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Shelf Life Report for NHS Scotland

Stability Study for
Clindamycin 9.52mg/mL in 0.9% w/v Sodium Chloride 50mL Infusion Bags

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1. PURPOSE OF STUDY

Stability testing of Clindamycin diluted in 0.9% w/v Sodium Chloride 50mL infusion bags to give a final concentration of 9.52mg/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 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

Samples:

Clindamycin 150mg/ml 4mL vial (as phosphate)
Manufacturer: Focus
Batch Number: 190539
Expiry: June 2021

Sodium Chloride 0.9% w/v 50mL Infusion Bag
Manufacturer: Baxter Healthcare Ltd.
Batch Number: 19L15G62
Expiry: February 2021

3. SAMPLE PREPARATION

Samples for testing were prepared at QCNW Stockport on the initial day of the study. For each bag prepared, the entire contents of one vial (4mL) of Clindamycin 150mg/mL was withdrawn into a 5mL syringe, the air expelled, and transferred to a 50mL Baxter 0.9% w/v NaCl bag via the port. No overages were removed and the final volume of the bag was approximately 63mL. 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 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.

4. STORAGE AND SAMPLING PROTOCOL

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 49 days. At each time point, samples were tested for pH, appearance, Clindamycin assay and monitoring of degradant peaks. At time 0, 14 and 28 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 analysis.

Results were reviewed following the originally proposed final time point (28 days) and it was agreed with the client that the study would be extended. An additional 49 day time point was, therefore, carried out.

[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

5. METHODS

5.1 HPLC Assay

Clindamycin was 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 Luna C18 (2) 100A column (250 mm x 4.6 mm) with 5µm particle size (R205). Elution was obtained using a mobile phase composition of 74% aqueous phase and26% acetonitrile (HPLC grade). The aqueous phase was prepared by dissolving approximately 3.58 g di-sodium hydrogen phosphate dodecahydrate and 1.20 g sodium dihydrogen phosphate in 2000mL HPLC grade water. The pH of the aqueous phase was recorded, ensuring a reading of 7.0, but no adjustment was performed.

An eluent flow rate of 1.0mL per minute, a detection wavelength of 210 nm and an injection volume of 10µL were used. Vials for analysis were maintained at a temperature of 12ºC.

5.1.1 Standard Preparation

Standard solutions were prepared at each time point and used to quantify sample concentrations using Clindamycin Phosphate (Manufacturer: Sigma-Aldrich, Batch No: LRAC4009, Expiry Date: 28/01/2022, Purity: 96.5%)

Clindamycin weights of approximately 102mg (stock solution 1) and approximately 125mg (stock solution 2) were transferred into 50mL volumetric flasks and diluted to volume with mobile phase to give stock standard concentrations of approximately 2.0 and 2.4mg/mL A calibrated analytical balance was used for standard weighing.

Working standards 1 and 2 were prepared by pipetting 5.0mL of the respective stock solution into 100.0mL volumetric flasks and diluting to volume with mobile phase.

5.1.2 Sample Preparation

At each time point, bags were removed from the refrigerator and samples prepared in duplicate from infusion 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 mobile phase.

5.2 pH

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 analysis of bags 1, 2, 3 and placebo was performed at all time points. Approximately 2mL 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.

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 a black background under appropriate lighting. Samples were observed for colour, clarity and particle formation.

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, 14 days and 28 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. A pre-run sample was also performed but the result was discarded.

6. VALIDATION

The method was quantitatively validated by standard and sample repeatability, standard and sample stability and intermediate precision. Accuracy, recovery and linearity tests were also performed. The method was validated qualitatively using forced degradation studies. All validation was performed on HPLC instrument LC 5 with column R205, unless otherwise stated.

6.1 PRECISION

6.1.1 Standard Repeatability

Two Clindamycin phosphate 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:

	Concentration (mg/mL)	**%RSD** Limit = 2.0%	**RF**
Standard 1 (n=6)	1.9580	0.5	106.8
Standard 2 (n=6)	2.4177	0.3	107.7
		RF%RSD Limit = 2.5%	0.6%

6.1.2 Sample Repeatability

A single infusion bag was prepared following the procedure described in section 3 to give a final concentration of Clindamycin 9.52mg/mL in 0.9% w/v NaCl. Six samples preparations were performed by diluting 2.0mL sample to 200mL with mobile phase. Sample precision was established by injecting each of the six samples in duplicate. The %RSD was within the limits of 2.0.

Sample	Clindamycin Phosphate Concentration (mg/mL)
1	12.0167
2	12.0207
3	12.0672
4	12.0294
5	12.0570
6	12.0287
%RSD Limit = 2.0%	0.2

6.1.3 Intermediate Precision

Additional testing was performed to ensure that the analytical method provides consistency of results between analysts and instruments. A second analyst prepared standards following the procedure in 5.1.1, and six samples from the same infusion bag used in section 6.1.2. Analysis was performed using a different HPLC (Chrom 6) and column (R188) to those used in Sample Precision. Results from section 6.1.2 and 6.1.3 were compared and it is evident that method precision has successfully been achieved. Varying factors of analysts, instruments and equipment do not impact sample results.

