PQM Promoting the Quality of Medicines

GSK Chlorhexidine Digluconate (7.1%) Gel Technology Transfer Report

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The Promoting the Quality of Medicines (PQM) program, funded by the U.S. Agency for International Development (USAID), is the successor of the Drug Quality and Information program implemented by the U.S. Pharmacopeial Convention (USP). PQM is USAID's response to the growing challenge posed by the proliferation of falsified and substandard medicines. By providing technical assistance to developing countries, PQM helps build local capacity in medicines regulatory, quality assurance and quality control systems, increase the supply of quality medicines to priority USAID health programs, and ensure the quality and safety of medicines globally.

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Acknowledgments

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Acronyms

4CA	4 chloroaniline
API	active pharmaceutical ingredient
CHX	chlorhexidine
CPP	critical process parameter
CQA	critical quality attribute
DoE	Design of Experiment
HPLC	high-pressure liquid chromatography
MRF	mean response factor
PAR	proven acceptable range
PDE	permitted daily exposure
QTPP	quality target product profile
RLD	Reference Listed Drug
RRF	relative response factor
UV	ultraviolet

1. Introduction

1.1 Product Description

Chlorhexidine (CHX) and its salts possess a high level of antimicrobial activity with a strong affinity for binding to the skin and mucous membranes. Chlorhexidine is used in a wide range of pharmaceutical and cosmetic products such as hand washes, preoperative cleansing products, wound care products, oral hygiene products, and general disinfection products.

Chlorhexidine digluconate (7.1%) gel (CHX gel) is a drug product used for prophylaxis of omphalitis (an infection of the umbilical cord stump). The drug product is available as a colorless to yellow translucent gel for topical use.

CHX gel formulation was developed by GSK based on considerations that the drug product would retain at the site of application and would be easier to apply. In addition to the active pharmaceutical ingredient (API), the CHX gel formulation includes components such as a thickener, a pH stabilizer, and the solvent.

1.2 Reference Product

CHX gel formulation was developed by GSK in direct response to the United Nations (UN) Commission Report (Sep 2012) on Life-Saving Commodities for Women and Children. GSK's CHX gel formulation development was based on information provided in the Program for Appropriate Technology and Health (PATH 004) Health Tech Report – Stability Data on Chlorhexidine Formulations (PATH, 2010). The formulation was simplified and optimized to minimize formation of impurities, in particular 4 chloroaniline (4CA), which has been shown to be genotoxic and carcinogenic in nonclinical studies. (CICAD 2003).

1.3 Scope

This technology transfer report is intended to serve as a guidance document on the development and manufacture of CHX gel. The report addresses the development of the formulation, including analytical testing along with manufacturing and primary packaging processes. The report also provides guidance for the commercial manufacturing and primary packaging of CHX gel into 3g sachets.

2. Pharmaceutical Development

CHX gel 7.1% w/w contains 4% w/w equivalent of chlorhexidine as the free base. The CHX gel formulation is packaged as a single-use 3g dose in a foil laminate sachet. Each 3g dose contains 213mg chlorhexidine digluconate (equivalent to 120mg of chlorhexidine). No overage is included in the CHX gel formulation composition. The target fill weight for sachets is 3.3g (range 3.0g–3.6g) to ensure that 3.0g gel is delivered from the sachet.

The Reference Listed Drug (RLD) for this product was based on information provided in the Program for Appropriate Technology and Health (PATH) Health Tech Report – Stability Data on Chlorhexidine Formulations (PATH, 2010).

2.1 Components of the Drug Product

The CHX gel drug product comprises an aqueous gel manufactured using chlorhexidine digluconate API (20% w/v aqueous solution), guar gum as a thickener, sodium acetate trihydrate as a pH modifier, and purified water as a solvent.

API Chlorhexidine Digluconate

The API used in this product is digluconate salt. Chlorhexidine is available commercially in different salt forms: dihydrochloride, diacetate, and digluconate. Digluconate salt was selected because it has higher aqueous solubility than the other salts. Dihydrochloride and diacetate have saturation solubilities of 0.2% and 2% respectively, whereas chlorhexidine digluconate is soluble in water to at least 50% w/v. Digluconate salt is available as a solution at 20% w/v (Zeng et al, 2009).

Chlorhexidine digluconate API used was a commercially available 20% w/v solution that complies with the requirements of the European Pharmacopeia (Ph. Eur.) monograph for chlorhexidine digluconate solution, 20% w/v. However, due to the need to minimize the 4CA impurity as far as reasonably practical, currently the 20% w/v solution is released with a 20 ppm limit, and GSK applies a 150 ppm limit upon receipt at site. This is much lower than the 500 ppm limit in the European Pharmacopoeia. It is suggested to procure API having lowest 4CA levels.

Physicochemical Attributes of Chlorhexidine Digluconate Solution, 20% W/V

Description of API

Chlorhexidine digluconate solution, 20% w/v is a clear to pale yellowish liquid conforming to the Ph. Eur. monograph for description.

Identity and Assay of API

Chlorhexidine digluconate is the only API added to this drug product formulation. There are two techniques for identification and assay of chlorhexidine digluconate: high-pressure liquid chromatography (HPLC)-based and ultraviolet (UV) spectroscopy-based technique.

The identity and assay of the API can be determined using the HPLC based method specified in the Ph. Eur. monograph for identity (identification) and content (assay).

pH of API Solution

The pH of chlorhexidine digluconate API solution is an important factor that contributes to the rate of formation of 4CA and other drug-related impurities and hence is important to its stability. To minimize levels of 4CA and other drug-related impurities in the drug product, the pH of the input chlorhexidine digluconate API solution should be controlled within the range of 5.5–7.0 in alignment with the Ph. Eur. monograph.

Impurities in API

- **4CA:** The drug-related impurity 4CA (referred to as Impurity P in the Ph. Eur. monograph for chlorhexidine digluconate solution, 20% w/v) is a critical quality attribute (CQA) for this product, as it has been found to be genotoxic and carcinogenic in nonclinical studies (CICAD 2003), and it is considered possibly carcinogenic in humans. For this reason, 4CA was identified as an API CQA to be controlled at the API manufacturer itself. The 4CA limit in the supplier specification for the API solution is 20 ppm to ensure that the 4CA level in the input API is minimized. An acceptance criterion of NMT 150 ppm was allowed for a holding period of the API solution before drug product manufacture. Both the USP Monograph and the Eur. Ph. Monograph have a limit of NMT 500 ppm for the chlorhexidine digluconate solution, 20% w/v API.

  The degradation mechanism involves hydrolytic formation of 4CA from chlorhexidine as a major pathway in both acidic and alkaline conditions (Zong & Kirsch, 2012). Literature reports also indicate that 4CA is formed under conditions of prolonged thermal stress (Revelle et al., 1993). For this reason, heat during processing should be minimized (e.g., chlorhexidine should not be present when hydrating the gel at high temperatures).

  The level for 4CA stability for the API solution should be controlled within the tighter acceptance limit of 150 ppm for the API at time of use. Starting with a chlorhexidine digluconate solution, 20% w/v of not more than 20 ppm, the solution will stay below 150 ppm for at least 6 months at Zone II climatic conditions as determined from 25°C/60% RH stability data. This strategy should be considered to balance operational flexibility during manufacturing (6 months in the warehouse) and achieving a 2-year shelf life of the commercial sachet product to keep the 4CA level as low as reasonably practical.

- **Other API-related impurities:** Drug-related impurities content is also a CQA of the specification for the API solution supplier. All drug-related impurities in the API must be controlled in accordance with the levels permitted in the Ph. Eur. Monograph.

  Degradation of chlorhexidine digluconate solution, 20% w/v, occurs via several different mechanisms, including hydrolysis and condensation reaction; these mechanisms are inherent and unavoidable in the input API, which is an aqueous solution.

  In addition to the 4CA impurity, several of the specified impurities (e.g., G, N, and K) are formed via hydrolysis in the aqueous solution form of the API.

  Additional impurities are formed through other mechanisms, e.g., impurity J, which is formed by condensation reaction with D-gluconic acid and is a side reaction intrinsic to chlorhexidine digluconate containing products (Revelle et. al., 1993; Zong & Kirsch, 2012).

All these drug-related impurities are to be controlled to within Ph. Eur. Monograph limits in the API solution.

Excipients

The CHX gel consist of the following excipients:

- Guar gum
- Sodium acetate trihydrate
- Purified water

These specific excipients and their concentrations were selected for their characteristic properties that can influence drug product performance (e.g., physical appearance, stability) or manufacturability and were considered relative to the respective function of each excipient. Selection of suitable excipients was based on compatibility of excipients with the API and other additives. The ability of excipients to provide their intended functionality throughout the intended drug product shelf life was also considered.

2.2 Drug Product Formulation Development

Selection of Excipients

The choice of excipients for formulating CHX gel formulation was based on prior available literature and on the RLD product. Changes in formulation excipients or their content from the composition of the RLD were made based on experimental studies performed to ensure the benefits or suitability of the excipients, or in their concentration levels for impact on the quality (stability, performance) of the final drug product.

The prior knowledge employed by GSK to initiate the formulation development for CHX gel was provided by the PATH report for PATH 004 formulations. The PATH 004 formulation (RLD) contained guar gum, sodium hydroxide, benzalkonium chloride, and water in the final drug product of CHX gel.

Experimental studies revealed that use of benzalkonium chloride as a preservative leads to discoloration of the drug product. Moreover, the active substance itself exhibited a self-preserving nature. Therefore, benzalkonium chloride was omitted as an additive from the GSK CHX gel formulation.

Excipient compatibility studies revealed that sodium hydroxide, which was used in the PATH004 formulation to adjust pH and other pH modifiers, resulted not only in the formation of turbid solution, but also decreased the chemical stability of the drug product in reference to 4CA and total impurities content. 0.1% w/w sodium acetate trihydrate was found to be better suited to adjust the pH of the gel to the desired pH range of 5.5–7.0, providing better stability with respect to 4CA content.

The content of the guar gum was optimized to match to the RLD product to achieve the desired physical consistency.

Role of Excipients

The excipients selected for use in chlorhexidine digluconate gel are listed in Table 1.

Table 1. Role of Excipients Used in CHX Gel

Excipient Name	Function
Guar gum, Ph. Eur.	Thickening agent
Sodium acetate trihydrate, Ph. Eur.	pH modifier
Purified water, Ph. Eur.	Solvent

Guar gum

Guar gum used in the drug product conforms to the Ph. Eur. and was selected as a thickening agent. Guar gum is of natural origin derived from the endosperm of guar beans and is widely used in foods and oral and topical pharmaceutical formulations. Guar gum is obtained from guar seeds via a series of mechanical processes. During these processes, the seeds are broken, the germ is separated from the endosperm, the endosperms are dehusked, and refined guar splits are obtained. The refined splits are then treated and finished into powders. As a result of processing/milling of the guar gum splits, the presence of small amounts of undehusked or colored splits in the form of particles as an impurity in the finished guar gum powder is common.

The structure of guar gum consists of a linear backbone of linked units of mannose with galactose. The ratio of mannose to galactose is 2:1. Guar gum in small amounts can increase viscosity, making it an efficient thickening agent. The other advantage of using guar gum is that it is nonionic, so it is stable over a wide pH range and compatible with the API (chlorhexidine digluconate solution, 20% w/v). Guar gum can be used within the drug product formulation to achieve a low viscosity gel in the range of 3000–8000 cPs.

Some variability in material properties of guar gum is expected, as it is an excipient of natural origin. It is acknowledged that some of these properties, such as particle size, molecular weight, and galactomannan levels, could have an impact on the hydration properties of guar gum and thus the viscosity achieved. GSK's risk assessment included an assessment of these guar gum attributes on the manufacturing process and on drug product viscosity. In addition to the microbiology requirements stated in the Ph. Eur, guar gum should also be tested for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in line with requirements for products for topical use.

The drug product viscosity range of 3000–8000 cPs allows for any variation in the sourcing of guar gum as a natural material and still achieves a suitable gel that would be appropriate for topical application.

To prevent lumps from forming, high shear mixing should be used during the addition and dispersion of the guar gum, and the guar gum should be added in a controlled manner via vacuum assist over a minimum of 2 minutes. The guar gum mix should be heated to 65 ± 5°C for hydration of the thickener for a period of 40–120 minutes, with transient excursions of up to 72°C permitted. Hydration of the guar gum continues during this phase and is accelerated by the application of heat. To minimize 4CA formation, chlorhexidine should not be present during the heated hydration period.

Sodium acetate trihydrate

Sodium acetate trihydrate is a buffering agent selected to maintain the pH of the drug product within the ideal range of 5.5–7.0. Sodium acetate trihydrate content was evaluated at different levels based

on the ability to maintain the product pH in the range of 5.5–7.0, control 4CA and other drug-related impurities content, and assay during the manufacture and upon storage. The formulation containing sodium acetate trihydrate at 0.1% w/w was shown to maintain the chemical stability of drug product in reference to total impurity and 4CA content in the CHX gel product (over the product shelf life of 2 years). Beyond the shelf life of 2 years, the level of 4CA exceeds recommended levels.

Purified water

Purified water is used as the vehicle for the continuous phase in CHX gel.

CHX Gel Manufacturing, Filling, and Primary Packaging Process Development

The manufacturing process for CHX gel consists of two unit operations:

- Gel manufacture
- Filling/sealing into the primary packaging sachets

CHX Gel Manufacture

The gel is manufactured by dispersing and hydrating guar gum with heating in an aqueous solution of sodium acetate trihydrate, prior to the addition and mixing of the chlorhexidine digluconate API solution.

Composition of CHX gel

The formulation of CHX gel consists of chlorhexidine digluconate (20% w/v solution), guar gum, sodium acetate trihydrate, and purified water. The detailed composition of the drug product is presented in Table 2.

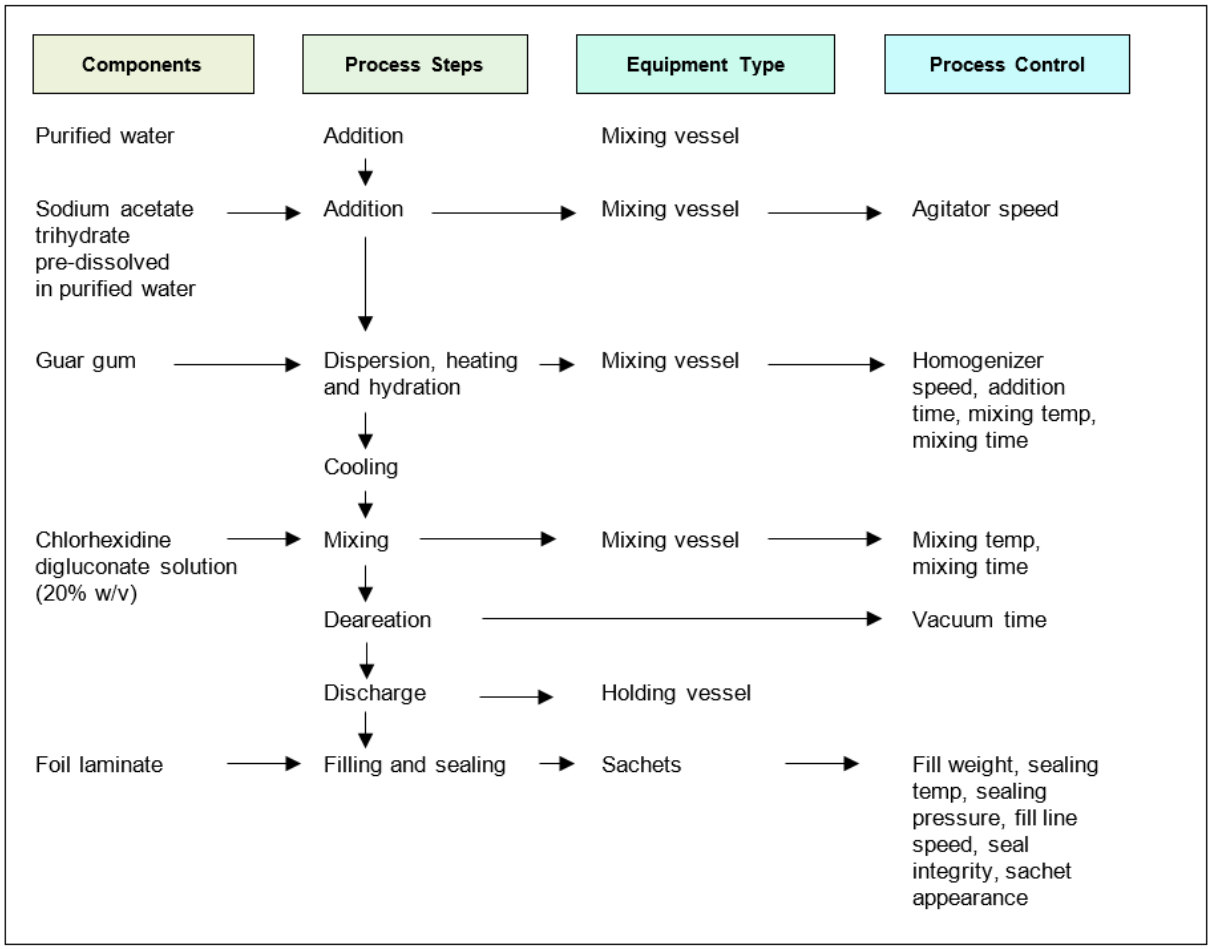
Table 2. Composition of the CHX Gel

Material	% w/w
Chlorhexidine digluconate solution, 20% w/v, Ph. Eur.	37.81*
Guar gum, Ph. Eur.	1.40
Sodium acetate trihydrate, Ph. Eur.	0.10
Water purified, Ph. Eur.	QS to 100

** May be adjusted for potency.*

The manufacturing process for CHX gel is represented in Figure 1.

