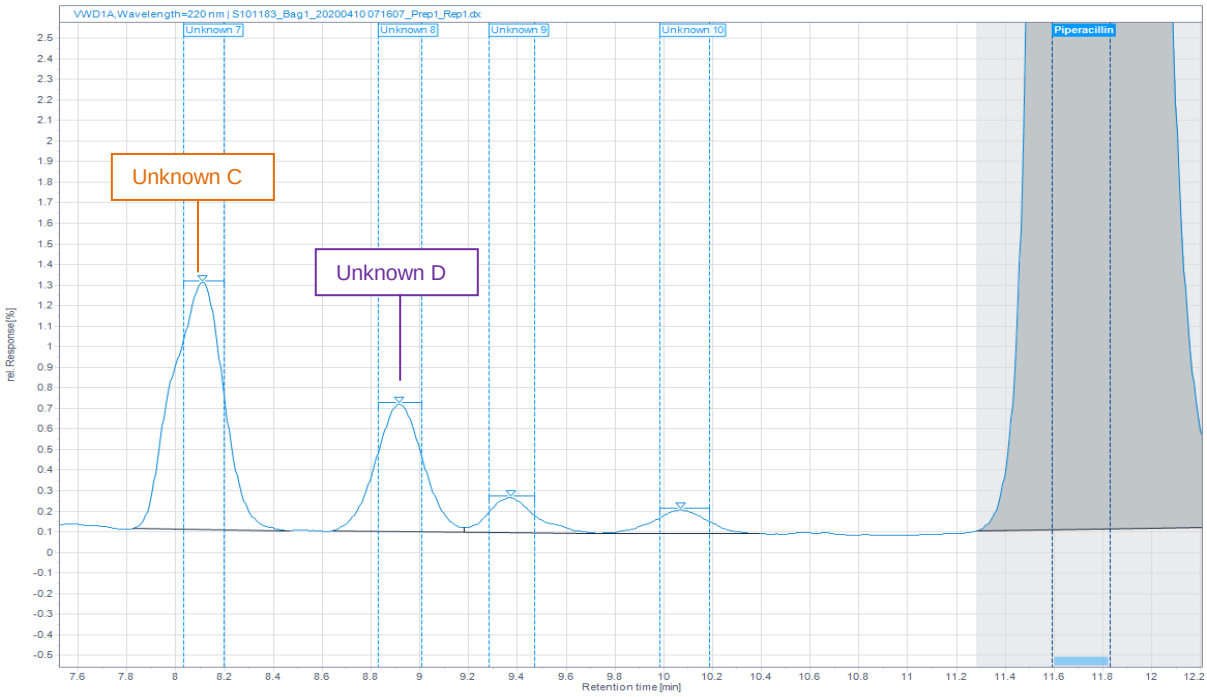
APPENDIX 4 – Final Chromatogram

Chromatogram of Related Substances at 17 Day Time Point (Bag 1)



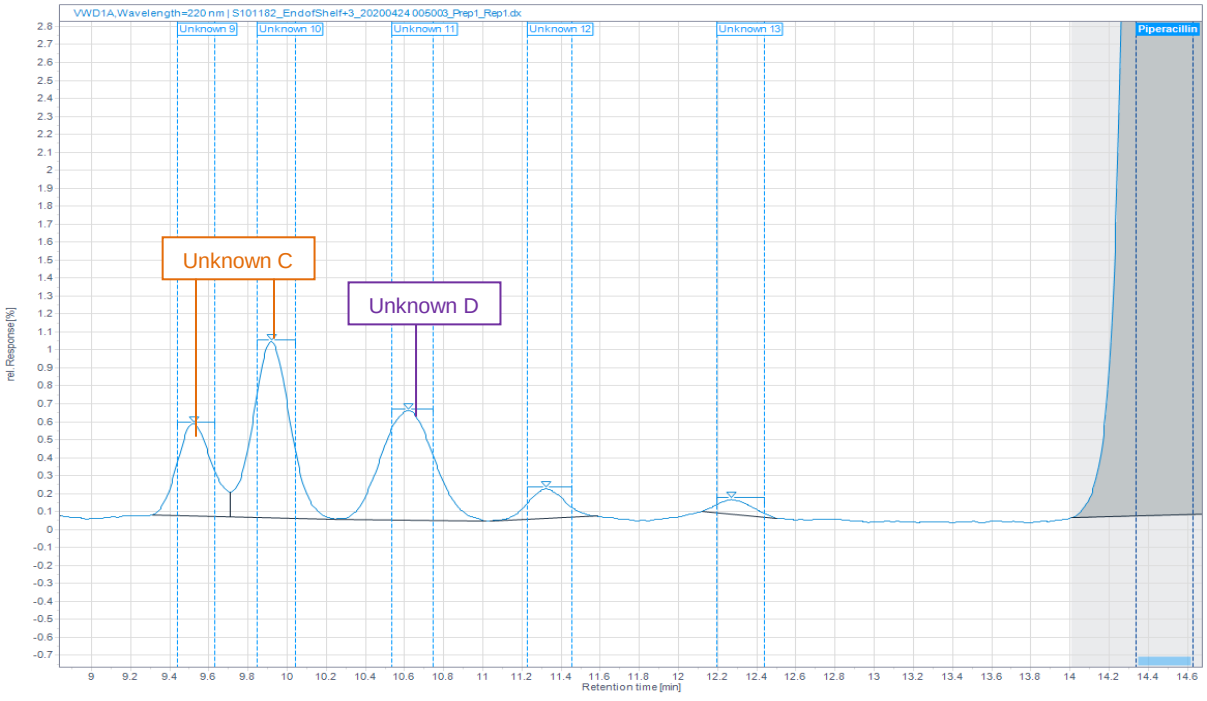
APPENDIX 5 – Final Chromatogram

Chromatogram of Related Substances at 17 Day Time Point (Bag 1)



APPENDIX 6 – Final Chromatogram (In-Use Study)

Chromatogram of Related Substances 17 Day (+3 hours) Time Point (In Use Bag)



13. DEVIATIONS

Time Point	Reasoning
10 Days	OOT result obtained for Tazobactam RF %RSD. Results for this time point included in stability study as they provided a worst-case scenario.
25 Days	OOT result obtained for Tazobactam assay. Results for this time point not included in stability study.