Sample	Clindamycin Phosphate Concentration (mg/mL)
1	11.9396
2	11.7989
3	11.9184
4	11.9858
5	11.9449
6	11.9966
%RSD (n=6) Limit = 2.0%	0.6
%RSD (n=12) Limit = 2.0%	0.6

6.2 Selectivity

A forced degradation study of Clindamycin phosphate was carried out to identify related substances for both standard and sample.

6.2.1 Standard Selectivity

A stock standard containing 125mg of Clindamycin phosphate was dissolved in 50mL mobile phase. From the stock standard, 4 separate 10mL intermediate stocks were prepared as follows:

1. 5.0mL sample + 1.0mL 1M hydrochloric acid, made to volume with water
2. 5.0mL sample + 1.0mL 1M sodium hydroxide, made to volume with water
3. 5.0mL sample + 1.0mL 30% hydrogen peroxide, made to volume with water
4. 5.0mL sample made volume with water.

Working standards were prepared by diluting 1.0mL intermediate stock into 10mL mobile phase and injected for analysis. All intermediate stocks were transferred to an oven and removed following 6 days storage at 70ºC. Working standards were prepared and injected for analysis. Initial and 6 day standards were compared to identify possible degradant products.

The Clindamycin analyte peak remained well resolved and no degradant peaks interfered with the main analyte. (See appendix 1 and 2).

6.2.2 Sample Selectivity

An infusion bag was prepared by adding one vial of Clindamycin 150mg/mL to a 50mL NaCl 0.9% w/v infusion bag and inverted several times to give a solution of 9.52mg/mL Clindamycin. This solution was used to prepare 4 separate 20mL stock samples as follows:

1. 2.0mL sample + 2.0mL 1M hydrochloric acid, made to volume with water
2. 2.0mL sample + 2.0mL 1M sodium hydroxide, made to volume with water
3. 2.0mL sample + 2.0mL 30% hydrogen peroxide, made to volume with water
4. 2.0mL sample made volume with water.

Working samples were prepared by diluting 1.0mL stock into 10mL mobile phase and injected for analysis. All stocks were transferred to an oven and removed following 6 days storage at 70ºC. Working samples were prepared and injected for analysis. Initial and 6 day samples were compared to identify possible degradant products.

The Clindamycin analyte peak remained well resolved and no degradant peaks interfered with the main analyte. (See appendix 3-4).

6.3 Stability

6.3.1 Standard Stability

From the stock standard prepared in section 6.2 (selectivity), a working standard was prepared by diluting 5.0mL to 100mL with mobile phase. The standard solution was immediately injected prior to aliquots of the working standard preparation being stored at 2-8ºC and room temperature in the dark (RTD) for 144 hours (6 days). Standards were then removed from storage conditions, assayed and compared to the initial results.

Time and Condition	Area	% of Initial Area
Initial	263.468	100.0
144 Hours RTD	266.367	101.1
144 Hours 2-8ºC	266.609	101.2

Results indicate that standards are stable when stored at room temperature and 2-8ºC for 144 hours (6 days).

6.3.2 Sample Stability

From the infusion bag prepared in section 6.2 (selectivity), a single sample was prepared by diluting 2.0mL to 200mL with mobile phase. The sample solution was immediately injected prior to aliquots of the sample preparation being stored at 2-8ºC and room temperature in the dark (RTD) for 144 hours (6 days). Samples were then removed from storage conditions, assayed and compared to the initial results.

Time and Condition	Area	% of Initial Area
Initial	245.263	100.0
144 Hours RTD	255.358	104.1
144 Hours 2-8ºC	256.227	104.5

Results indicate that samples are stable when stored at room temperature and 2-8ºC for 144 hours (6 days).

6.4 Accuracy, Linearity and Recovery

A 200% stock sample of Clindamycin was prepared by diluting 1.12780g Clindamycin phosphate to 50mL with 0.9% w/v sodium chloride. Samples were then diluted down as follows:

40%	2.0mL sample diluted to 10mL with 0.9% NaCl	
60%	3.0mL sample diluted to 10mL with 0.9% NaCl	
80%	4.0mL sample diluted to 10mL with 0.9% NaCl	Triplicate
100%	5.0mL sample diluted to 10mL with 0.9% NaCl	Triplicate
120%	3.0mL sample diluted to 5mL with 0.9% NaCl	Triplicate

All stock solutions were diluted to working concentrations by diluting 2.0mL to 200mL with mobile phase.