Figure 1. Manufacturing Process for CHX Gel Flow Chart



CHX Gel Filling and Sealing into the Primary Packaging of Sachets

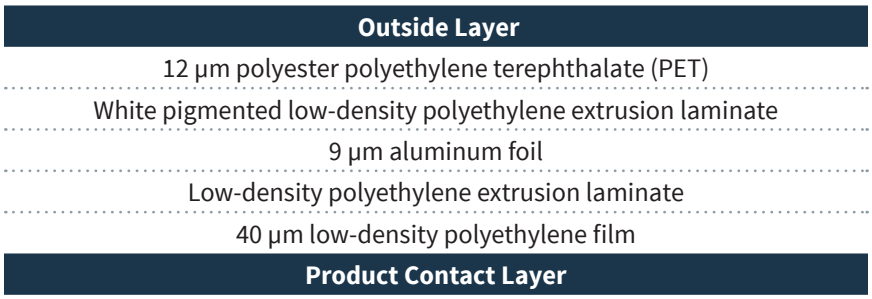
The resulting gel is filled into foil laminate sachets by an automated filling and sealing piece of equipment. The fill weight of the dispensed gel was determined experimentally. The filled sachets are opened, the product is squeezed out, and the delivered weight is measured. The fill weight filling operation should be controlled to 3.0g–3.6g delivered. The target fill weight for sachets was developed to be 3.3g (range 3.0g–3.6g) to ensure that a minimum of 3.0g gel is delivered from the sachet.

Primary Packaging Sachet Material of Construction

Aluminum foil laminate sachets should be used for packaging CHX gel. The selected laminate should provide good compatibility with liquid products, adequate moisture, and light protection, as well as have an acceptable safety profile. The foil laminate sachet should provide the unit dose presentation that will maintain drug product integrity over the shelf life, encompassing storage, transportation (shipping), and use of the product, and must be able to be opened without scissors. The sachet size should be enough to hold usage/pictorial information.

The sachet material selected by GSK had a structure that consisted of five laminated layers of material as illustrated in Figure 2. The foil laminate sachet material selected complies with the applicable European Directive No. 10/2011 for food contact and Ph. Eur. 3.1.3 Polyolefins.

Figure 2. Foil Laminate Structure and Materials of Construction



Evaluation of Primary Packaging for Extractables and Leachable

A risk assessment of all potential sources of leachables entering the drug product should be undertaken. This risk assessment should evaluate potential leachables from the container closure system and any potential contribution from the manufacturing process. Specifically, the following areas should undergo a risk assessment: foil laminate material, filling and sealing processing of foil laminate, and gel bulk manufacture.

Critical Quality Attributes and Their Control Strategy for CHX Gel Manufacturing

The drug product's CQAs are the measurable properties of the drug product that could impact efficacy and/or patient safety. The critical process parameters (CPPs) are processing conditions that impact the CQAs.

Chlorhexidine digluconate degrades via hydrolysis with multiple degradation pathways and generates a range of impurities. The most critical of these potential impurities is 4CA as the final degradation product, which has been shown to be genotoxic and carcinogenic in nonclinical studies. The 4CA impurity, which is also referred to as impurity P, is known to increase with time and temperature and is impacted by the pH of the gel. 4CA content in the drug product can be minimized via the following measures:

- Controlling pH and 4CA level in the input API
- Selection of compatible excipients
- Minimizing heat exposure of CHX
- Suitable storage instruction
- Assurance of drug product quality by release of product within a specified pH range and against a specification for a maximum 4CA level

The maximum levels of 4CA in a drug product at release should be less than 800 ppm and at the end of the 24-month shelf-life, 4CA should be less than 3500 ppm.

The Design of Experiment (DoE) approach was followed for the drug product development to increase understanding of the limits and variables of the established manufacturing and filling processes.

The quality target product profile (QTPP) for this product was developed based on the PATH 004 gel formulation. The QTPP for chlorhexidine digluconate gel, 7.1% w/w, is presented in Table 3.

Table 3. Quality Target Product Profile for CHX Gel

Dosage form and strength	A single-unit dose topical gel, 3g of a 7.1% w/w gel containing 213mg of chlorhexidine digluconate API for a single application for 1 to 7 days. A low-viscosity gel that is easy to apply to the affected site and able to be filled into suitable single-use containers.
Drug product CQAs	Description, identity, content, minimum fill, drug related impurities, 4CA, and pH.
Drug delivery and release criteria	The drug needs to be delivered topically to the surface of the skin.
Container closure system	A container closure that facilitates single-dose use containing the topical gel and provides protection to the product from light and moisture.
Stability criteria	Components of the drug product (active and inactive ingredients) must be physically and chemically compatible with the requisite functional characteristics to ensure appropriate stability of the drug product over the shelf life of not less than 2 years under climatic zones II to IV.

The manufacturing process for CHX gel 7.1% w/w involves dissolution of sodium acetate trihydrate in water followed by dispersion and hydration of guar gum in it. The solution must be heated at this stage to aid hydration of the guar gum. The resultant gel is then cooled to room temperature. This is followed by the addition and mixing of chlorhexidine digluconate API solution. Subsequently, the gel is deaerated using a vacuum and then discharged into a holding vessel prior to being filled into foil laminate sachets using suitable form-fill-seal packaging equipment. The drug product CQAs for CHX gel, together with their corresponding control strategies, are provided in Table 4.

Table 4. Summary of the CQA Control Strategy for the Manufacture of CHX Gel

Input Materials	Gel Manufacture	Filling and Sealing	Drug Product Release Tests	Drug Product CQAs
Chlorhexidine digluconate solution description	Mixing temperature (guar gum)	Sachet appearance	Description	Description
Chlorhexidine digluconate solution identity			Identification	Identity
Chlorhexidine digluconate solution content	Mixing temperature (guar gum)	Fill weight, seal integrity, sealing temperature, sealing pressure, filling line speed	Content	Content
	Mixing temperature (guar gum)	Fill weight, seal integrity, sealing temperature, sealing pressure, filling line speed	Minimum fill	Minimum fill
Chlorhexidine digluconate solution drug-related impurities chlorhexidine digluconate solution pH			Drug-related impurities	Drug-related impurities
Chlorhexidine digluconate solution 4CA level Chlorhexidine digluconate solution pH			Drug-related impurities	4CA
Chlorhexidine digluconate solution pH			pH	pH

The drug product CQA is impacted by a CPP and/or an input or in-process material CQA and/or drug product release testing is performed.

The drug product CQA is not impacted by a CPP and/or an input or in-process material CQA.

Control Strategy of 4CA Levels in CHX Gel

In agreement with the principles of a risk-based approach, the primary focus must be control of the level of the hydrolysis-mediated impurity, 4CA. This impurity has been demonstrated to be genotoxic and carcinogenic in nonclinical studies. It is considered possibly carcinogenic in humans, and its level in the final drug product is a drug product CQA. Formation rate of 4CA is closely linked to pH, increasing significantly outside of the 5.5–7.0 pH range and with exposure to high temperatures. The release specification for pH of this drug product was set at a tighter level of 5.5–6.5, and the shelf life specification was set at 5.5–7.0.

Control Strategy of Other Drug-Related Impurities in CHX Gel

Other drug-related impurities content is an identified CQA of the drug product. It should be consistent with the known impurities in the API being controlled in the drug product release specification to the limits specified in the Ph. Eur. It has been found that the levels of other drug-related impurities also increase with increasing pH, as is the case with 4CA. Due to the mechanism of formation and the nature of the drug product (an aqueous gel), formation of the specified impurities (G, N, K, and J) is inherent and unavoidable and will therefore increase on storage. Thus, shelf life specification for these impurities was developed to take into account the increase in these impurities on storage over the proposed shelf life at 30°C/35% RH, as well as the release acceptance criteria for the drug product.

To control the above two CQAs, the following two control measures should be kept in compliance to minimize and control the levels of 4CA and other drug-related impurities in the drug product:

- The pH of the drug product at release is controlled to pH 5.5–6.5. The input API in this product, chlorhexidine digluconate solution, 20% w/v, should comply with Ph. Eur. pH specification of 5.5–7.0, and its shelf life stability data should be provided by the supplier.
- The acceptance criterion for 4CA for API solution manufacture was set at “Not Greater Than 150 ppm” for chlorhexidine digluconate API (more stringent than the 500 ppm as defined in the Ph. Eur.).

Risk Assessment and Risk Mitigation of Process CQAs

The principal risks identified during development are presented in Table 5. The risks are listed against the relevant unit operation, with the drug product CQA potentially impacted. Table 5 also presents the outcome of the risk assessment (i.e., the mitigation of the risk), with a discussion of the controls implemented to reduce the risk to acceptable levels, and includes a reference to the section where the pertinent process step and development of these controls is presented.

Table 5. Principal Risks Identified During Development of CHX Gel and Their Mitigations

Risks identified	Drug product CQA	Risk mitigation
Process Step: Gel manufacture		
Insufficient mixing time, shear, and/or inadequate temperature, combined with an uncontrolled and/or inappropriate guar gum addition procedure, may result in a nonhomogeneous gel with lumps, which will only slowly hydrate, thus impacting the drug product CQAs of description and (by potentially impacting the filling process) the drug product CQAs of content and minimum fill.	Description content minimum fill	The target was to develop a robust, controlled, and appropriate addition procedure for guar gum, followed by its rapid dispersion and subsequent hydration. To ensure rapid and sustained dispersion of the guar gum, thus facilitating its subsequent hydration, turbulent mixing conditions must exist and be maintained in the vessel system. For this reason, a vessel fitted with a high shear mixing capability was selected. To further provide thorough mixing and ensure temperature uniformity in the vessel during the addition, dispersion, and hydration stages, a vessel with a low-intensity mixer was selected and was kept running throughout to ensure adequate mixing. The most operationally effective method of addition of guar gum that minimizes batch processing times, involves addition of guar gum using vacuum, either directly into the base of the homogenizer (where it would be rapidly dispersed) or into a recirculation loop (where it would be rapidly returned to the vessel and be dispersed by the homogenizer). In addition, during initial dispersion, to reduce the propensity for guar gum particles to adhere together and thus form lumps, the sodium acetate trihydrate in purified water solution vehicle within the manufacturing vessel was to be maintained at ambient temperature. To accelerate hydration time of the guar gum and reduce propensity to form lumps, an elevated mixing temperature of 65 ±5°C, with transient excursions to 72°C allowed, was maintained and was found to be effective. Mixing temperature (guar gum) is identified as a CPP. The time that the guar gum was held at 65 ±5°C, with transient excursions to 72°C, was not identified as a CPP, but it has a role in ensuring hydration of the guar gum. A mixing time of 40–120 mins was developed as a proven acceptable range (PAR). The mixing temperatures and time in the gel manufacturing process were found to be scale independent. The defined mixing times were found to be effective in hydrating the guar gum. Overall, the risk associated with the gel manufacture unit operation is mitigated by the manufacturing process controls defined.

Risks identified	Drug product CQA	Risk mitigation
Air entrapment in and/or foaming of the gel during manufacturing, causing variability in the quantity of gel delivered by the filling nozzles during filling, resulting in fill weight uniformity issues and thus impacting the drug product CQAs of content and minimum fill.	Description content minimum fill	The target is to develop a robust gel manufacturing process that would ensure a minimum of foaming and air entrapment during manufacture. In initial campaigns with less operationally capable equipment, adding the API, chlorhexidine digluconate solution, 20% w/v, prior to the guar gum where less liquid overall was present in the vessel, led to an increased propensity for foaming. The order in which APIs was added was adjusted so that the chlorhexidine digluconate solution, 20% w/v, is added after dispersion and hydration of the guar gum, thus ensuring that foaming of the surface-active chlorhexidine digluconate API can be minimized. The final stage of the gel manufacture unit operation involves a deaeration step to further minimize any air entrapment and foaming. Overall, the aeration risk associated with the gel manufacture unit operation was mitigated by adjusting the order of addition in the manufacturing process controls.
Insufficient mixing time and/or shear to ensure complete mixing of the chlorhexidine digluconate solution, 20% w/v, with the hydrated guar gum, resulting in variable content of chlorhexidine digluconate in the filled sachets due to nonhomogeneous distribution of chlorhexidine digluconate in the gel.	Content	The target was to develop a robust gel manufacturing process that would ensure the homogeneity of chlorhexidine digluconate content. For the commercial site, the process must use both the agitator system and the homogenizer with recirculation through a recirculation loop to deliver an operationally effective method to ensure chlorhexidine digluconate homogeneity. Overall, the risk associated with the gel manufacture unit operation of mixing was mitigated by the manufacturing process controls in the optimization in mixing technology.

Risks identified	Drug product CQA	Risk mitigation
Degradation of the chlorhexidine digluconate, principally to 4CA, upon exposure to temperatures above ambient during the manufacturing process.	4CA	Chlorhexidine digluconate was found to be stable at the temperatures exposed during the manufacturing process. Manufacturing should be carried out in a temperature-controlled vessel, which ensures that the temperature is maintained within a narrow range (25°C ±2°C, with transient excursions to 33°C allowed) during and following addition of the chlorhexidine digluconate solution. In addition, consideration was given to the potential exposure of chlorhexidine digluconate to elevated localized temperatures during the manufacturing process, particularly from the homogenizer. It should be noted that heat will be dissipated into the product as it passes through the homogenizer head and a temperature rise will be seen in equipment with no or inadequate cooling and/or where the homogenizer was run for long periods at high speeds. Hence, a homogenizer equipped with a recirculation loop should be used during mixing of the chlorhexidine digluconate solution, e.g., the defined process at the commercial site. The high flow rate of product through the homogenizer, coupled with a very short residence time, ensures that any localized product temperature increases are limited and therefore that thermal degradation of the active substance does not occur. Recirculation of the product being homogenized will ensure that the temperature stays within appropriate limits.
Process Step: Filling and sealing		
Inappropriate sealing conditions to ensure a robust seal of the sachet, potentially resulting in leaking sachets, thus impacting the drug product CQAs of description, content and minimum fill, or in extreme cases, potentially impacting foil delamination and the drug product CQA of drug-related impurities to potential exposure to the environment.	Description content minimum fill and drug-related impurities	The risk mitigation strategy was to develop a robust sachet sealing process design space development via DoE that provided the optimized balance of CPPs to seal the foil laminate materials, resulting in an integral sachet. Sealing temperature, filling line speed, and sealing pressure have been confirmed as CPPs, with a design space proposed for sealing temperature (140°C–160°C) and filling line speed (20–40 cpm), with a minimum limit defined for pressure (NLT 4.2 bar).

3. Commercial Manufacturing Including Primary Packaging

3.1 Commercial Bulk Manufacturing Process

Based on the optimized formulation process described in the development section, scaled-up bulk manufacturing process for 210 kg batches of commercial scale can be summarized as follows:

- A portion of purified water is to be added to the vessel.
- The sodium acetate trihydrate is to be dissolved in a further portion of purified water and added to the manufacturing vessel at ambient temperature and mixed (an agitator speed of 30 rpm should be used, with the agitator remaining at this setting throughout manufacture).
- The guar gum should then be added to the vessel in a controlled manner while mixing with the agitator and with the homogenizer in the “on” position. The guar gum should be added via a recirculation loop over a period of not more than 5 minutes using a vacuum of −0.6 to −0.2 bar (note that vacuum level can fall during guar gum addition), with the homogenizer being operated at 3000 rpm, to achieve turbulent flow for adequate mixing.
- The suspension with dispersed guar gum should be heated to 65 ±5°C, with the agitator and the homogenizer turned on at a higher speed (4000 rpm) for a minimum of 40 minutes for the hydration of the guar gum.
- The hydrated guar gum solution should then be cooled to 25 ±2°C.
- The API chlorhexidine digluconate 20% solution is then added with the temperature controlled during addition and mixing to 25 ±2°C, with transient excursions allowed to 33°C.
- The resultant CHX gel should be mixed through a recirculation loop for a period of at least 20 minutes to ensure homogeneity.
- The CHX gel should be deaerated under vacuum.
- The batch is discharged into a suitable holding vessel prior to filling.

Critical Processing Parameters for the Bulk-Manufacturing Process

The CPPs of the above briefly described bulk manufacturing process of CHX gel are detailed below:

- The guar gum powder must be added to the aqueous phase (sodium acetate trihydrate dissolved in purified water) in the vessel system in a controlled manner, i.e., via an appropriate method of entry into the vessel system and at a controlled rate of addition. During the addition stage, mixing conditions in the vessel system must be maintained in a turbulent state to ensure that the powder entering the system is rapidly dispersed, minimizing the potential of powder aggregates to form. It is at this stage that the guar gum will start to hydrate.
- Once addition of the guar gum into the vessel system is complete and a homogenous dispersion achieved, the guar gum rate of hydration is accelerated by heating the product. The guar gum aqueous dispersion should be typically heated to a temperature of 65 ± 5°C, with transient excursions up to 72°C allowed. During this guar gum hydration stage, turbulent mixing conditions should prevail within the vessel system, ensuring that the guar gum stays well dispersed as it continues to hydrate. For CHX gel at the commercial 220 kg batch size, a homogenizer speed of 4000 ±200 rpm during the hydration step is recommended.

