SI 1 – Pilot study methods – Analysis of the stability of commercial aqueous CHX eye drops

Commercially purchased aqueous 0.2% CHX solutions (C_{Aq}) were either refrigerated at 4 °C or incubated at 40 °C at 70% relative humidity in quadruplet. At time intervals 0, 6 and 12 months the pH was determined and an aliquot was analysed by high performance liquid chromatography fitted with dioade array detection (HPLC-DAD).

Each sample was injected onto the HPLC column, and the quantity of CHX present was measured using Thermo Scientific™ Dionex™ Ultimate™ 3000 HPLC-DAD system (Thermofisher, Hemel Hempstead, UK) and separation achieved using a GENESIS® AQ 4 µm column (150 x 4.6 mm, Grace Materials Technologies, Cranforth, UK); eluting with ammonium formate (10 mM, pH 2.7) and acetonitrile (v/v; 60:40) flowing at 2 mL/min through the DAD set at 239 nm. The authenticity of the detected peak for CHX, at 1.56 mins, was determined by comparison of retention time, spectral extraction at 239 nm and spiking the sample with commercially available CHX. A calibration curve of CHX was generated by Chromeleon (Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System) using known amounts of the standard CHX injected onto the column.

SI 2 – Chlorhexidine 0.2% production protocol

Chlorhexidine Digluconate Eye Drops 0.2% w/v 10 mL

Batch No:	Date of Manufacture:
Authority:	Authorisation date:

Equipment Required Checklist – Tick off items as assembled	
Equipment	**Location**
Water filtration assembly	
1000 mL measuring cylinder	
Freshly distilled water, 2.5 L	
Metal jug	
20 mL measuring cylinder	
Volumetric flask 2 L	
Volumetric flask 20 mL and 5 mL	
Glass pipettes + fillers	
10 mL measuring cylinder	

Formula		
Ingredients	**Quantity for standard batch**	**Quantity required if different**
Chlorhexidine digluconate 20% solution	20.0 mL	
Sodium Acetate	23.198 g	
Acetic Acid 20%	4.46 mL	
Sodium hydroxide 10M	1.938 mL	
Freshly distilled water	2 L	

Precautions	
Chlorhexidine digluconate	Irritant to soft tissues, irritates eyes, skin and respiratory surfaces. Wear gown, gloves, hat, mask and safety glasses.
Acetic acid	Corrosive to tissues and flammable. Wear gown, gloves, mask and safety glasses.
Sodium hydroxide	Corrosive. Causes burns to tissues. Wear gown, gloves, mask and safety glasses.

Ingredient details				
Ingredient	**Supplier**	**Catalogue number**	**Batch number**	**Expiry Date**
Chlorhexidine digluconate 20%				
Acetic Acid 20%				
Sodium acetate				
Sodium hydroxide 10 M				

Preparation of containers and materials			
No.	**Step**	**SOP No.**	**Operator**
1	Count bottles into clean bowl		
2	Wash bottles and place in sterilisation drum	04	
3	Autoclave drum for sterilisation	12	
4	Collect 2.5 L of freshly distilled water	03	
5	Ensure production room and equipment is clean	02, 05	
6	Rinse 2 L volumetric flask with distilled water		

Preparation of buffer			
No.	**Step**	**SOP No.**	**Operator**
7	Ensure the preparation room is clear of previous batch		
8	Weigh 23.198 g of sodium acetate into a weigh boat	06	
9	Dissolve sodium acetate in 2 L of freshly distilled water in mixing beaker		
10	Add 4.46 mL of 20% acetic acid	18	
11	Measure pH. **pH measured:**	07	
12	Adjust pH to 6.75. Add 1.938 mL of 10 M NaOH in small portions to allow the pH to equilibrate before next addition. **Final pH:**		