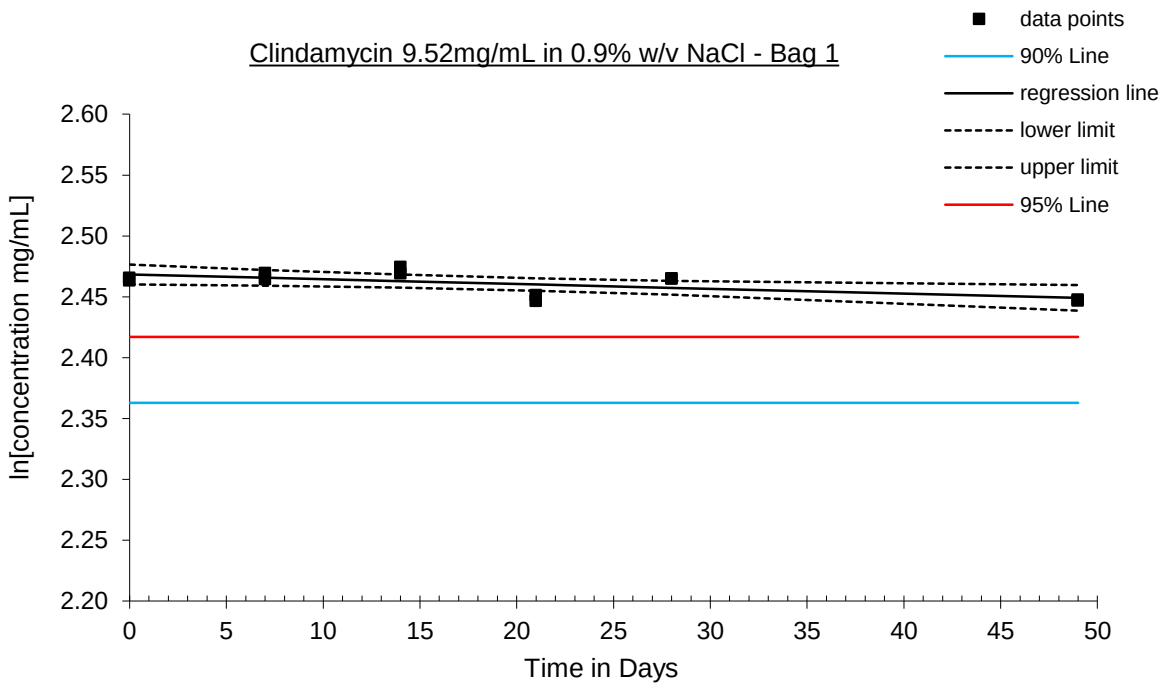
	Limit	Result
Recovery 100% level (n=3)	Mean = 95-105%	95.5%
Accuracy All levels (n=11)	Mean = 95-105% (%RSD = less than 5)	95.3% (0.7%)
Linearity	R^2 Greater than 0.999	0.9999

7. RESULTS: REFRIGERATED STUDY

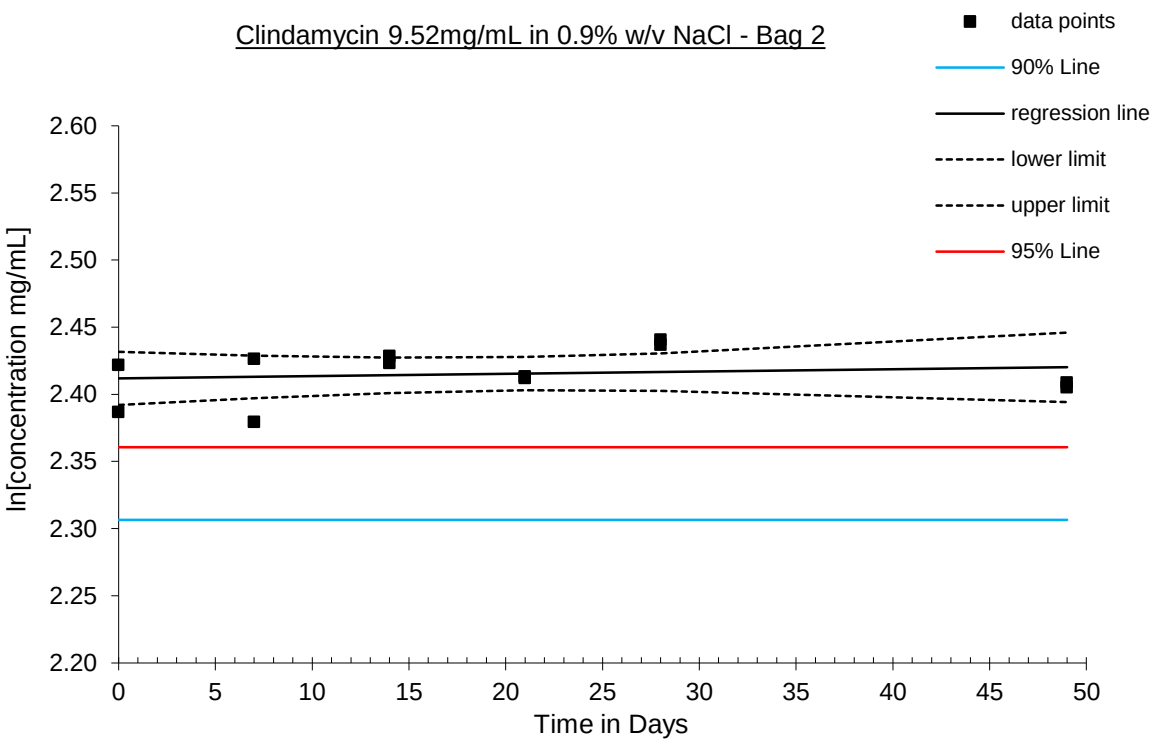
The results for individual bags for the Clindamycin assay content and pH are tabulated below. Assay results are a mean of two results and are expressed in terms of Clindamycin concentration as a percentage of the initial assay. (See appendix 5 -7 for chromatograms).