- Once at 65 ±5°C (with transient excursions allowed up to 72°C), the product continues to be mixed under turbulent/high shear conditions for a defined time period; a PAR for the mixing time of 40–120 minutes has been found to be qualified.
- After this defined time period of heated mixing, the resulting bulk product should be a homogenous and lump-free hydrated guar gum aqueous gel. The product should be cooled to 25 ±2°C (with transient excursions allowed to 33°C), to avoid exposing the API chlorhexidine digluconate to increased temperatures when it is added.
- The chlorhexidine digluconate solution, 20% w/v, is then added and mixed. Mixing should be carried by employing a low-intensity bulk agitator and the homogenizer operating at a lower shear setting, facilitating an efficient mixing through the recirculation loop. The CPPs for this critical mixing step should be qualified during the scale-up process, customized to the equipment employed.
- A low vacuum is applied on the head space during all of the above stages, once addition of the guar gum is complete, to minimize air entrainment, followed by a final deaeration using the applied vacuum step once chlorhexidine digluconate solution, 20% w/v, is incorporated. The vacuum level and final deaeration time period CPPs for this critical deaeration process during the API incorporation step should be qualified during the scale-up process, customized to the equipment employed.
- The resultant CHX gel product should then be discharged from the vessel system into the holding tank, in readiness for filling. The CPPs for the bulk product holding time in the holding vessel during the filling process should be qualified during the scale-up process, customized to the equipment employed.

Manufacturing Process Developed at Scale of 210kgs

The CPPs for manufacturing of CHX gel for 210 kg batch size, based on equipment employed by GSK, are presented in Table 6. However, the specific CPPs will vary depending on the batch size and the equipment available at the manufacturing site.

Table 6. Gel Manufacturing Process Scale-Up Controls

Variables	CPPs for 210kg Batch Size
Agitator speed	30 +5 rpm
Homogenizer (guar gum addition)	3000 +150 rpm
Addition time (guar gum)	Minimum of 2 mins
Homogenizer (guar gum mixing)*	4000 +200 rpm
Mixing temperature (guar gum)	65 + 5°C, with transient excursions allowed to 72°C
Mixing time (guar gum)*	40–120 mins
Homogenizer (chlorhexidine digluconate mixing)*	3000 +150 rpm
Chlorhexidine digluconate solution addition and mixing temperature	25°C ±2°C, with transient excursions to 33°C
Chlorhexidine digluconate solution mixing time	Minimum of 20 mins
Deaeration vacuum level	Minimum of −0.5 bar
Deaeration vacuum time	Minimum of 15 mins

** critical steps to be considered while batch manufacturing*

3.2 Commercial Filling and Sealing Processes of CHX Gel in Sachets

Each sachet is sealed on three sides immediately prior to filling. Following gel manufacture, the sachets were filled and sealed into foil laminate sachets at GSK, as discussed and detailed in section 3.2.2.3, using an automated form-fill-seal packaging machine. The sachets were secondary packaged in sachets or boxes according to the marketing requirements.

The CHX gel product was transferred from the conical holding vessel to the filling machine receiving vessel using a transfer pump. The automated filling machine dispensed product evenly through a six-syringe/needle assembly into the six foil laminate sachets (primary package) at a time. The sachets were then sealed and further packaged into Correx boxes (secondary package).

The sachets were filled to achieve a fill weight target of 3.3g (range 3.0g–3.6g). The amount of gel filled into each sachet was checked at regular intervals during the filling runs. The sachets were also checked at regular intervals during the filling runs for the in-process material CQAs of *seal integrity* and *sachet appearance*. Sealing pressure was maintained above a minimum level not less than (NLT) 4.2 bar, defined through equipment qualification studies using a representative foil laminate.

Filling performance was evaluated and optimized across all the filling heads on the two development batches, three stability batches, and registration exhibit batches, all manufactured at the commercial site using commercial equipment.

An online check weighing system was used to determine the fill weight of the gel in the sachet. Sachets were sampled from the filling line throughout the batch from each filling head. Each filling head was sampled at a minimum of every 15 minutes for the defined CQAs, i.e., the fill weight and seal integrity.

An at-line integrity tester was used to determine the seal integrity of the CHX gel sachets. Sachets were loaded into the integrity tester machine nests, and a minimum pressure of 0.4 MPa was applied for 10 seconds. After each cycle, the sachets were examined for seal integrity.

The sachets with satisfactory CQAs viz. fill weight, seal integrity, and sachet appearance were achieved using filling and sealing processes controlled with the CPPs listed in Table 7.

Table 7. CPPs for Filling and Sealing Process Controls Using **Marchesini MS235 Sachet Filling Machine**

Variables	CPPs Value Range
Fill weight	3.0 g–3.6 g
Filling line speed	20–40 cycles per minute (cpm)
Sealing temperature	140°C–160°C
Sealing pressure	NLT 4.2 bar
Sachet integrity	Confirm sachet integral
Sachet appearance	Confirm satisfactory sachet appearance

4. Manufacturing Premises and Equipment

4.1 Premises

Since 4CA is a carcinogenic impurity, premises controls should be defined. The measures employed to control the formation of this impurity for incoming API and during manufacturing processes have already been addressed in this technology transfer report. The manufacturer would need to assess the toxicological aspects such as permitted daily exposure (PDE) and additional equipment and facility controls after consulting the appropriate regulatory authorities.

4.2 Manufacturing Equipment for Compounding and Filling CHX Gel in Sachets

The manufacturing process for CHX gel should utilize a suitably sized manufacturing vessel, e.g., for a batch of 210 kg, a 250 L Becomix manufacturing vessel was used at GSK. The Becomix manufacturing vessel was a sealable stainless steel vessel fitted with a variable speed, bidirectional homogenizer, and variable speed agitator. The jacket of the vessel was supplied with water for heating and cooling purposes. The vessel had a semiautomatic PLC control system to control the operation of the vessel viz. a Siemens PCS7 controller. The vessel was supplied with purified water at 80°C via a metering valve. The vessel was also supplied with a vacuum and compressed air for material transfer purposes. Intermediate bulk storage stainless steel conical holding vessels with a capacity of 200–300 kg for storage were employed to store the CHX gel product prior to filling. GSK is in the process of validating a 1-ton scale manufacturing process.

CHX GEL Bulk Product Manufacturing Equipment Attributes

All product contact equipment used during manufacture of the bulk CHX gel product should have the following attributes:

- Geometry to promote good mixing throughout the entire vessel.
- Ability to use a vacuum to add solid or liquid raw materials in a controlled manner and for deaeration.
- Homogenizer designed for the dispersion of one material phase into another.
- Mixing system that ensures good mixing within the vessel while minimizing vortices.
- External heating and cooling jacket.
- Temperature control.
- Appropriate vessel base design, typically spherical or conical, to eliminate dead spaces during mixing and ensure good product recovery on discharge.
- Pressure rating appropriate to manufacturing conditions (e.g., vacuum).

The equipment attributes considered and optimized for the development of CHX gel bulk product manufacture were as follows.

Raw material addition routes

- The vessel was designed to allow raw materials to be added under controlled conditions by using a vacuum assisted addition system.
- Powder addition is typically added to the material under turbulent mixing conditions to ensure dispersion of material into the bulk product. This objective was achieved by adding powder directly through the homogenizer with a vacuum assist, where it was then rapidly dispersed into a fast-flowing aqueous stream. The fast-flowing aqueous stream was achieved by recirculating the aqueous medium through an external recirculation loop.
- The bulk agitation and homogenizer mixing systems were optimized to ensure rapid dispersal of the added material throughout the entire bulk product, ensuring product homogeneity during the hydration stage of the guar gum.

Vacuum system

- The vacuum system creates a negative in the containers for raw material transfer into the compounding vessel. The vacuum system is also utilized to deaerate the product during bulk manufacture.
- A control system that allows a specific vacuum to be pulled to match the requirements of the product and the process.

Homogenizer high-intensity mixing system

- A rotor-stator-based “homogenizer” designed to disperse one material phase (e.g., solid) into a second (e.g., liquid) was used. Mixing conditions within the homogenizer will be highly turbulent (high intensity), and the associated high shear rate will drive the dispersion processes.
- The homogenizer will provide product recirculation within the manufacturing vessel assisted. External recirculation loops will distribute the product from the bottom to the top of the vessel. This will ensure the bulk product is well mixed and passes through the homogenizer.
- The homogenizer design and size should be selected by the manufacturer to match the capacity of the vessel, both from providing sufficient shear/turbulence to a semisolid preparation at maximum capacity of the manufacturing vessel, as well as to affect a sufficiently high flow of semi-solid through a recirculation line, if present and if used during product manufacture. It should be noted that the heat will be dissipated into the product as it passes through the homogenizer head. The flow of the product (and residence time in the homogenizer) will limit temperature rise coupled with temperature jacket control of the bulk vessel.

Bulk low-intensity mixing system

- A mixing system that ensures gentle bulk mixing within the vessel while minimizing vortices that could entrain air into the product should be used. This is typically achieved through the use of counter-rotating agitators or a single agitator and vortex/flow breakers or baffles.
- Wall scrapers mounted on the agitation system would need to be employed, which would promote effective transfer of heat from the vessel jacket into the product. The scrapers also would prevent the build-up of material on the jacket wall (e.g.. viscous material during cooling).

Vessel heating/cooling system

- The vessel should be fitted with an external jacket, allowing the product to be heated and cooled by circulating hot or cold water through the jacket as per the requirements of the manufacturing process.

Filling Equipment Attributes

- Filling for CHX gel is to be performed on an automated sachet filling machine designed to fill sachets in the weight range of 3.0g–3.6g. An example of this automated sachet filling equipment is the Marchesini MS235 filling and bulk packaging line or equivalent. The product would be transferred from the conical holding vessel to the filling machine receiving vessel using a transfer pump like the Avery Berkel make.

5. Analytical Specification and Methods for CHX Gel

The quality of the manufactured CHX gel formulation should be assessed based on the below mentioned tiered specifications at different stages of manufacturing and during the shelf life of the product.

Table 8. Specifications for CHX Gel Drug Product

CQA parameters	In-process specifications (acceptance criteria)	Release specification (acceptance criteria)	Shelf life specifications (acceptance criteria)
Description		A colorless to yellow, translucent gel essentially free from visible particles	A colorless to yellow, translucent gel essentially free from visible particles
Identification of chlorhexidine digluconate by: HPLC UV		a. Retention time of chlorhexidine peak in the sample solution should be within ± 3% of retention time of chlorhexidine in reference solution preparation b. UV maxima of chlorhexidine should be within ±3 nm relative to reference standard.	
pH		5.5–6.5	5.5–7.0
Apparent viscosity by viscometer (cPs)		3000–8000 cPs	3000–8000 cPs
Minimum fill	Target: 3.3 g Range: 3.0 g–3.6 g	As per USP <755> Minimum Fill	As per USP <755> Minimum Fill
Chlorhexidine digluconate content by HPLC (%w/w)		6.75–7.45 % w/w (95.0% to 105.0% of label claim)	6.39–7.45% w/w (90.0% to 105.0% of label claim)
Seal integrity	Confirm sachet integral (pass/fail)		
Sachet appearance	Confirm satisfactory sachet appearance		

CQA parameters	In-process specifications (acceptance criteria)	Release specification (acceptance criteria)	Shelf life specifications (acceptance criteria)
Drug-related impurities content by HPLC (% w/w)			
4CA (Impurity P)		NMT 0.08%	NMT 0.35%
1-(6-aminohexyl)-5-(4-chlorophenyl) biguanide (Impurity G)		NMT 0.3%	NMT 0.6%
1-[6-(carbamimidoylaminohexyl] -5-(4chlorophenyl) biguanide (Impurity N)		NMT 1.0%	NMT 2.0%
1-(4-chlorophenyl)-5-[6-[[4-[(4-chlorophenyl) amino]-6[(1S,2R,3R,4R)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl] amino] hexyl] biguanide (Impurity J)		NMT 0.4%	NMT 0.7%
1,1-[iminobis(carbonimidoyliminohexane-6,1diyl) bis [5-(4-chlorophenyl) biguanide] (Impurity H)		NMT 0.5%	NMT 0.5%
1-(4-chlorophenyl)-5-[6[cyanocarbamimidoyl)amino] hexyl] biguanide (Impurity A)		NMT 0.4%	NMT 0.4%
Sum of Impurity (I+O), 5-(2-chlorophenyl)-5'-(4chlorophenyl)-1&1'-(hexane-1,6-diyl) dibiguanide		NMT 0.4%	NMT 0.4%
N-(4-chlorophenyl)-N'-[[6[[[(4chlorophenyl) carbamimidoyl] carbamimidoyl] amino] heyxl] carbamimidoyl] urea (Impurity K)		NMT 0.4%	NMT 0.7%
N-(4-chlorophenyl) urea (Impurity F)		NMT 0.2%	NMT 0.3%
Any other specified impurities*		NMT 0.2%	NMT 0.2%
Any other unspecified impurities		NMT 0.1%	NMT 0.2%
Total Impurities		NMT 2.0%	NMT 4.0%
Microbial content		Complies with harmonized pharmacopoeia	
Total aerobic microbial count (TAMC)		$\leq 10^2$cfu/g	$\leq 10^2$ cfu/g
Total combined yeasts/mold count (TYMC)		$\leq 10^1$ cfu/g	$\leq 10^1$ cfu/g
Staphylococcus aureus		Absent in1g	Absent in1g
Pseudomonas aeruginosa		Absent in1g	Absent in1g

** Any other specified impurities include:*

1. *1-[[6-[[[(4-chlorophenyl) carbamimidoyl] carbamimidoyl] amino] hexyl] carbamimidoyl] urea (Impurity B)*
2. *(5R,6S)-2-[(4-chlorophenyl) amino]-5-hydroxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]-5,6-dihydro-4H-1,3-oxazin-4-one (Impurity L)*
3. *Impurity Q*

5.1 Justification of Product Specifications

Release and shelf life specifications are provided for CHX gel. The release specification is applied at the time of manufacture, and the shelf life specification is applied throughout the product life. Both specifications include tests for description, chlorhexidine digluconate content, pH, apparent viscosity, drug-related impurities content (including 4CA), and microbial limits test. In addition, the release specification includes a test for identification and minimum fill.

The justification for the finished product release and shelf life specification acceptance criteria proposed for CHX gel is described below.

The proposed drug product specifications were defined by taking into consideration ICH Q6A and ICH Q3B (R2), as well as available release and stability data on CHX gel by GSK. The specification is an integral part of the overall control strategy for the manufacture of the CHX gel.

Description

Each batch of gel should be visually examined to determine the appearance of the material. The acceptance criterion is a colorless to yellow translucent gel, essentially free from visible particles. The acceptance criteria for release and shelf life specifications are set to ensure the gel has an acceptable appearance for use. The acceptance criteria for description are based on data from development batches manufactured by GSK and fully describe the visual appearance of the product containing the materials used in the formulation.

Identification of Chlorhexidine Digluconate by HPLC Retention Time and HPLC UV Maxima

Identification of the active ingredient in the CHX gel product should be carried out during batch release. Based on ICH Q6A, one specific method or two methods utilizing different principles are acceptable for identification. The first method for identification of chlorhexidine can be by HPLC by comparing the retention time of chlorhexidine peak in reference standard and sample solutions.

The second method for identification of chlorhexidine can be carried out by comparing the UV spectrum of standard and sample solutions using the HPLC diode array detector.

pH

The acceptance criteria for pH are set to ensure maximum stability of the active ingredient, chlorhexidine digluconate. Chlorhexidine digluconate stability is optimal between pH 5.5 and 7.0, as the rate of degradation of chlorhexidine digluconate increases above pH 7.0 and below pH 5.5.

The shelf life specification for the pH of the drug product is set at 5.5–7.0. The stability data of the development batches made at GSK for drug product indicates a slight increase in the pH following storage under accelerated conditions. Thus, the pH of the drug product is controlled within a range of 5.5–6.5 at the time of release to allow for the increase in pH over the shelf life of the product.

Apparent Viscosity

The acceptance criteria for apparent viscosity for the drug product is set to ensure the product is of a suitable consistency for use by patients. The drug product at release should be controlled within a viscosity range of 3000–8000 cPs. The viscosity range of 3000–8000 cPs is typical for low viscosity pharmaceutical gels (Buhse, 2013., David B. Troy, 2006). No change in the apparent viscosity was observed

during the stability data of the development batches made at GSK for the drug product at the long-term storage or accelerated storage conditions. Therefore, the shelf life specification for viscosity can also be controlled within the range of 3000–8000 cPs.

Minimum Fill

Since the minimum fill test is designed to ensure that an appropriate quantity of gel is filled into the sachet, the minimum fill test was performed at release only. The acceptance criteria were set in accordance with the requirements of USP <755. For primary and supportive stability studies, however, the sachet weight change upon storage should be monitored.

Chlorhexidine Digluconate Content

A specific, stability-indicating HPLC method was used to determine the chlorhexidine digluconate content in the CHX gel formulation. The release limits were set at 95.0% to 105.0% of the nominal concentration of chlorhexidine digluconate, while the shelf life specification was set at 90.0% to 105.0% of the nominal concentration. The stability specification was to allow for a reduction in the chlorhexidine digluconate content expected over the shelf life of the product due to hydrolysis of the API. The GSK development batches release data indicate that typical assay levels at the time of release fell in the range 99.0%–102.0% of the label claim, while after storage for 24 months at 30°C/35% RH the chlorhexidine digluconate content results were in the range of 98%–104% of the label claim.