Solution preparation			
No.	**Step**	**SOP No.**	**Operator**
13	Add 1.5 L of acetate buffer to volumetric flask		
14	Measure 20 mL of 20% chlorhexidine digluconate in 20 mL volumetric flask		
15	Transfer chlorhexidine into 2 L volumetric flask, rinse 20 mL flask 3 times with buffer and transfer to 2 L flask.		
16	Fill flask to 2 L mark with sodium acetate buffer.		
17	Place lid on flask, invert flask and shake to stir		
18	Filter solution through glass sinter over vacuum	09	
19	Transfer filtrate into jug		

Solution Filling			
No.	**Step**	**SOP No.**	**Operator**
20	Lay out bottles on clean surface		
21	Fill the bottles with 10 mL solution	16	
22	Place the filled and capped bottles into the water bath trays. Record number of bottles filled and trays to be sterilised below and on white label.		

	No. of bottles filled: **No. of trays for sterilisation:**		
23	Clean all equipment used.	15, 17	

Sterilisation			
No.	**Step**	**SOP No.**	**Operator**
24	Confirm the waterbath or autoclave is empty, safe and ready for use.		
25	Sterilise by waterbath or autoclave	11, 12	
26	Remove the batch from the waterbath or autoclave	11, 12	

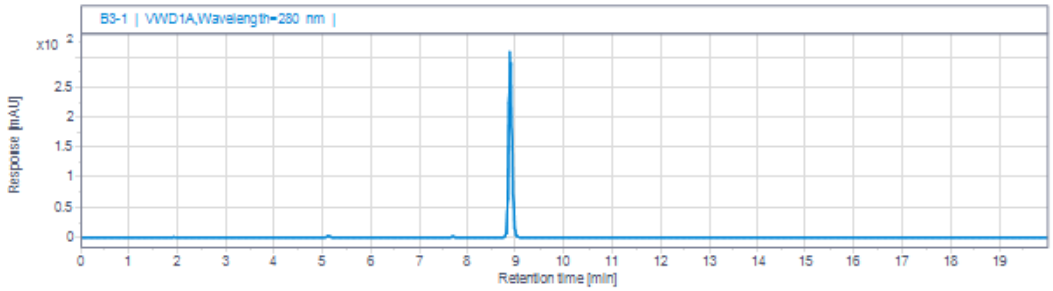
Inspection			
No.	**Step**	**SOP No.**	**Operator**
27	Visually check the bottles for an particles or debris. Check lids cannot be tightened further. Check correct fill volume. Reject any bottles that do not conform to these criteria.	13	

Labelling			
No.	**Step**	**SOP No.**	**Operator**
28	Check correct labels are correct	14	
29	Label each pass bottle.		
30	Clean down bench.		

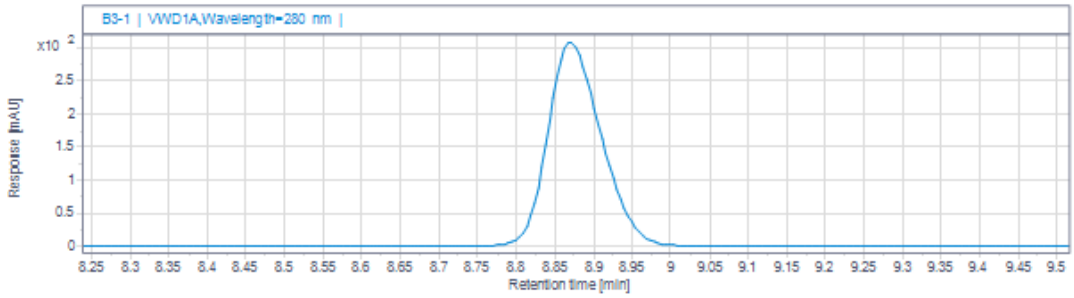
No. of containers filled		
No. of rejects	Leaking	
	Particles/fibres	
	Caps	
	Others	
No. Destroyed		
Certified that the above information is correct and that the batch may be passed to holding room. ……. Batch approved for release: Date:		

SI3- HPLC chromatogram of CHX eye drops prepared in Uganda after 30 months storage at room temperature. A and B: Compounded buffered CHX eye drops: CHX appeared at retention time 8.87 minutes and no degradation peak was observed (A), B is enlargement of CHX peak. C is control CHX sigma: CHX appeared at retention time 8.87 min. D is enlargement of control CHX peak.