Time Point (Days)	Percentage of Initial (%) (mg/mL Clindamycin Phosphate)			pH		
	Bag 1	Bag 2	Bag 3	Bag 1	Bag 2	Bag 3
0	100.0 (11.76mg/mL)	100.0 (11.07mg/mL)	100.0 (11.75mg/mL)	6.44	6.44	6.42
7	100.3	99.9	98.5	6.45	6.45	6.43
14	100.7	102.2	99.8	6.43	6.43	6.44
21	98.5	100.8	98.7	6.42	6.43	6.41
28	100.1	103.5	101.1	6.46	6.45	6.45
49	98.0	100.4	99.3	6.42	6.41	6.40

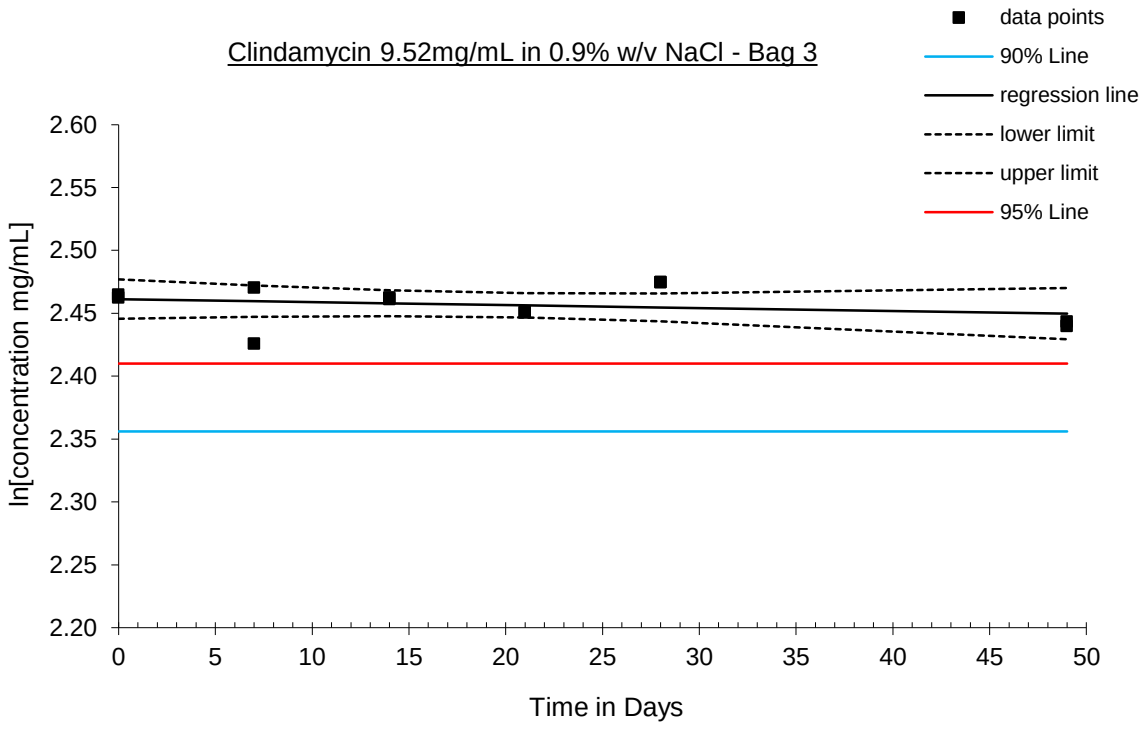
7.1.1 Clindamycin Stability Graphs



T (0.9) = 147.76 days, T (0.95) = 72.19 days



T (0.9) = 172.78 days, T (0.95) = 84.42 days



T (0.9) = 123.35 days, T (0.95) = 60.26 days

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 (63mL)	
	10 Micron	25 Micron	10 Micron	25 Micron
0	3	1	177	7
14	6	1	338	10
28	7	1	416	7

7.3 Appearance

The appearance of the solutions in all bags remained clear, colourless and free from visible particulates throughout the study for the 49 day study period.

7.4 Degradation Products

No HPLC peaks relating to degradation products were identified throughout the 49 day period study.

8. RESULTS: IN USE STUDY

Three additional infusion bags were prepared and sampled at time zero and following 49 days after refrigerated storage. Infusion bags were then placed at 25°C/60%RH for 24 hours to replicate a bag being stored at room temperature during infusion.