Drug-Related Impurities Content

The drug-related impurities content of CHX gel was determined using a reversed-phase gradient HPLC method, shown to be stability indicating. Chlorhexidine digluconate is known to degrade principally by hydrolysis in the absence of light, both in the API (chlorhexidine digluconate solution, 20% w/v) and in the drug product, CHX gel (Revelle et al., 1993., Zong and Kirsch, 2012). The impurities generated by the hydrolysis mechanism are well characterized. The hydrolysis mechanism is known to be impacted by pH, which is why both the API and the drug product pH are controlled between 5.5 and 7.0 (5.5–6.5 at release for drug product), where hydrolysis is known to be minimum. The relevant Ph. Eur. acceptance criteria, which are also in line with this pH range, should be applied to ensure the quality of the incoming API. This will help ensure the quality of CHX gel at release and throughout the shelf life. A tighter specification for 4CA content is proposed for the input API, which is aligned with the proposed 4CA impurity content specification for the drug product at the time of release. The specification is supported by the product and process knowledge presented pharmaceutical development, including formulation and manufacturing risk assessments and the control strategy.

Specified Identified Impurities

4 Chloroaniline (4CA)

4CA is a genotoxic impurity that is formed via hydrolysis of chlorhexidine. Both the input API, chlorhexidine digluconate solution, and the drug product, CHX gel, are aqueous based. 4CA was observed at quantifiable levels in the drug product batches throughout development. The 4CA release and shelf life specifications for CHX gel are based on:

- Controlling the 4CA level in the input chlorhexidine digluconate solution, 20% w/v, to as low as reasonably practicable.
- Accounting for 4CA levels in the input chlorhexidine digluconate solution, 20% w/v, after 6 months storage at 25ºC/60%RH, which provides for an appropriate and robust supply chain from the supplier to GSK and warehouse storage until use in manufacturing the CHX gel product.
- Accounting for statistical projections of 4CA levels after 24 months of storage at 30ºC/35%RH in the drug product (based on long-term stability data).

Release specification

A tighter level of 150 ppm of 4CA was established in the chlorhexidine digluconate solution, 20% w/v API specification, whereas the USP monograph for the API has a limit of 500 ppm. The product release limit was set at 800 ppm (0.08% w/w) by GSK, since 150 ppm in the chlorhexidine digluconate solution, 20% w/v, with respect to the mass of the active substance solution is equivalent to 800 ppm (0.08% w/w) in CHX gel with respect to the chlorhexidine digluconate salt in the CHX gel product.

Shelf life specification

Based on the proposed initial release specification of 0.08% w/w (800 ppm) for the drug product and accounting for the increase in 4CA content forecast for the 24-month shelf life period when stored at 30ºC/35%RH, the level of the impurity statistically predicted at the 99% confidence limit was 0.312% w/w (3120 ppm).

Impurity G

Impurity G is primarily formed via hydrolysis of chlorhexidine. At release, the impurity G content is controlled by a specification limit of not greater than (NGT) 0.3% w/w (3000 ppm), which is aligned with the chlorhexidine digluconate solution specification and was supported by primary stability studies carried out. From the supportive stability data, it was observed that the content of impurity G after 24-month storage at 30ºC/35% RH increased to a maximum of 0.27%w/w (2700 ppm), from an initial time point value of 0.08% w/w. Taking this increase into account and the release specification for CHX gel of NGT 0.3% w/w (3000 ppm), a shelf life specification of NGT 0.6% w/w (6000 ppm) was therefore proposed by GSK. Impurity G has no structural alerts for genotoxicity.

Impurity N

Impurity N is primarily formed by hydrolysis of chlorhexidine. At release, the impurity N content is controlled by a specification limit of NGT 1.0% w/w (10000 ppm), which aligns with the chlorhexidine digluconate solution specification and was supported by the primary stability. From the supportive stability data, it was observed that impurity N content after 24-month storage at 30°C/35% RH increases to a maximum of 0.83% w/w (8300 ppm), from an initial time point value of 0.15% w/w (1500 ppm). Taking this increase over the shelf life into account and the release specification for CHX gel of NGT 1.0% w/w (10000 ppm), a shelf-life specification of NGT 2.0% w/w (20000 ppm) was proposed by GSK. Impurity N has no structural alerts for genotoxicity.

Impurity J

Impurity J is a gluconic acid adduct of chlorhexidine. At release, the impurity J content level is controlled by a specification limit of NGT 0.4% w/w (4000 ppm), which aligns with the chlorhexidine digluconate solution specification and was supported by the primary stability data. From the supportive stability data, it was observed that impurity J content after 24-month storage at 30°C/35% RH conditions is at a maximum of 0.23% w/w (2300 ppm), from an initial time point value of 0.09% w/w (900 ppm). Taking this increase over the shelf life into account and the release specification for CHX gel of NGT 0.4% w/w (4000 ppm), a shelf-life specification of NGT 0.7% (7000 ppm) w/w was therefore proposed by GSK. Impurity J has no structural alerts for genotoxicity.

Impurity K

Impurity K is formed mainly due the hydrolysis of chlorhexidine. At release, the impurity K content level is controlled by a specification limit of NGT 0.4% w/w (4000 ppm), which aligns with the chlorhexidine digluconate solution specification and was supported by primary stability data. In the supportive stability data, it was observed that impurity K content after 24-month storage at 30°C/35% RH conditions was at a maximum of 0.39% w/w (3900 ppm), from an initial time point value of 0.25% w/w (2500 ppm). Taking this increase over the shelf life into account and the release specification for CHX gel of NGT 0.4% w/w (4000 ppm), a shelf-life specification of NGT 0.7% w/w (7000 ppm) was therefore proposed by GSK. Impurity K has no structural alerts for genotoxicity.

Impurity F (N-(4-chlorophenyl) urea)

Impurity F is formed by hydrolysis of chlorhexidine. At release, impurity F is controlled by a specification limit of NGT 0.2% w/w (2000 ppm), and the shelf life specification is set to NGT 0.3% w/w (3000 ppm) by GSK. These limits are supported by the primary stability data. Impurity F has no structural alerts for genotoxicity.

Impurity A, H, and I+O & Other Specified Impurities

At the time of CHX gel product release, these impurities limits are controlled by a specification limit supported by the chlorhexidine digluconate solution specification and stability studies. Stability studies show no increase in levels of impurities A, H, I+O, and other specified impurities following storage for up to 24 months at the long-term storage conditions (30ºC/35%RH). Hence, the shelf life specification limits for these impurities were set as the same drug product release specification by GSK.

Any Unspecified Impurities

The acceptance criterion for “any unspecified impurities” can be derived from the threshold for identification of degradation products in new drug products specified in ICH Q3B. Since the maximum daily dose of chlorhexidine is in the range of >10mg to −2g, the applicable threshold is “0.2% or 2mg TDI (total daily intake), whichever is lower.” For CHX gel, the lower threshold is 0.2% w/w.

From the stability study data, the maximum amount of any other unspecified impurities was observed to be <0.1% w/w (1000 ppm). For any other unspecified impurity, at the time of release a specification can be set at NGT 0.1% w/w (1000 ppm), and over the product shelf life a specification can be set at NGT 0.2% w/w (2000 ppm).

Total Impurities

Release specification

In setting the release specification for the drug product, both the performance of the primary batch stability studies and also API solution stability were considered. It was known from the supplier's Active Substance Master File that the total impurities content also increased during storage of the API solution. Therefore, the starting point for total impurities content in the drug product can be higher if aged API solution was used. Based on the drug product release data and knowledge of the rate of degradation of aged API solution, a 2.0% w/w (20000 ppm) specification was justified by GSK.

Shelf life specification

Stability data have shown that total impurities are NGT 2.7% w/w (27000 ppm) after 18 months of storage at the long-term storage condition of 30ºC/35% RH. Considering the release specification of NGT 2.0% w/w (20000 ppm) and the projected increase due to degradation of ~2% w/w (20000 ppm), an NGT 4% w/w (40000 ppm) shelf life specification limit was considered to be appropriate for controlling the total impurities in CHX gel based on the available data set by GSK. A review of the full-term stability data should be conducted, for the primary batches and supportive batches manufactured at the proposed commercial site to justify the proposed specification limit.

Microbial Limits Test

CHX gel was required to comply with the requirements of Ph. Eur. <2.6.12> and <2.6.13> for microbial testing by GSK.

5.2 Non-Specification Tests: Elemental Impurities

A risk assessment should be conducted to evaluate the potential for elemental impurities to be present in CHX gel. This risk assessment can be performed in line with ICH Q3D "Guideline for Elemental Impurities" and should include the following: the input API, excipients, processing aids (water), manufacturing equipment, and container closure system. ICH Q3D does not specifically list the topical administration route, so the guidance associated with the oral route should be applied when the risk assessment is to be performed for CHX gel. No ICH Q3D elements from Class 1, 2A, 2B, or 3 should be deliberately used at any stage of the manufacturing of the components, packaging, or the finished product itself.

The risk assessment therefore should be focused upon the Class 1 elemental impurities (Cadmium, Lead, Arsenic and Mercury) and the Class 2A elemental impurities (Cobalt, Vanadium and Nickel). Class 2B and Class 3 can be discounted from the rest of the assessment as recommended by the ICH Q3D guidance.

The likelihood of Class 1 and 2A elements reaching the final dose delivered to the patient should be deemed to be low considering the following:

- Information from the suppliers on how the components of the drug product formulation was produced, the secondary manufacturing process which converted the components into the finished product and the primary packaging into an aluminum foil sachet.

Considering that the suppliers had complied with earlier regulations and pharmacopoeia standards rather than the more extensive range in ICH Q3D, it is recommended that several batches of drug product over the course of product development be tested for the relevant elemental impurities described above, using an ICP-AES method. Very low levels of the relevant elements, <10% of the PDE limit, can then be approved in the final product. If more up to ICH Q3D data can be obtained from the suppliers, then testing of the final product may not be necessary.

5.3 Analytical Methods

The analytical methods used to characterize CHX gel can be categorized as physical tests and chemical tests.

Physical tests

The following physical tests are applied for controlling the drug product:

- Description
- pH
- Viscosity
- Minimum fill

pH, viscosity, and minimum fill are performed as described in the relevant pharmacopeial monographs.

Chemical tests

The following chemical tests were applied for controlling the drug product CHX gel:

- Identification of chlorhexidine in the CHX gel by:
  - HPLC retention time
  - HPLC UV spectrum
- Chlorhexidine digluconate content
- Drug-related impurities content in CHX gel by HPLC

Physical Tests

Description

Perform the description test for CHX gel, visually examining 1.0 g of gel product against a white background. Examine and record the description of the sample, paying particular attention to color, clarity, consistency, physical state, and presence of any foreign matter.

pH

Equipment

Use a pH probe containing a gel-filled reference, such as the Mettler Toledo Inlab ExpertPro. Standard liquid filled probes will not accurately measure the pH in the gel product.

Procedure

Test the pH of the CHX gel sample at the time of release and during stability analysis, maintaining a sample temperature of 25 ±2°C and using the pH probe described in the Equipment section, above. No sample preparation or dilution step is necessary for the pH measurement. This test method is as described in the current Ph. Eur.

Apparent Viscosity by Viscometer

Equipment

Use a Brookfield viscometer with S92 TB spindle to obtain comparable viscosity results to the registered specifications. Using alternative equipment may produce different results for semi-solid products which do not behave like Newtonian fluids. The specifications and the testing process are intimately connected.

Procedure

Test 45 g of sample of the CHX gel at the time of release and during stability testing, using the S92 TB spindle operated at 30 rpm while maintaining the sample temperature at 25 ±2°C and measuring after 5 minutes of rotation (non-helipath) time. For packaged material, mix the required number of packs together to generate a 45 g sample and then equilibrate it to achieve the stated temperature. This test method is performed as per the current Ph. Eur. Procedure.

Minimum Fill

This test will be performed as per current USP monograph <755> for minimum fill.

Chemical Tests

Identification of Chlorhexidine in the CHX Gel by HPLC Retention Time

The method utilizes a reverse-phase chromatographic separation to provide a means of identification. This means of identification requires the concordance of the retention time of the principal peak in each sample chromatogram to that in the reference chromatogram. This procedure was developed in accordance with ICH Guideline Q6A, *Specifications: Test Procedures and Acceptance Criteria for New APIs and New Drug Products: Chemical Substances*. The identity of chlorhexidine in the drug product is confirmed when the retention time of the principal peak in the sample chromatogram corresponds (±3%) to that of the major elution peak in the working standard chromatogram similarly prepared and measured.

Example chromatograms are shown in the figures below.

Figure 3. Typical Diluent Solution (Example Chromatogram)

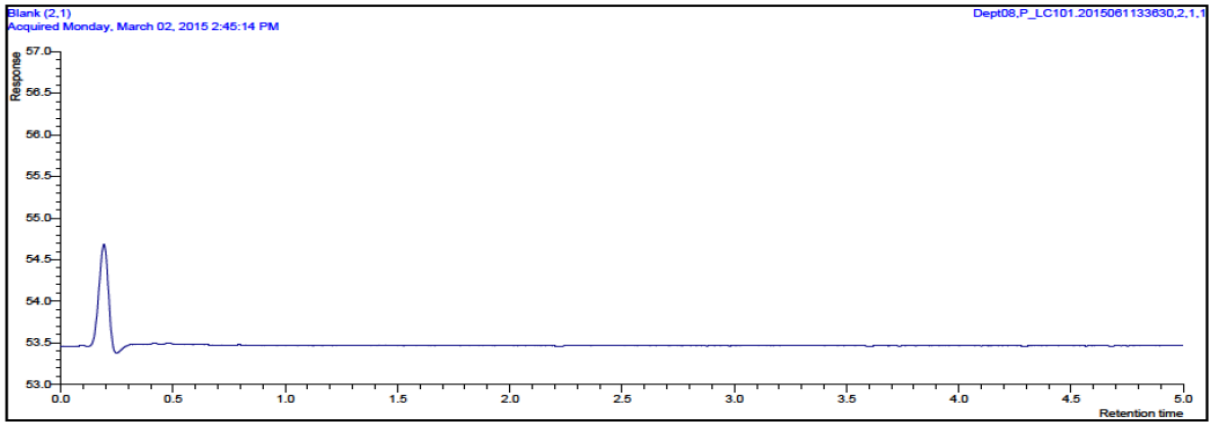


Figure 4. Typical Standard Solution (Example Chromatogram)