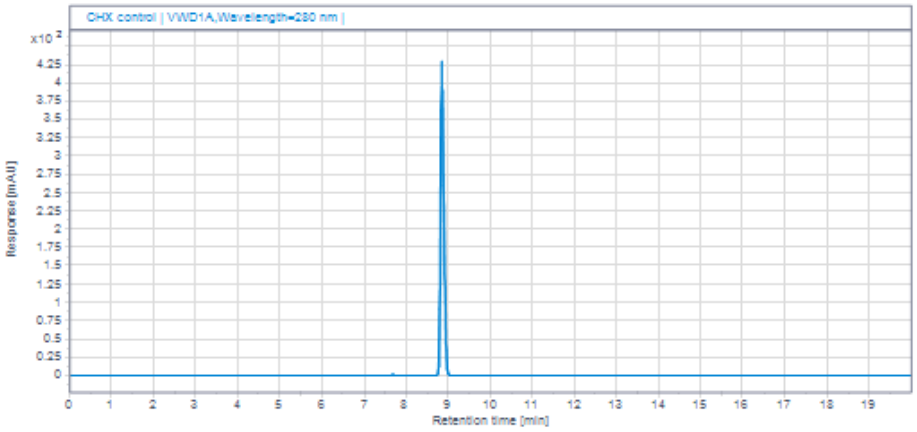
A. Compounded buffered CHX eye drops



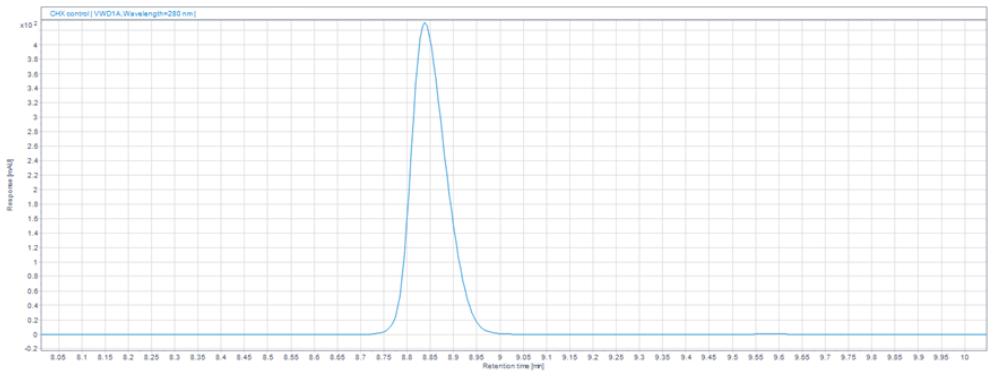
B. Enlarged peak of compounded buffered CHX eye drops



C. Control aqueous CHX



D. Enlarged peak of aqueous control CHX



SI4-References to support HPLC method

1. A. Mohamed-Ahmed, A. Lockwood, H. Fadda, S. Madaan, P.T. Khaw, S. Brocchini, K. Karu: “LC-MS analysis to determine the biodistribution of a polymer coated ilomastat ocular implant”(J. Pharm. Biomed.Anal, 2018, 16 (157):100-106).

2. A. Mohamed-Ahmed, A. Lockwood, H. Li, M. Bailly, P.T. Khaw, S. Brocchini: “An Ilomastat-CD eye drop formulation to treat ocular scarring”: (Invest. Ophthalmol. Vis. Sci, 2017, 58(9):3425-3431).

SI 5 Glossary

AcB	Compounded buffered CHX eye drops
Aq	Compounded unbuffered aqueous CHX eye drops
CAq	Unbuffered commercial aqueous CHX eye drops
CAcB	Buffered commercial CHX eye drops