8.1 In Use Study – Assay and pH

Time Point (Days)	Percentage of Initial (%) (mg/mL Clindamycin Phosphate)	pH
0	100.0 (11.62mg/ml)	6.42
49	98.8	6.44
49 (+24 hours at 25°C)	99.1	6.39

The assay and pH results obtained support the stability study performed that demonstrated approximately 1% decrease in assay over 49 days at 5°C. The pH also mirrors the stability study samples at 6.4 to 1 decimal place). Assay and pH showed no significant difference following 24 hours storage at 25°C.

8.2 In Use Study - Particle Count

Time Point (Days)	Counts per mL		Counts per container (63mL)	
	10 Micron	25 Micron	10 Micron	25 Micron
49 (+24 hours at 25°C)	5	1	284	19

Particle count results support the results obtained in the stability study. No true trend can be obtained, however, compliance with the British Pharmacopeia monograph is demonstrated throughout the stability study and in-use test. In use particle count results suggest that no rapid particulate increase is observed during storage at 25°C/60%RH for 24 hours.

9. DISCUSSION

- The appearance of all three bags remained clear and colourless with no visible particulate matter throughout the duration of the study
- The pH of all three bags remained constant at 6.4 – 6.5 (1 d.p).
- The mean assay results for all three bags were found to be in the range of 98 - 104% of initial concentration for Clindamycin over the 49 day study period. Small variations from the original concentration over all time points tested were due to inherent variance in the analytical method.
- No degradation products were noted in the HPLC chromatograms throughout the duration of the study.
- In use testing demonstrated that storing the finished product at 25°C/60%RH for 24 hours does not compromise the physical or chemical stability of the product, as percentage assay and pH of solution remains consistent.

10. CONCLUSION

In conclusion, Clindamycin 9.52mg/mL in 0.9% w/v sodium chloride exhibited chemical and physical stability under refrigerated storage conditions (2-8ºC) for 49 days.

Chemical and physical stability was also demonstrated following 24 hours storage at 25ºC/60%RH.

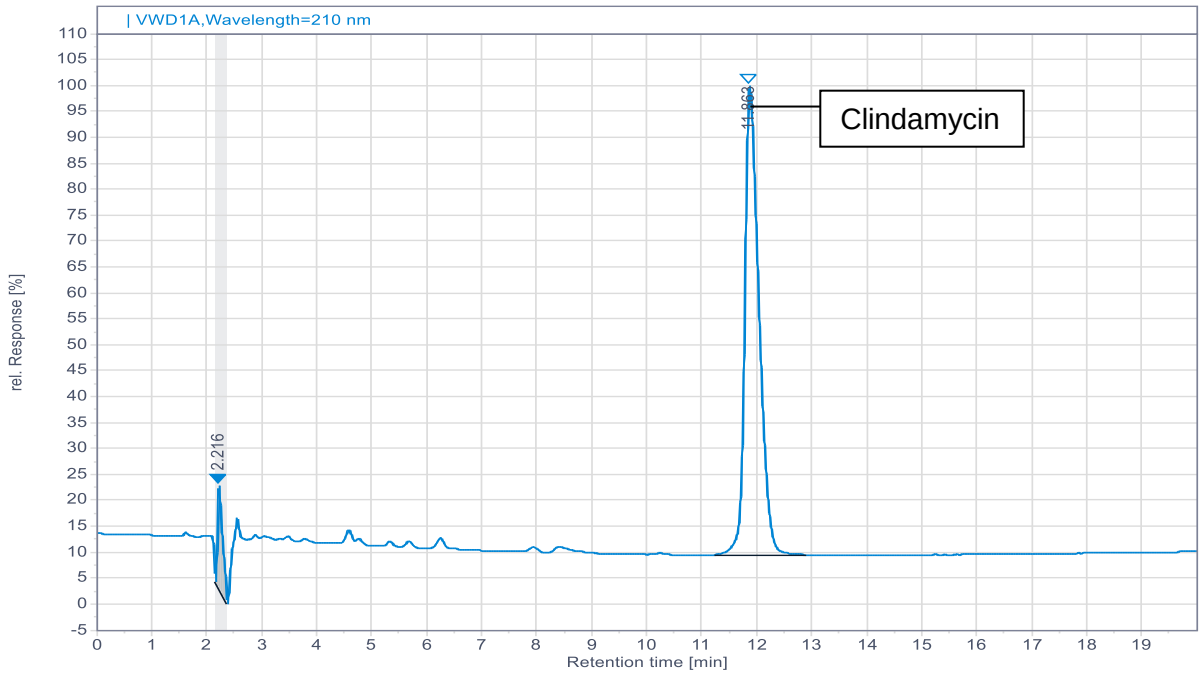
11. CHANGE CONTROL

Document Reference	Date	Description of Change
SCN-2020-01	19/05/2020	Initial Version
SCN-2020-01v2	17/12/2020	Removal of 'Private and Confidential' at request of client