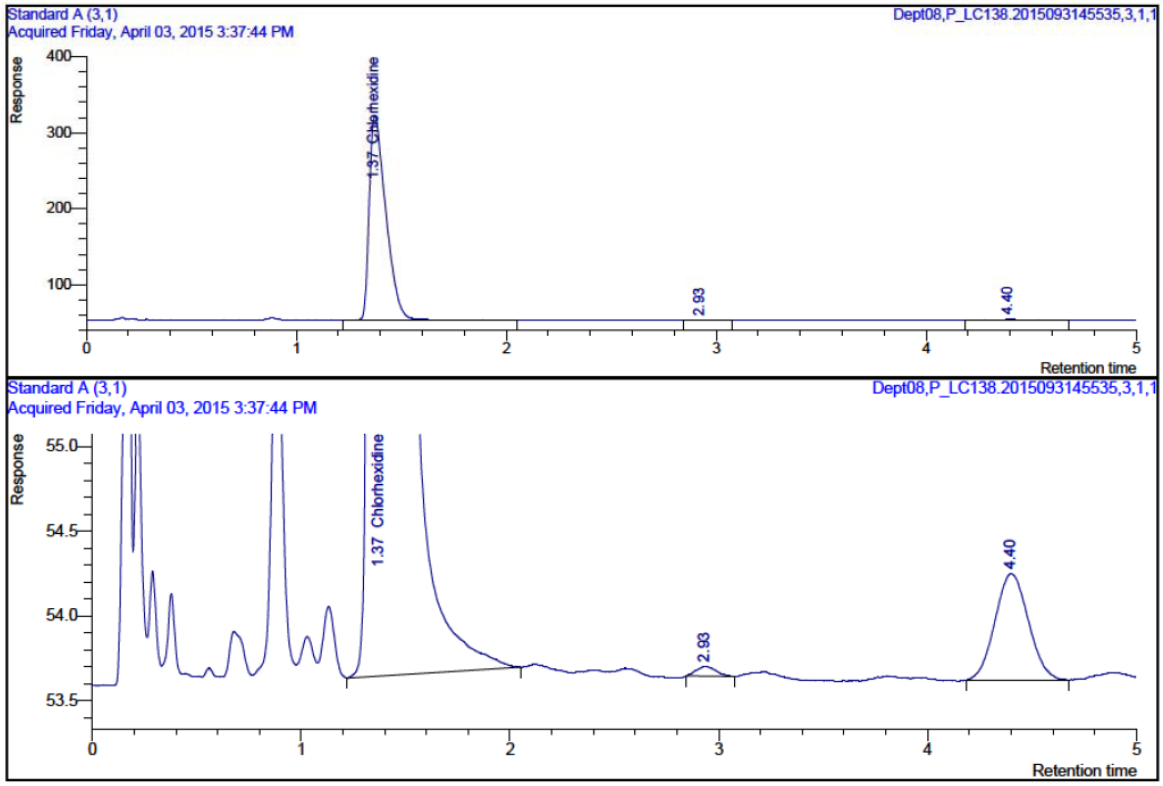
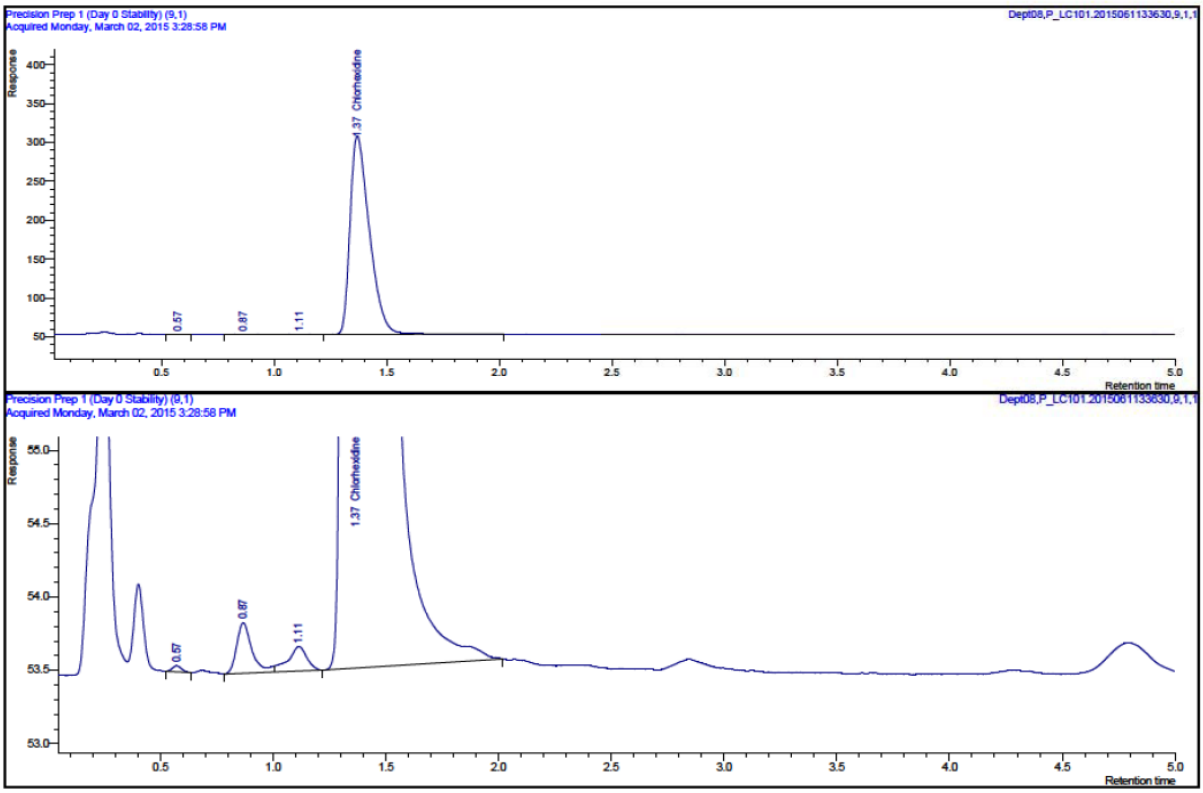


Figure 5. Typical Sample Injection (Example Chromatogram)



Identification of Chlorhexidine in the CHX Gel by HPLC UV Spectrum

The method utilizes a reverse phase chromatographic separation to provide a means of identification. This means of identification requires the concordance of the UV spectrum of the principal peak in the sample chromatogram, to that in the reference chromatogram (which is due to chlorhexidine), following separation by reverse phase HPLC. This was developed in accordance with ICH Guideline Q6A, *Specifications: Test Procedures and Acceptance Criteria for New APIs and New Drug Products: Chemical Substances*.

Examples of UV spectra for standard and sample solutions are given below.

Figure 6. Spectra of Chlorhexidine Peak in Reference Standard Solution

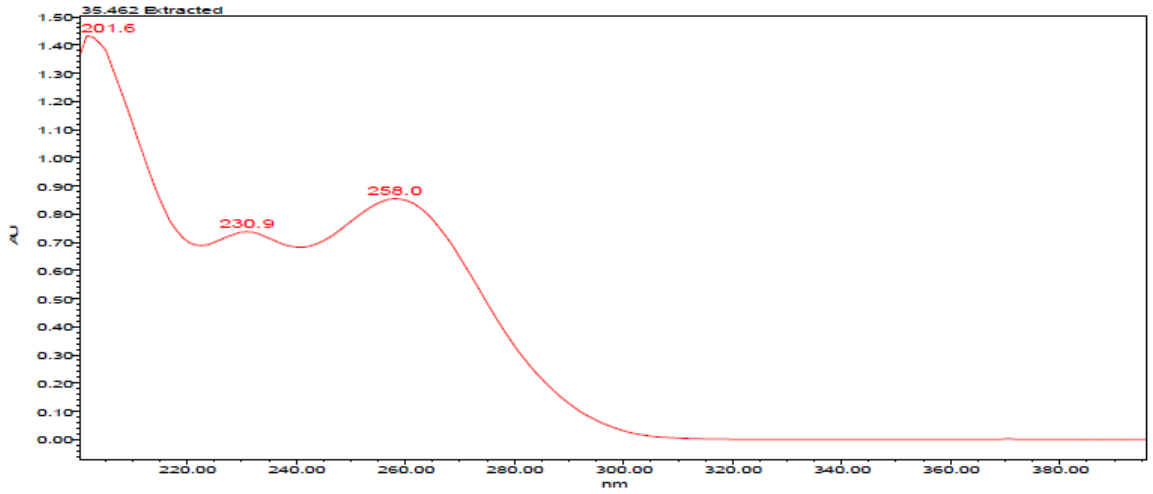
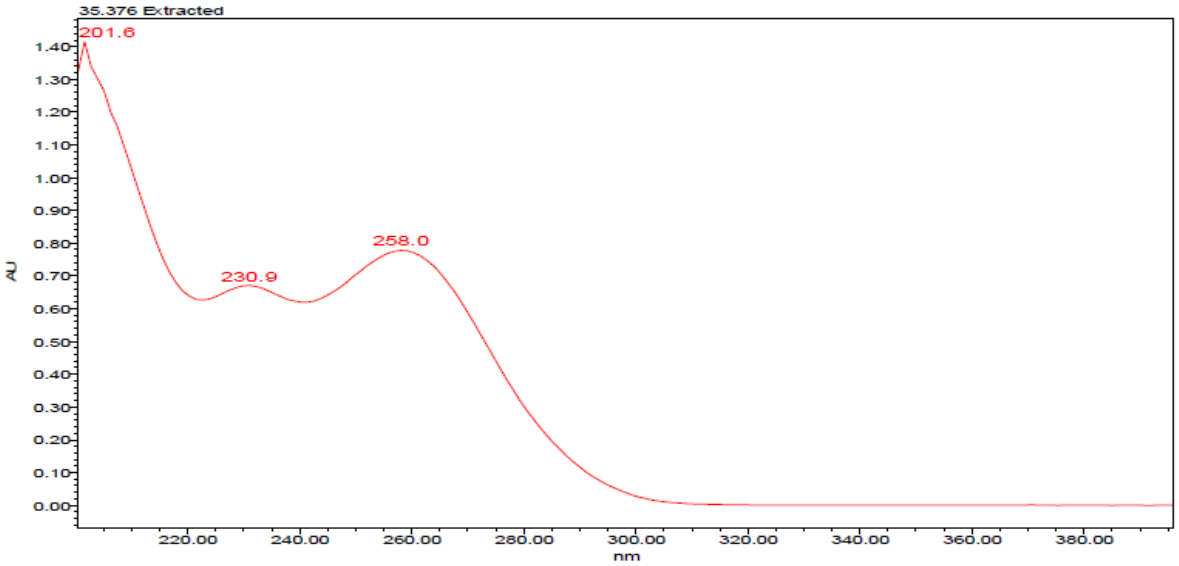


Figure 7. Spectra Chlorhexidine Peak in Sample Solution



Determination of Chlorhexidine Digluconate In CHX Gel by HPLC

A reversed phase isocratic HPLC method is used to determine the chlorhexidine digluconate content of CHX gel. All method parameters may be varied within the ranges described in the validation data.

1. **Preparation of Solutions**
   - Preparation of Diluent 1

     Prepare a mixture of acetonitrile, water, and trifluoroacetic acid in the ratio 10:90:0.1(v/v/v). For example, add 100 mL of acetonitrile and 1 mL of trifluoroacetic acid to 900 mL of water and mix well.

   - Preparation of Diluent 2

     Prepare a mixture of acetonitrile and water in the ratio 50:50 (v/v). For example, add 500 mL of acetonitrile to 500 mL of water and mix well.

   - Preparation of Mobile Phase A

     Prepare suitable quantity of 0.1% v/v trifluoroacetic acid in water. For example, add 1.0 mL of trifluoroacetic acid to 1000 mL of water and mix well.

   - Preparation of Mobile Phase B

     Prepare suitable quantity of 0.1%v/v trifluoroacetic acid in acetonitrile. For example, add 1.0 mL of trifluoroacetic acid to 1000 mL of acetonitrile and mix well.

   - Preparation of Working Standard Solution

     Prepare, in duplicate, solutions of chlorhexidine diacetate in Diluent 1 at a concentration of about 0.11 mg/mL (equivalent to 0.16 mg/mL of chlorhexidine digluconate). For example, transfer approximately 5.8 mg of chlorhexidine diacetate reference standard, accurately weighed into a 50 mL volumetric flask. Add about 25 mL of Diluent 1 and sonicate to dissolve the solid material, dilute to the volume with Diluent 1, and mix. This solution is equivalent to 100% of product formulation concentration of chlorhexidine digluconate. This working standard solution is stable for up to 5 days when stored at refrigerated conditions and protected from light.

   - Preparation of Blank Solution

     The blank solution is the Diluent 1.

   - Preparation of Sample Solutions

     Prepare, at least in duplicate, solutions of chlorhexidine digluconate gel in diluent at a concentration of about 0.16 mg/mL of chlorhexidine digluconate. For example, transfer approximately 1.13g of CHX gel (corresponding to approximately 80mg of chlorhexidine digluconate), accurately weighed into a 100 mL volumetric flask. Add about 50 mL of Diluent 2 and sonicate for 60 minutes with occasional shaking (alternatively, the sample can be prepared

by vortexing for 5 minutes followed by sonication for 15 minutes with intermittent shaking). Please note that all the samples will not be dissolved after sonication. Bring to volume with Diluent 2and mix well. Further dilute 10.0 mL to 50 mL with Diluent 1. Wait at least 15 minutes before analysis. Filter through a 0.45µm nylon filter directly into an HPLC vial.

This sample solution is stable for up to 7 days when stored at USP refrigerated conditions (2°C–8°C) and protected from light.

2. Experimental Procedure

- Instrument Parameters

  Minor changes to the instrument parameters may be necessary to ensure compliance with system suitability criteria. All changes must, however, should be within the validated parameters of the method.

Table 9. Instrument Parameters

Column	Waters X select CSH C18 2.5 µm, 2.1 mm x 30 mm P/N 186006100
Mobile Phase-A	0.1% TFA in water
Mobile Phase-B	0.1% TFA in acetonitrile
Flow rate	1.0 mL/min
Isocratic	76% MP A: 24% MP B
Column temperature	35°C
Wavelength of detection	254 nm
Injection volume	5 µL
Run time	5min
Retention time	Chlorhexidine, about 2 min
Needle wash solution	Diluent 2

- Assay Procedure

  Using the described instrument conditions, equilibrate the chromatographic system with the mobile phase. Record the chromatograms for the blank, standard, and sample solutions. Record the chromatograms required to meet any system suitability criteria and for the standard and sample solutions.

- System Suitability Criteria

  The peak corresponding to chlorhexidine in the standard chromatogram should elute in around 2 minutes.

  The tailing factor of the chlorhexidine peak should not be more than 2. The calculated % RSD of the response factor for replicate injections of the standard solutions should not be more than 1.5%. The calculated standard agreement for replicate injections of the standard solutions should not be more than 1.5%.

3. Example Calculation of Results

Report results according to site practices. The following are examples that show how the calculations may be performed manually. They do not show how specific computer programs work. Alternative expressions may be used.

- Response Factor

  For each standard injection calculate a response factor (RF) as follows:

$$RF = \frac{Ws \times P}{As \times P}$$

  Where:

  Ws = Weight of standard (mg)
  As = Peak area of main component in standard injection
  P = Purity of standard (% w/w)

Calculate the mean response factor (MRF) for all standard injections and determine the % RSD.

- Assay

  Using the MRF, calculate the assay for each sample injection.

$$Assay\ of\ Chlorhexidine\ Digluconate\ (\%\,w/w) = \frac{MRF \times Au \times DFu \times MW1 \times 100}{Wt\ Gel \times DFs \times MW2}$$

  Where:

  MRF = Mean response factor
  Au = Peak area of main component in sample injection
  DFu = Dilution factor for sample (mL)
  100 = Factor to convert to %
  Wt gel = Weight of sample (mg)
  DFs = Dilution factor for standard (mL)
  MW1 (Molecular weight of chlorhexidine digluconate) = 897.76
  MW2 (Molecular weight of chlorhexidine diacetate) = 625.55

Validation of the Determination of Chlorhexidine Digluconate in CHX Gel by HPLC

Demonstration of the specificity of the method for the determination of chlorhexidine in CHX gel

To demonstrate the specificity of the method and that the method is stability indicating, the known potential drug-related impurities of synthesis and degradation are chromatographed using the defined method. The specificity of the analytical procedure is demonstrated by the absence of interference with the peak due to any known drug-related impurities and from the excipients from the chlorhexidine peak. The figures below demonstrate that all commonly occurring impurities of synthesis and degradation are resolved from chlorhexidine.

Representative chromatograms

To demonstrate the absence of interference from the formulation excipients, the following chromatograms were obtained using the defined method for the determination of chlorhexidine digluconate content for sample solutions of:

- Freshly manufactured CHX gel
- Active-free product
- Aged/stressed CHX gel

Inspection of the following figures demonstrates sufficient selectivity between chlorhexidine and potential synthetic impurities/degradation products. This can also be confirmed by diode array spectroscopy using peak purity analysis.

Figure 8. **Chromatogram Showing the Separation of Chlorhexidine from known Potential Impurities of Synthesis and Degradation in CHX Gel**

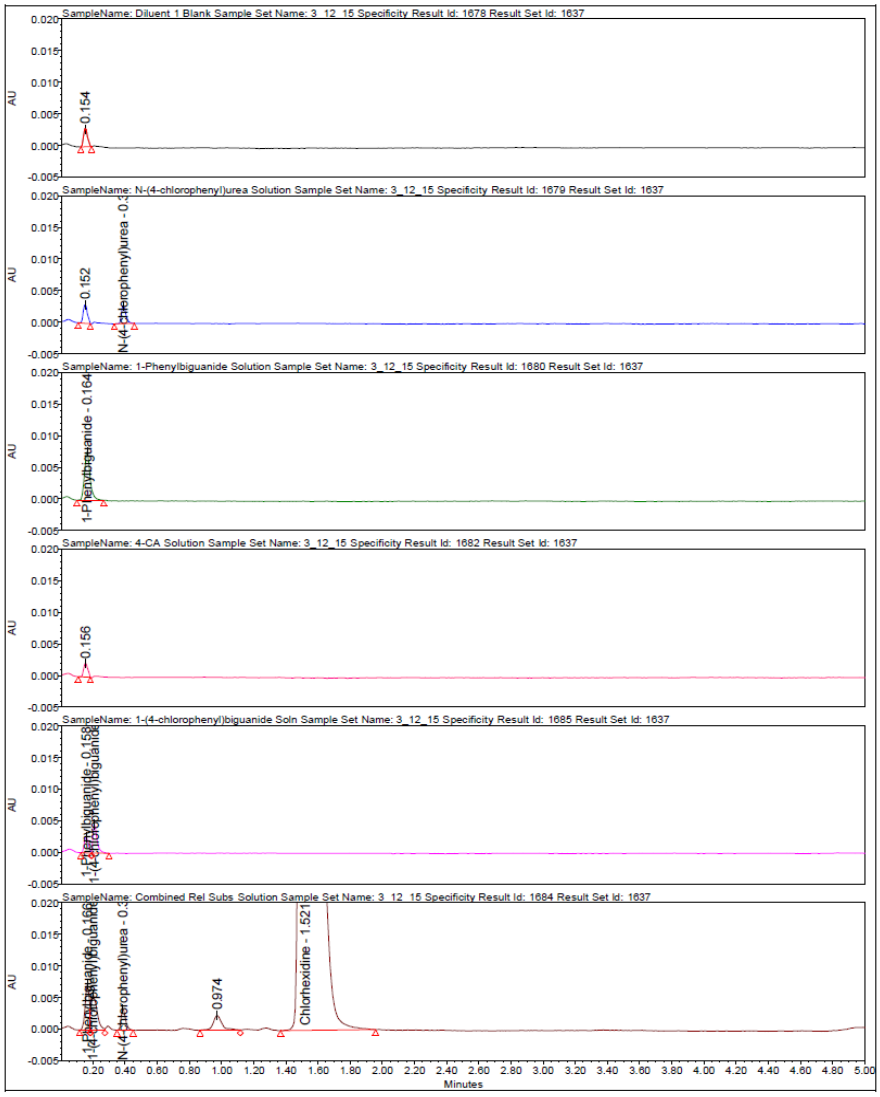
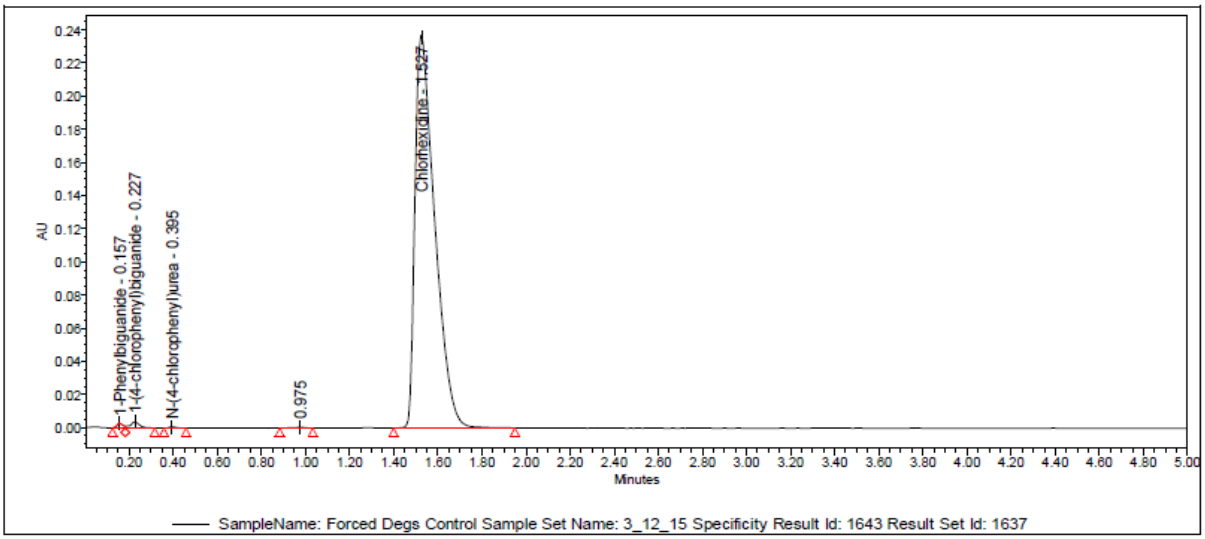