12. APPENDICES

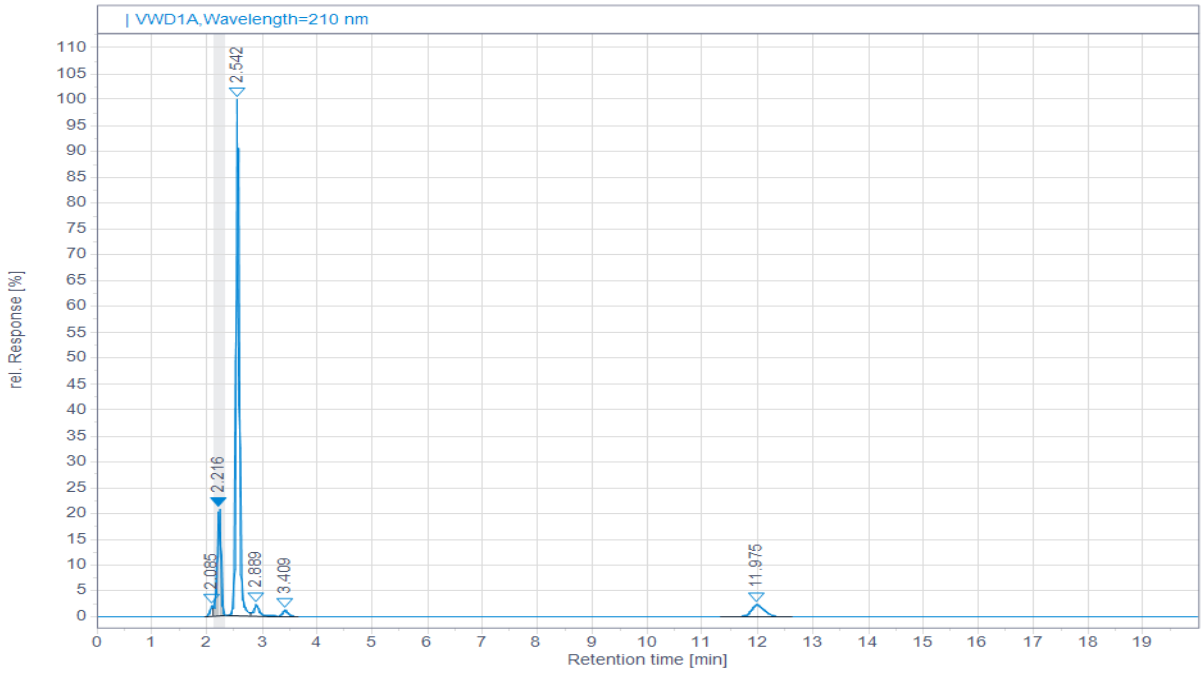
APPENDIX 1 – Standard Selectivity (Acid)

Chromatogram - Standard Selectivity (HCl – 6 Days)



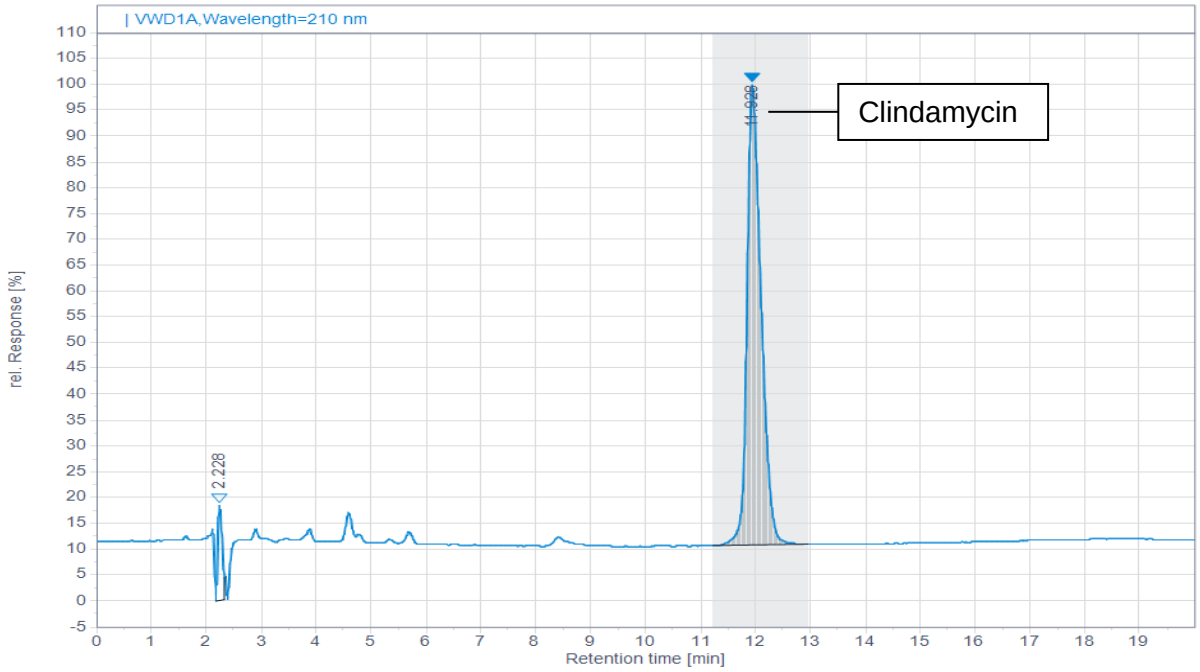
APPENDIX 2 – Standard Selectivity (Base)