Figure 9. Chromatogram of CHX Gel Sample Solution



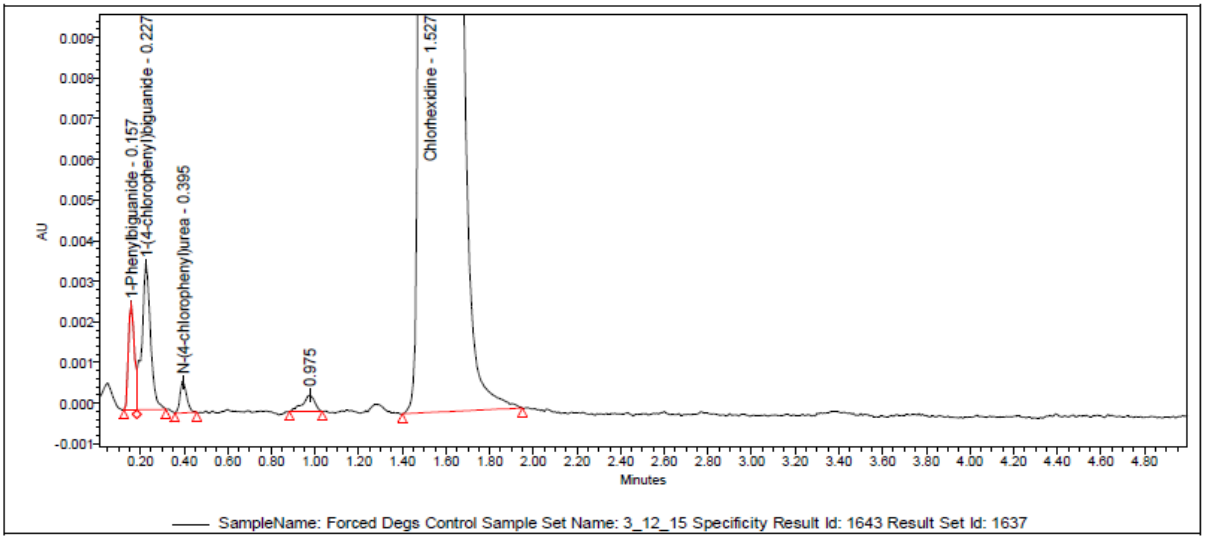


Figure 10. Chromatogram of Placebo Gel

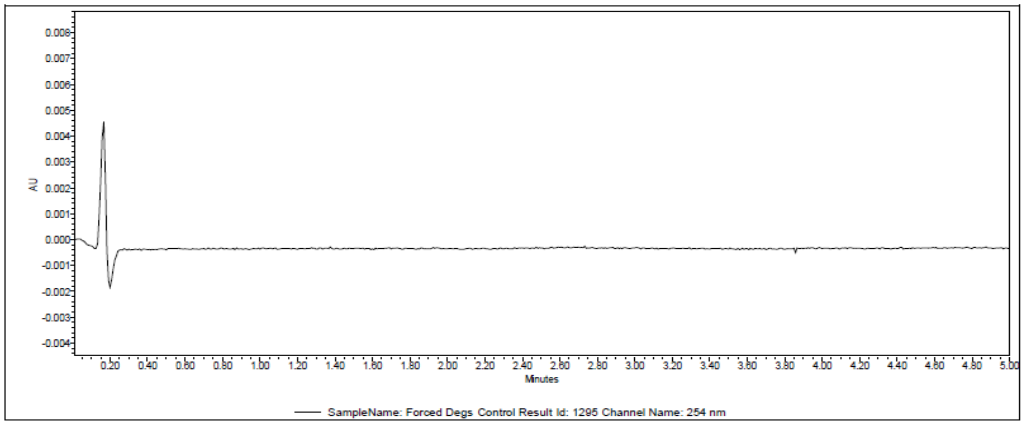
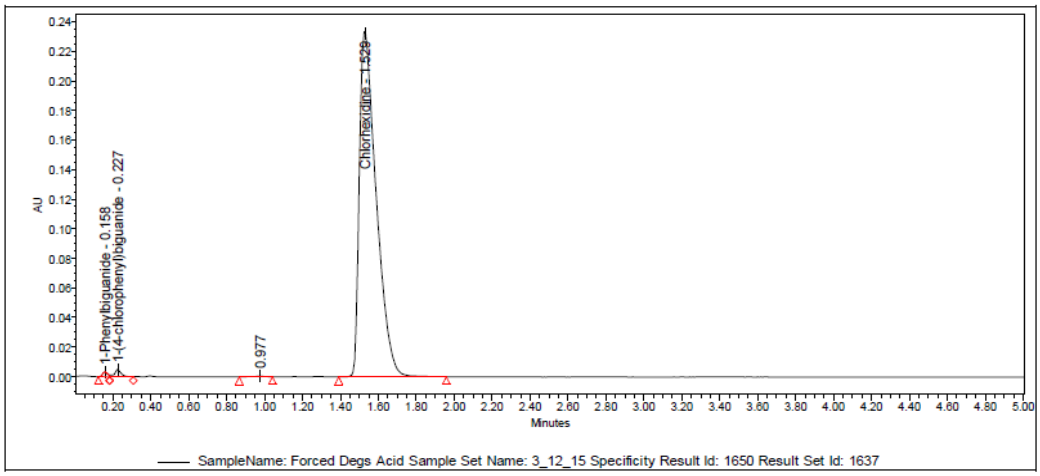


Figure 11. Chromatogram of Aged/Stressed Product (Acid hydrolysis/1NHCl/24 hours/Room Temperature)



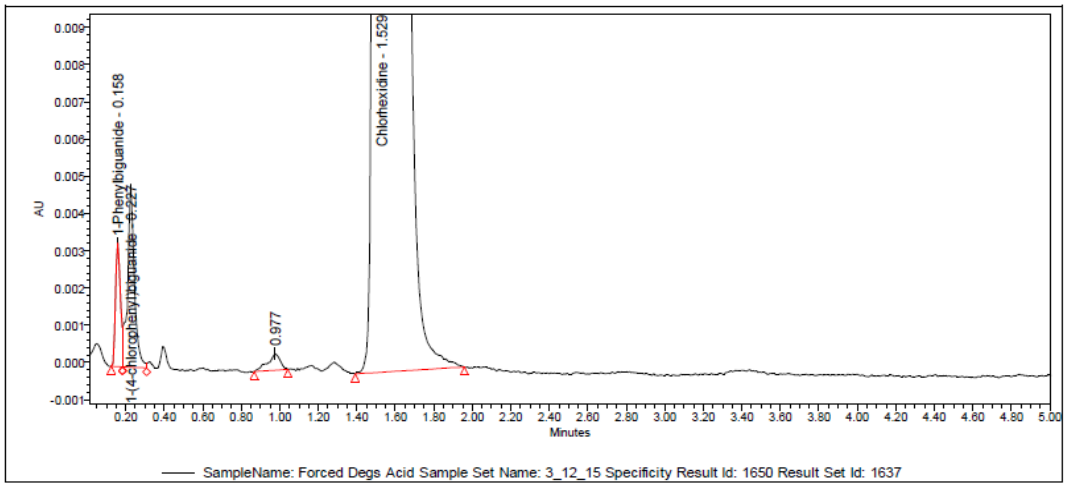


Figure 12. Chromatogram of Aged/Stressed Product (Base Hydrolysis/1NNaOH/24 Hours/Room Temperature)

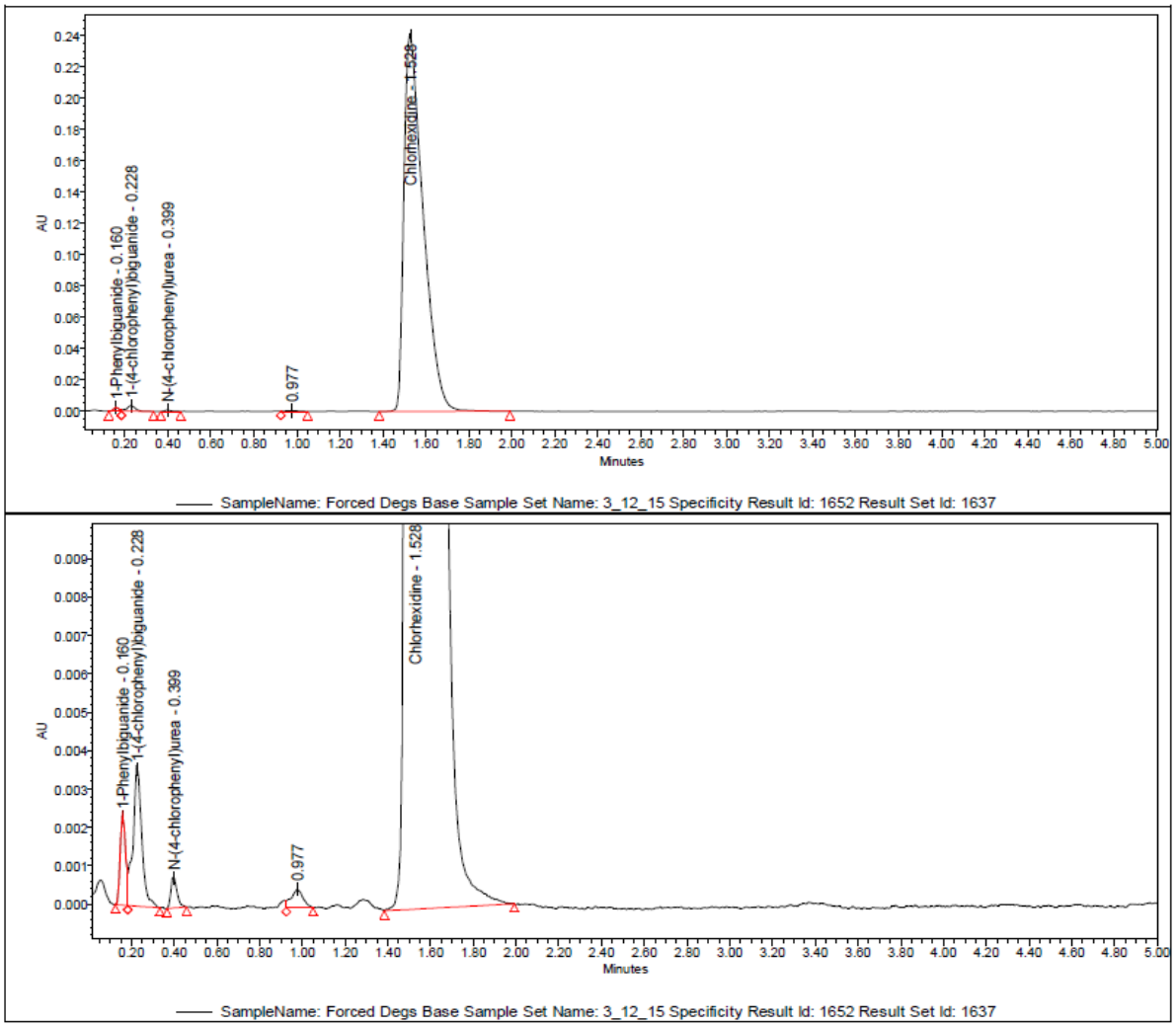


Figure 13. Chromatogram of Aged/Stressed Product (Oxidation/30% H2O2/24 Hours/Room Temperature)

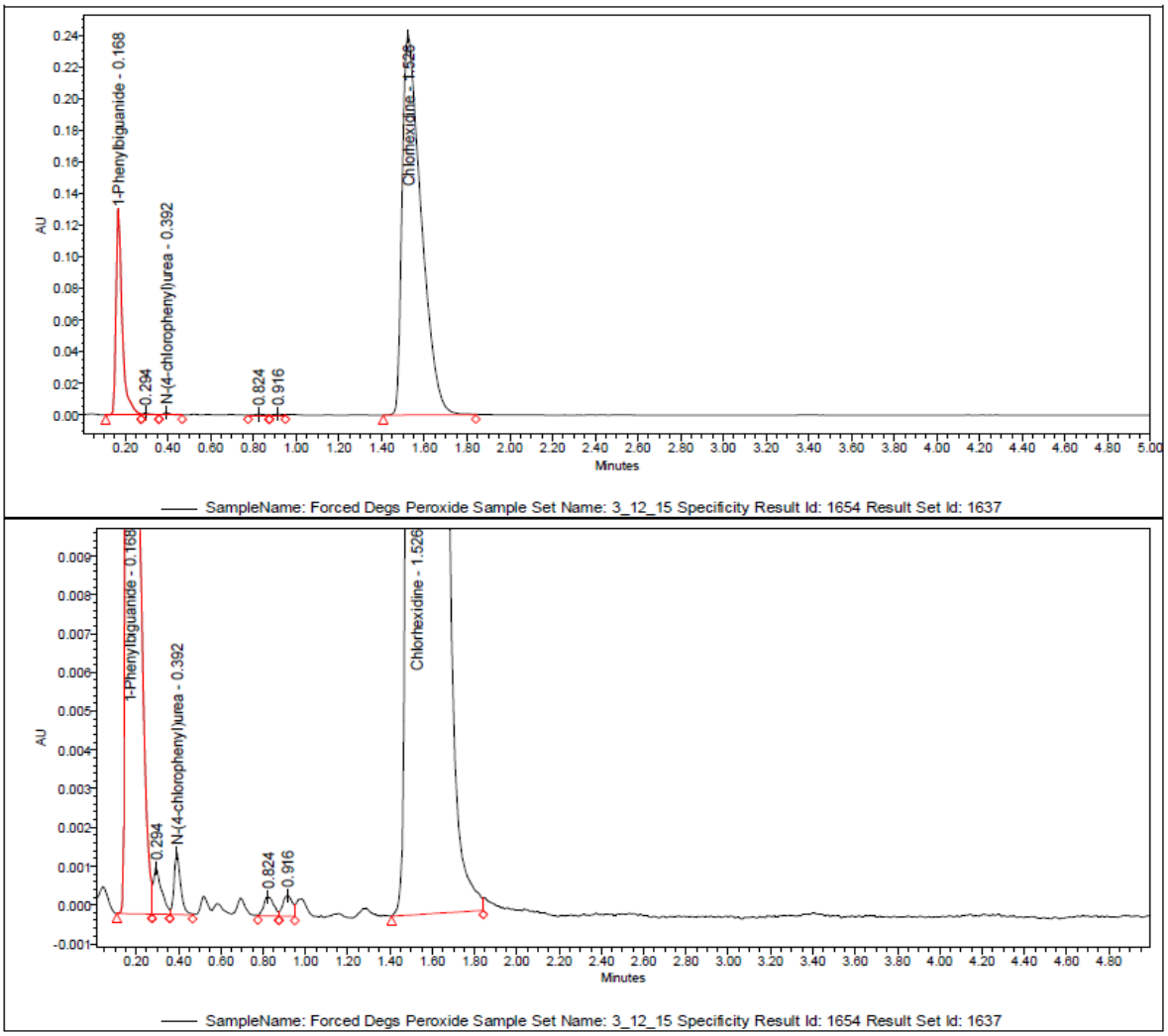
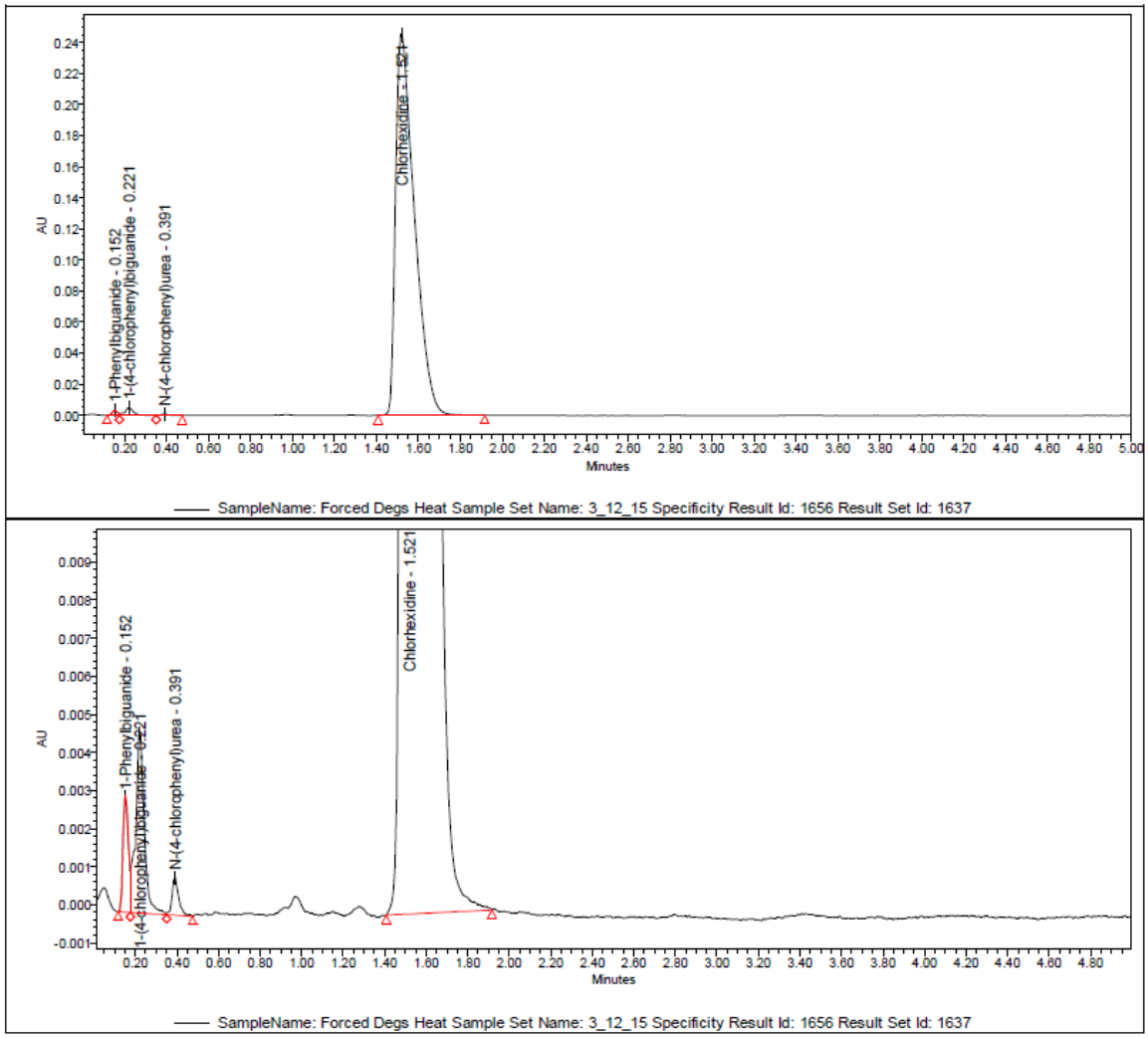