Chromatogram - Standard Selectivity (NaOH – 6 Days)



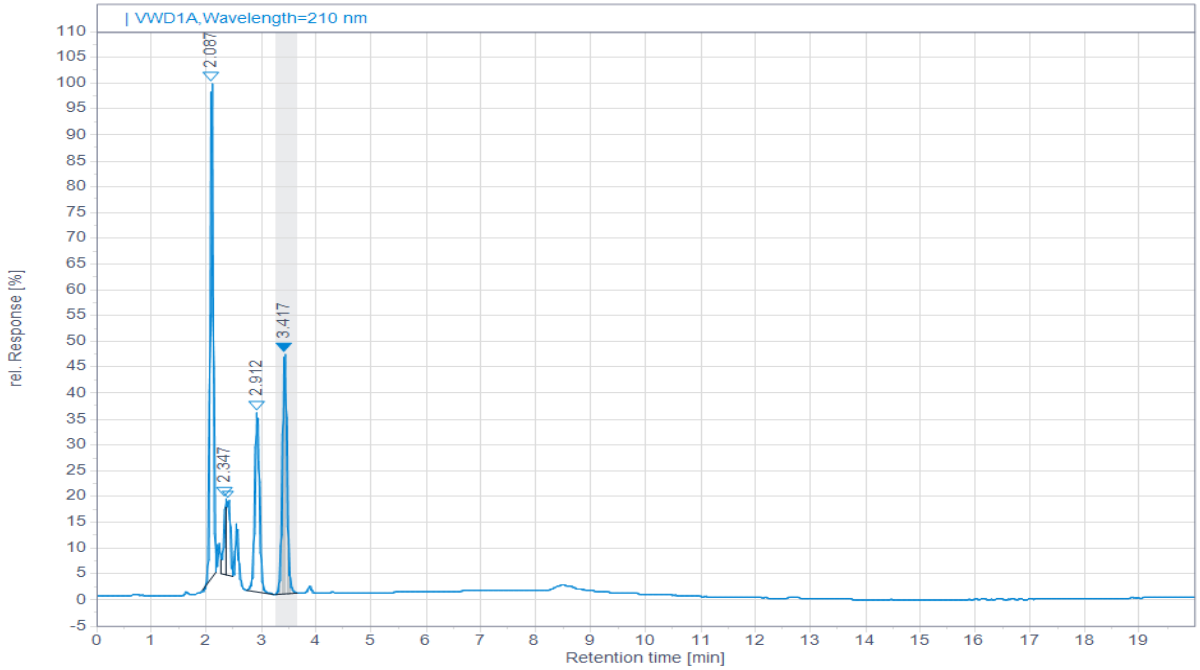
APPENDIX 3 – Sample Selectivity (Acid)

Chromatogram - Sample Selectivity (HCl – 6 Days)



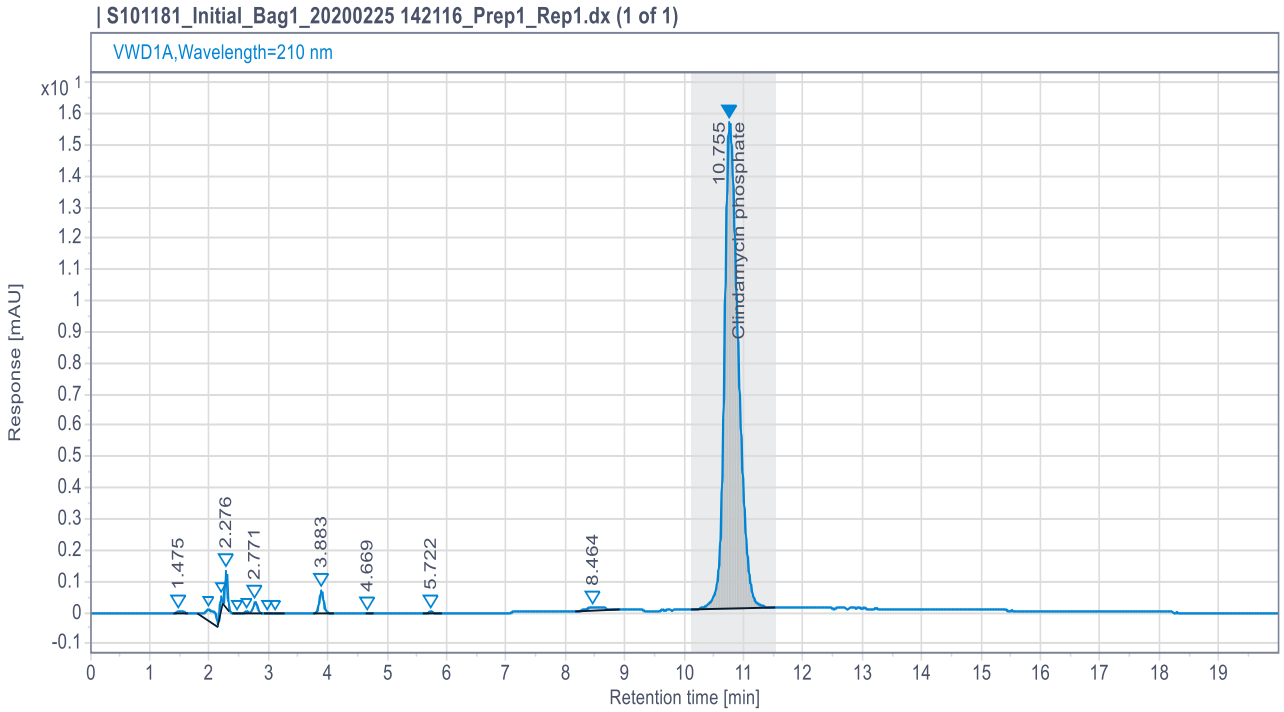
APPENDIX 4 – Sample Selectivity (Base)