Figure 14. Chromatogram of Aged/Stressed Product (Heat Stress/60°C/24 Hours)



Demonstration of the linearity of response for chlorhexidine in the method for determining chlorhexidine digluconate in CHX gel

The linearity of the response obtained for chlorhexidine diacetate was investigated over the range 30% to 130% of the nominal level (0.11 mg/mL of chlorhexidine diacetate equal to 0.16 mg/mL of chlorhexidine digluconate) as recommended in the analytical method.

Demonstration of the range of the method for determining chlorhexidine digluconate in CHX gel

The method was demonstrated to be suitable over the range from 80%–120% of the label claim, based on the results for linearity, accuracy, and precision.

Demonstration of accuracy of the method for determining chlorhexidine digluconate in CHX gel

The accuracy of the analytical method was investigated using known quantities of chlorhexidine diacetate as a reference standard. Known quantities of chlorhexidine diacetate as a reference standard were spiked into active-free (placebo gel) samples. The individual recovery values obtained were found to vary from 99.3% to 99.9%.

Demonstration of precision of the method for determining chlorhexidine digluconate in CHX gel

- Repeatability

  The repeatability of the preparation of sample solutions was determined by preparing six sample solutions at 100% nominal concentration. The content of chlorhexidine digluconate for the sample solutions was then calculated.

- Intermediate precision

  The intermediate precision of the analytical procedure was determined by measuring the content of chlorhexidine digluconate for one batch of CHX gel on different equipment and by different analysts on a different day.

Demonstration of the robustness of the method for determining chlorhexidine digluconate in CHX gel

The robustness of the analytical method was investigated using deliberate variations (e.g., column temperature, organic content, flow rate, sonication time) of the chromatographic parameters.

Demonstration of stability of solutions prepared for the method for determining chlorhexidine digluconate in CHX gel

Standard and sample solutions were prepared, and the percentage difference in the content of chlorhexidine digluconate from the initial values was determined using the defined analytical method. After storage at refrigerated conditions (2°C–8°C), the concentration of chlorhexidine digluconate content was redetermined at predetermined intervals for 7 days using freshly prepared standard solutions.

Validation Summary for Method for the Determination of Chlorhexidine Digluconate Content in CHXGEL by HPLC

To demonstrate the suitability of the HPLC assay method for determining chlorhexidine digluconate, the experiments summarized in the table below were conducted using the defined procedure.

Table 10. Summary of Validation Experiments for Determining Chlorhexidine Digluconate Content in CHX Gel by HPLC

Experiment	Acceptance criteria	Result
Specificity of the chromatographic system	None of the known related substance or any excipients' peaks should interfere with the chlorhexidine peak.	Resolution was demonstrated for chlorhexidine from its known potential impurities of synthesis and degradation and excipients of the formulation. Acceptable specificity was demonstrated with less than 1.0% label claim response from excipients of the formulation with chlorhexidine digluconate at 254 nm.
Linearity of detector response to for chlorhexidine digluconate (range equivalent to 30%–130% of nominal 0.16 mg/mL of chlorhexidine digluconate)	Correlation coefficient (R) must be > 0.9997 and the y-intercept of the linear regression must be < 2.0% of nominal column loading/concentration	Linearity was demonstrated for the response due to chlorhexidine digluconate. Correlation coefficient (R^2) = 0.9999 intercept = 0.4% of nominal column loading/concentration.
Range	Report results.	Chromatographic linearity, accuracy and precision was demonstrated from 30 to 120% of the nominal column loading/concentration employed during the analytical procedure.
Accuracy	Mean recovery at each level should be 98.0%–102.0%. The % RSD of the % recovery at each level must be < 2.0%.	Accuracy was demonstrated by recovery from spiked active-free dosage form. Mean % accuracy values for chlorhexidine digluconate: 80% = 99.5% (n=3) 100% = 99.8% (n=3) 120% = 99.4% (n=3)
Precision: repeatability	The % RSD must be < 2.0% for the six assay results	RSD = 0.2% (n = 6) Repeatability was demonstrated for 6 replicate preparations of the above solutions.
Intermediate precision	The % RSD must be < 2.0% for the six assays. **Results** The % RSD of the 12 assay results must be < 2.0%.	Intermediate precision was demonstrated for different analysts using different equipment in the same laboratory to analyze the same batch of CHX gel on different days. %RSD = 0.5% (n=6) Overall % RSD for two analysts = 0.4% (n=12)

Experiment	Acceptance criteria	Result
Reproducibility	The % RSD must be < 2.0% for the six assays. **Results** The % RSD of the 12 assay results must be < 2.0%.	Reproducibility was demonstrated by different analysts in different laboratories to analyze the same batch of CHX Gel
Robustness	The mean sample result at the varied condition must be 98.0%–102.0% of the result at the nominal condition.	Robustness was demonstrated by experiments that focused on amendments to chromatographic parameters, including sample preparation procedure, percentage composition of organic component in mobile phase, column temperature, and flow rate.
Stability of solutions of chlorhexidine digluconate	Recovery values at different day compared to the initial should be 98.0%–102.0%.	Solutions of standards were demonstrated to be stable for up to 5 days when stored at refrigerated condition and protected from light (2°C–8°C). Sample solutions are stable for up to 7 days when stored at refrigerated condition and protected from light (2°C–8°C).

Determination of Chlorhexidine Digluconate 4CA Content in CHX Gel By HPLC

A reversed phase gradient HPLC method is employed to determine the chlorhexidine content and drug-related impurities content of CHX gel. All method parameters are allowed to be varied within the ranges described in the validation data.

1. Preparation of Solutions

Preparation of Diluent

- Preparation of Diluent 1

  Prepare a mixture of acetonitrile, water, and trifluoroacetic acid in the ratio 10:90:0.1(v/v/v). For example, add 100 mL of acetonitrile and 1 mL of trifluoroacetic acid to 900 mL of water and mix well.

- Preparation of Diluent 2

  Prepare a mixture of acetonitrile and water in the ratio 50:50 (v/v). For example, add 500 mL of acetonitrile to 500 mL of water and mix well.

Preparation of Mobile Phase

- Preparation of 0.1% trifluoroacetic acid in water

  Prepare a suitable quantity of 0.1% v/v trifluoroacetic acid in water. For example, add 1.0 mL of trifluoroacetic acid to 1000mL of water and mix well.

- Preparation of 0.1% trifluoroacetic acid in acetonitrile

  Prepare a suitable quantity of 0.1% v/v trifluoroacetic acid in acetonitrile. For example, add 1.0 mL of trifluoroacetic acid to 1000 mL of acetonitrile and mix well.

- Preparation of mobile phase A

  Prepare a mixture of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroacetic acid in water in the ratio 20:80 (v/v).

  For example, add 200 mL of 0.1% v/v trifluoroacetic acid in acetonitrile to 800 mL of 0.1% v/v trifluoroacetic acid in water and mix well.

- Preparation of mobile phase B

  Prepare a mixture of 0.1% trifluoroacetic acid in acetonitrile and 0.1% trifluoroacetic acid in water in the ratio 90:10 (v/v). For example, add 900 mL of 0.1% v/v trifluoroacetic acid in acetonitrile to 100 mL of 0.1% v/v trifluoroacetic acid in water and mix well.

Preparation of chlorhexidine diacetate stock standard solution

Prepare solutions of chlorhexidine diacetate in diluent (0.0007 mg/mL). For example, transfer approximately 7.2 mg of chlorhexidine diacetate reference standard, accurately weighed into a 100 mL volumetric flask. Add about 50 mL of Diluent 1 and sonicate to dissolve the solid material, dilute to the volume with Diluent 1, and mix. Further dilute 1. 0mL of the above solution to 100 mL with Diluent 1.

Preparation of 4CA stock standard solution

Prepare solutions of 4CA in diluent (0.0001 mg/mL). For example, transfer approximately 10 mg of 4CA reference standard, accurately weighed into a 100 mL volumetric flask. Add about 50 mL of acetonitrile and sonicate to dissolve the solid material, dilute to the volume with acetonitrile, and mix. Further dilute 5.0 mL to 100 mL with Diluent 1 to get a 0.005 mg/mL solution. Dilute 2.0 mL of 0.005 mg/mL solution to 100 mL with Diluent 1.

Preparation of Sensitivity (QL) Check Solution

Prepare a solution of 0.07 µg/mL of chlorhexidine diacetate (0.05% w/w with respect to chlorhexidine digluconate nominal sample concentration) and 0.03 µg/mL of 4CA. For example, dilute 1 mL of chlorhexidine diacetate stock standard solution and 3 mL of 4CA stock standard solution to 10 mL with Diluent 1 and mix well. This solution is stable for up to 5 days when stored at USP refrigerated conditions (2°C–8°C) and protected from light

Preparation of Resolution Solution (Chlorhexidine for System Suitability Solution)

Prepare a solution of 0.2mg/mL of chlorhexidine for system suitability CRS. For example, transfer approximately 2 mg of chlorhexidine for system suitability CRS, accurately weighed into a 10 mL volumetric flask. Add about 5 mL of Diluent 1 and sonicate to dissolve the solid material, dilute to volume with Diluent 1, and mix. Store the resolution solution at 2°C–8°C. This solution is considered to be stable if the known impurity peaks are clearly identified in the chromatogram.

Preparation of Blank Solution

The blank solution is the Diluent 1.

Preparation of Sample Solutions

Prepare, in duplicate, solutions of chlorhexidine digluconate gel in diluent at a concentration of about 0.2 mg/mL of chlorhexidine digluconate. For example, transfer approximately 1.13 g of CHX gel (corresponds to approximately 80 mg of chlorhexidine digluconate) sample, accurately weighed into a 100 mL volumetric flask. Add about 50 mL of Diluent 2 and sonicate to dissolve. This will take about 30 minutes with intermittent shaking. Bring to volume with Diluent 2 and mix well. Further dilute 5.0 mL to 20 mL with Diluent 1. Wait at least 1 hour before analysis. Filter through a 0.45 µm Nylon filter directly into an HPLC vial.

2. Experimental Procedure

2.1 Instrument Parameters

Minor changes to the instrument parameters may be necessary to ensure compliance with system suitability criteria. All changes must, however, be within the validated parameters of the method. Please note that the method is robust with respect to changes in the chromatographic conditions, including changes in the column oven temperature up to +5°C, mobile phase flow rate changes up to +0.2 mL/min, and an increase of up to 5% v/v in organic content of the mobile phase. Decreasing the organic content of the mobile phase, from the target concentration of 20% v/v for mobile phase A and 90% v/v for mobile phase B, will increase the risk of not meeting the system suitability criteria.

Table 11. HPLC Method

Parameter	Specification		
Analytical column	Luna C18 (2), 250 x 4.6 mm, 5 µm, P/N 00G-4252-E0 or validated equivalent		
Mobile Phase A	20% acetonitrile: 80% water, 0.1% trifluoroacetic acid (v/v)		
Mobile Phase B	90% acetonitrile: 10% water, 0.1% trifluoroacetic acid, v/v		
Mobile phase gradient	Time (Minutes)	% of Mobile Phase A	% of Mobile Phase B
	0.0	100	0
	2.0	100	0
	32.0	80	20
	37.0	80	20
	47.0	70	30
	54.0	70	30
	55.0	100	0
	60.0	100	0
Flow rate	1.0 mL per minute		
Column temperature	30˚C		

Parameter	Specification
UV detection wavelength	For 4CA: 215 nm For all other impurities: 254 nm
Data acquisition rate/peak width	At least 0.1 (2 sec)
Detector bandwidth and slit width	8 nm (Agilent systems)
Detector filter response	0.5 (Waters systems)
Injection volume	50 µL
Approximate run time	60 minutes
Auto sampler wash solvent	Diluent 2

- Related Substances Procedure

  Using the described instrument conditions, equilibrate the chromatographic system with a blank (Diluent 1). Record the chromatograms required to meet any system suitability criteria and for the standard and sample solutions.

  Example chromatograms are shown below.