Chromatogram – Sample Selectivity (NaOH – 6 Days)



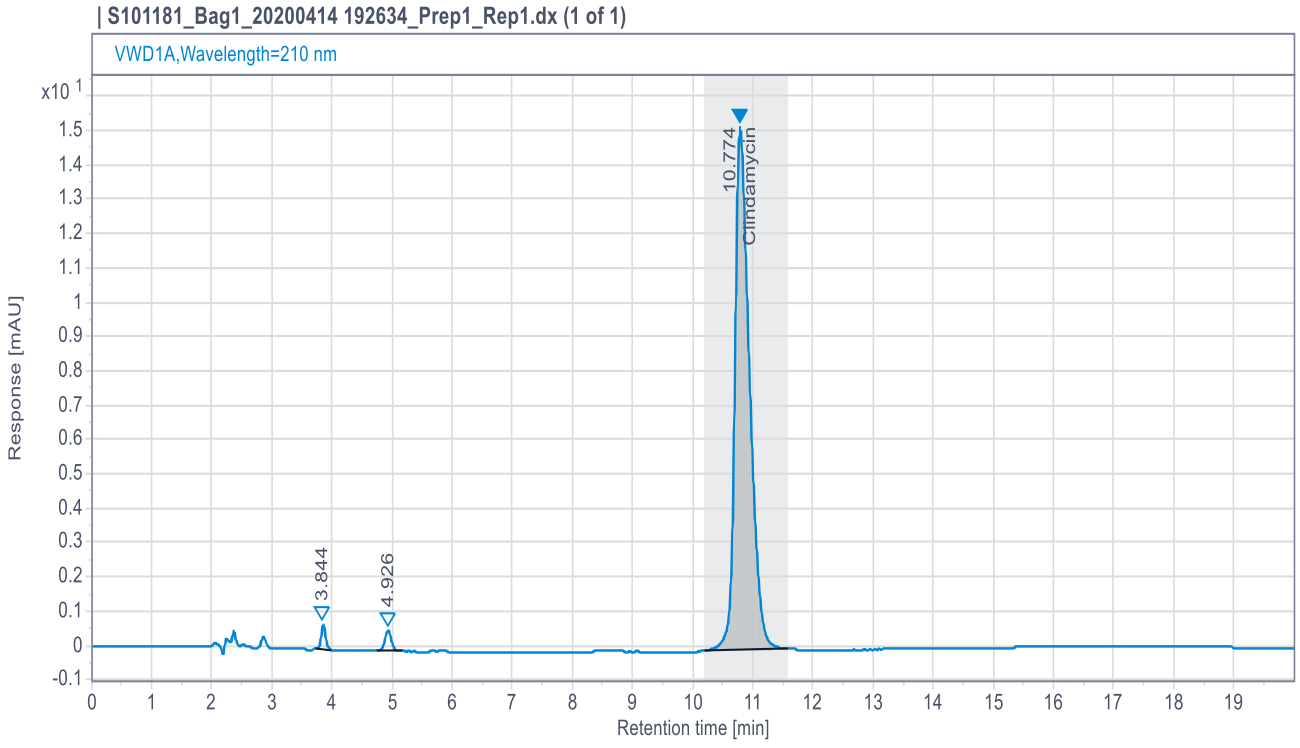
APPENDIX 5 – Initial Chromatogram

Chromatogram of Clindamycin at Initial Time Point (Bag 1)



APPENDIX 6 – Final Chromatogram

Chromatogram of Clindamycin at 49 days (bag 1)



APPENDIX 7 – Standard Chromatogram

Chromatogram of Clindamycin Standard at 49 days