Figure 15. Typical Diluent Solution at 254nm

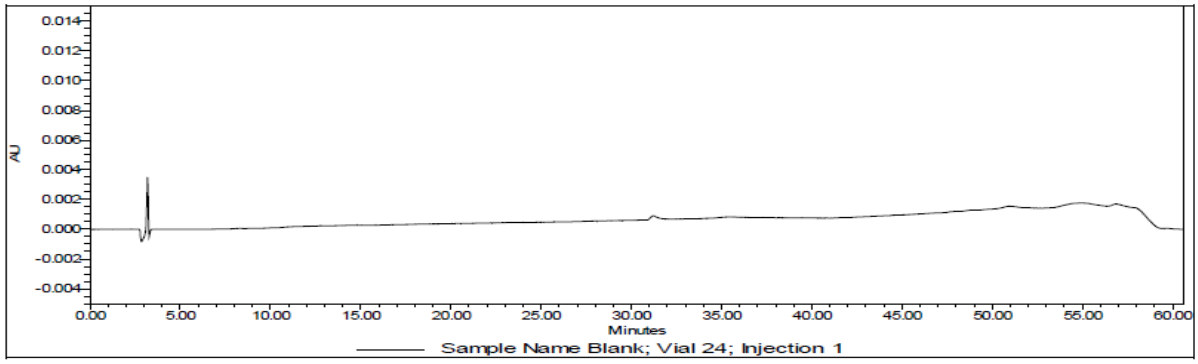


Figure 16. Typical Sensitivity Check Injection at 254nm

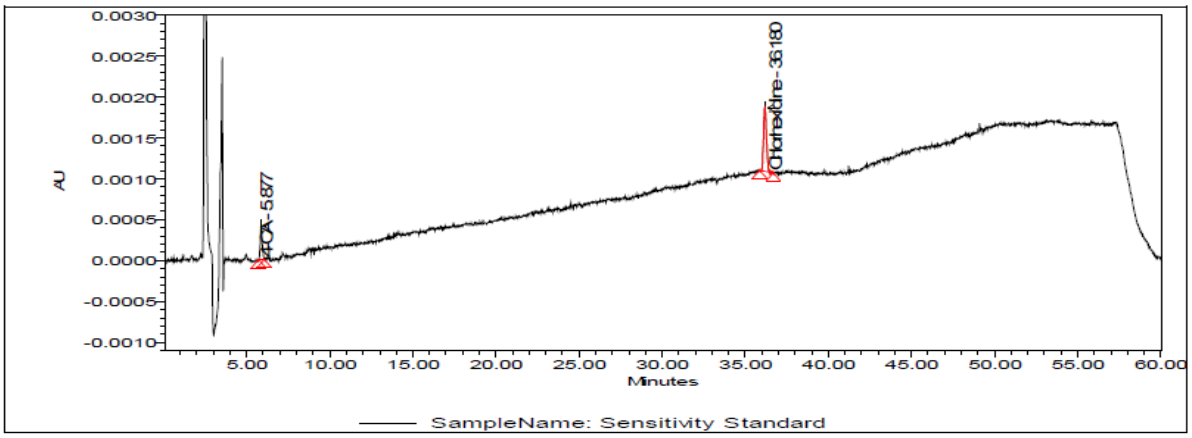


Figure 17. Typical Resolution Solution at 254nm

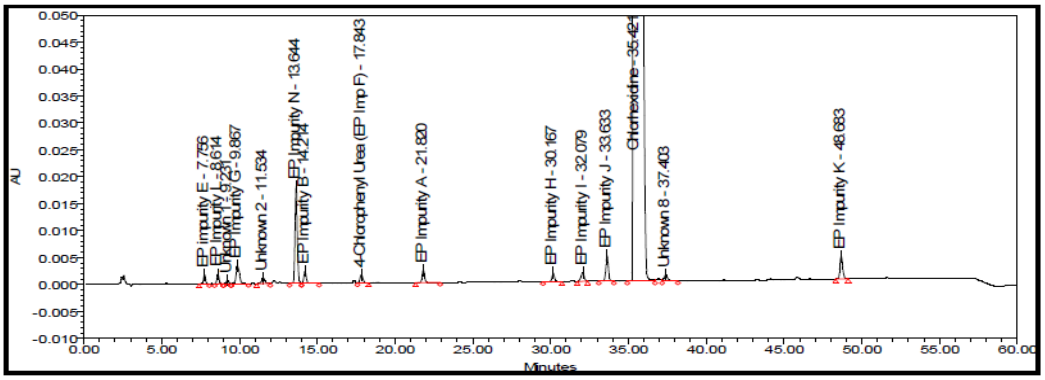


Figure 18. Typical Sample Solution at 254nm

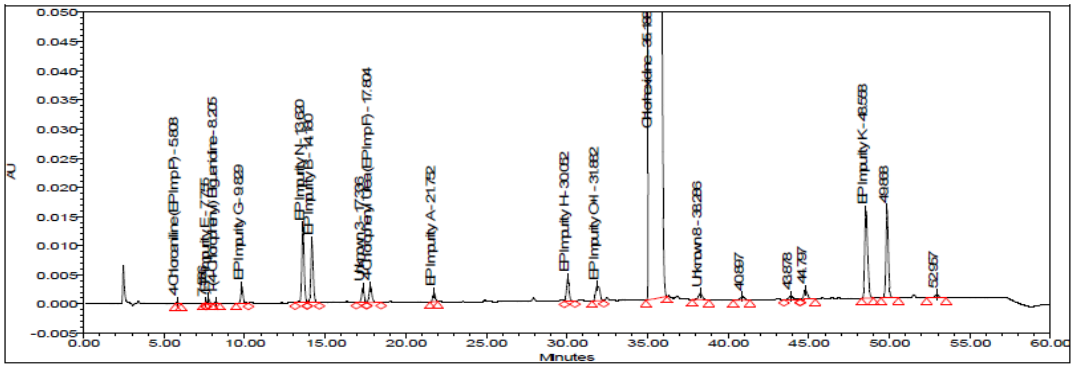


Figure 19. Typical Diluent Solution at 215nm

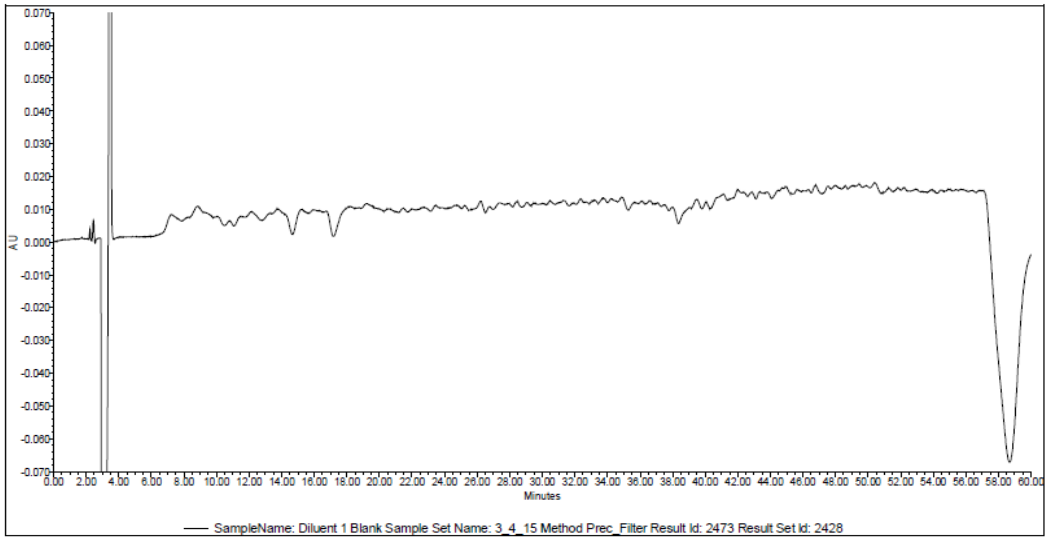


Figure 20. Typical Sensitivity Check Injection at 215nm

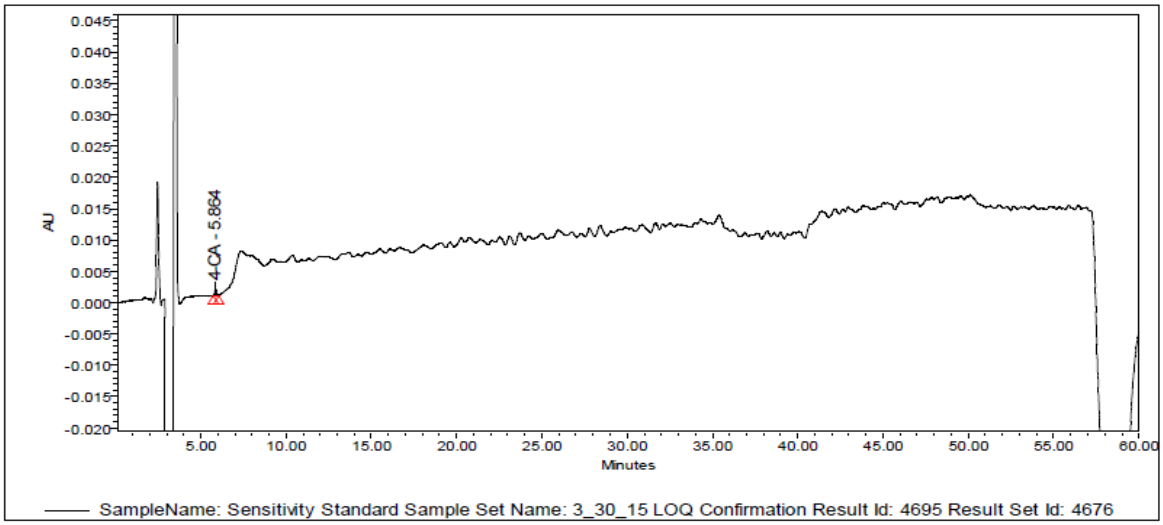
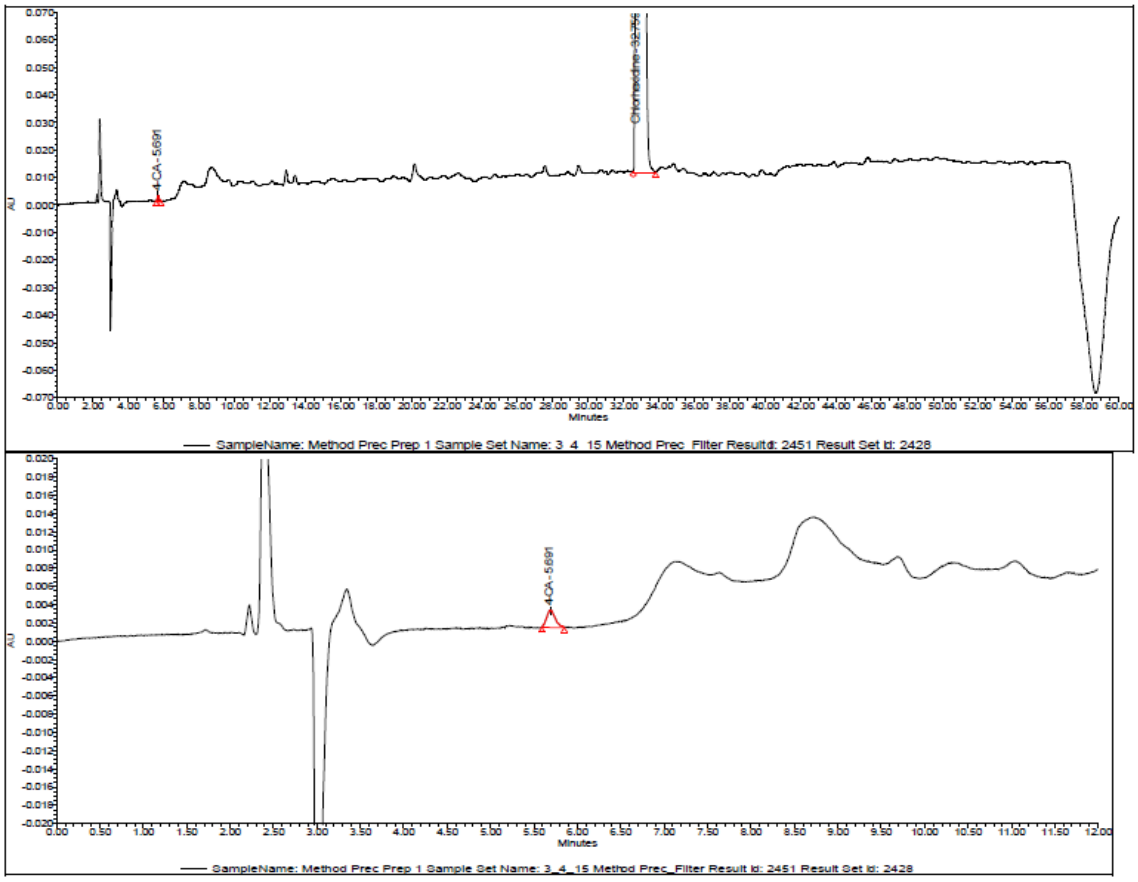


Figure 21. Typical Sample Solution at 215nm



2.2 System Suitability Criteria

The following system suitability requirements should be met prior to reporting results. The retention time for the peak corresponding to chlorhexidine in the resolution solution chromatogram should elute at about 35 minutes. The chlorhexidine peak in the sensitivity check chromatogram should have an EP signal-to-noise ratio of not less than 10 at 254 nm. The 4CA peak in the sensitivity check chromatogram should have an EP signal-to-noise ratio not less than 10 at 215 nm. All peaks in the resolution solution chromatogram should be similar to that of the chromatogram provided with the chlorhexidine for system suitability CRS batch at 254 nm.

Table 12. Relative Retention Time (RRT) and Relative Response Factors (RRF)

Related substance	RRT	RRF
1-phenylbiguanidine (1PB)	0.10	1.18
4-chloroaniline (4CA) (Impurity P) *	0.15	0.56
1-(4-Chlorophenyl) biguanide (4-CB)	0.22	1.12
(5R,6S)-2-[(4-chlorophenyl) amino]-5-hydroxy-6-[(1R,2R)-1,2,3- trihydroxypropyl]-5,6-dihydro-4H-1,3-oxazin-4-one (Impurity L)	0.23	1.00
Impurity Q	0.24	1.00
1-(6-aminohexyl)-5-(4-chlorophenyl) biguanide (Impurity G)	0.25	1.00
1-[6-(carbamimidoylaminohexyl]-5-(4-chlorophenyl) biguanide (Impurity N)	0.35	1.00
N- [[6- [[[(4-chlorophenyl) carbamimidoyl] carbamimidoyl] amino] hexyl]-carbamimidoyl] urea, (Impurity B)	0.36	1.00
N-(4-chlorophenyl) urea (4CU) (Impurity F)	0.50	1.34
1-(4-chlorophenyl)-6-[6[(cyanocarbamimidoyl)amino] hexyl] biguanide (Impurity A)	0.60	1.00
1,1-[iminobis(carbonimidoyliminohexane-6,1-diyl) bis[5-(4-chlorophenyl) biguanide] (Impurity H)	0.85	1.00
5-(2-chlorophenyl)-5′-(4-chlorophenyl)-1,1′-(hexane-1,6-diyl) dibiguanide (Impurity O)	0.90	1.00
Impurity I	0.91	1.00
1-(4-chlorophenyl)-5-[6-[[4-[(4-chlorophenyl) amino]-6-[(1S,2R,3R,4R)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl] amino] hexyl] biguanide (Impurity J)	0.96	1.00
Chlorhexidine	1.00	1.00
N-(4-chlorophenyl)-N'-[[6[[[(4-chlorophenyl) carbamimidoyl] carbamimidoyl] amino] hexyl]- carbamimidoyl] urea (Impurity K)	1.40	1.00

** Relative response factor for 4-chloroaniline is determined at 215 nm*

2.3 Data Analysis and Reporting

All impurities are reported as % w/w. At 254 nm wavelength, integrate all peaks, except 4CA. At 215 nm, integrate only 4CA and chlorhexidine peaks.

3. Example Calculation of Results

The following are examples which show how the calculations may be performed manually. They do not show how specific computer programs work. Alternative expressions may be used locally.

3.1 Impurity Content Calculation

Calculate the impurity content for each sample injection.

$$Impurity\ content\ (\%\, w/w) = \frac{Ai \times 100 \times RRF}{\Sigma[Ai] + Am}$$

Where:

Ai = Area of impurity peak

Am = Area of main analyte peak

[Ai] = Sum of all sample related impurity peaks

RRF = Relative response factor

Note that the total area ($\Sigma[Ai]$) should include all peaks arising from the CHX gel, except those arising from excipients/dissolving solvent.

$$RRT = \frac{RTi}{RTm}$$

Where:

RRT = Relative retention time

RTi = Retention time of impurity peak

RTm = Retention time of main analyte peak

3.2 Total Impurities Content

Total impurities are equal to the sum of 4CA (greater than or equal to 0.015%) and all other impurities (greater than or equal to 0.05%).

Validation of the CHX Gel Product 4CA (Impurity) Content in CHX Gel By HPLC

Demonstration of the specificity of the method for determining 4CA in CHX gel

It is essential to demonstrate the specificity of the method. To demonstrate that the method is stability indicating, the known potential drug-related impurities of synthesis and degradation should be chromatographed using the defined method. The specificity of the analytical procedure is demonstrated by the absence of interference with the peak due to 4CA from the other related substances.

The absence of interference from the formulation excipients is also demonstrated by the chromatograms obtained using the defined method for the determination of 4CA content for sample solutions of:

- Freshly prepared sample solution of CHX gel
- Active-free product (placebo gel)

Inspection of the chromatograms demonstrates sufficient selectivity between 4CA and potential synthetic impurities/degradation products. This can also be confirmed by diode array spectroscopy. LC-MS data can also confirm absence of co-eluting impurities. Example chromatograms are shown below.

Figure 22. Chromatogram Showing the Separation of 4CA from Known Potential Impurities of Synthesis and Degradation in CHX Gel

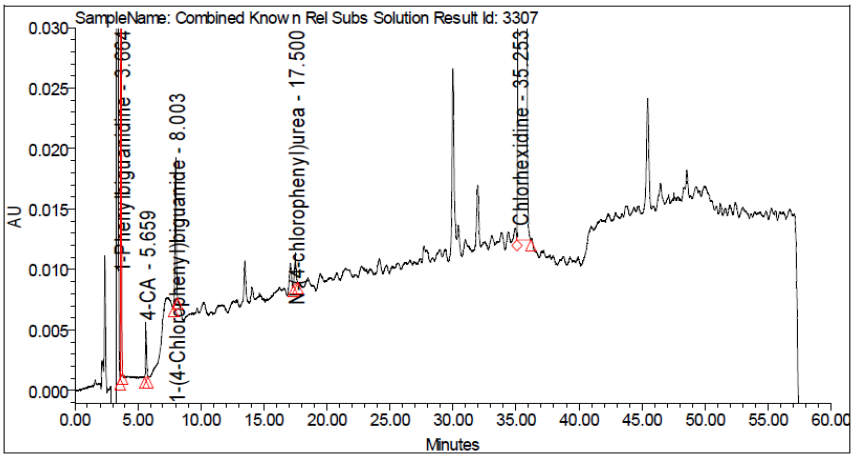


Figure 23. Chromatogram of Freshly Prepared Sample Solution of CHX Gel

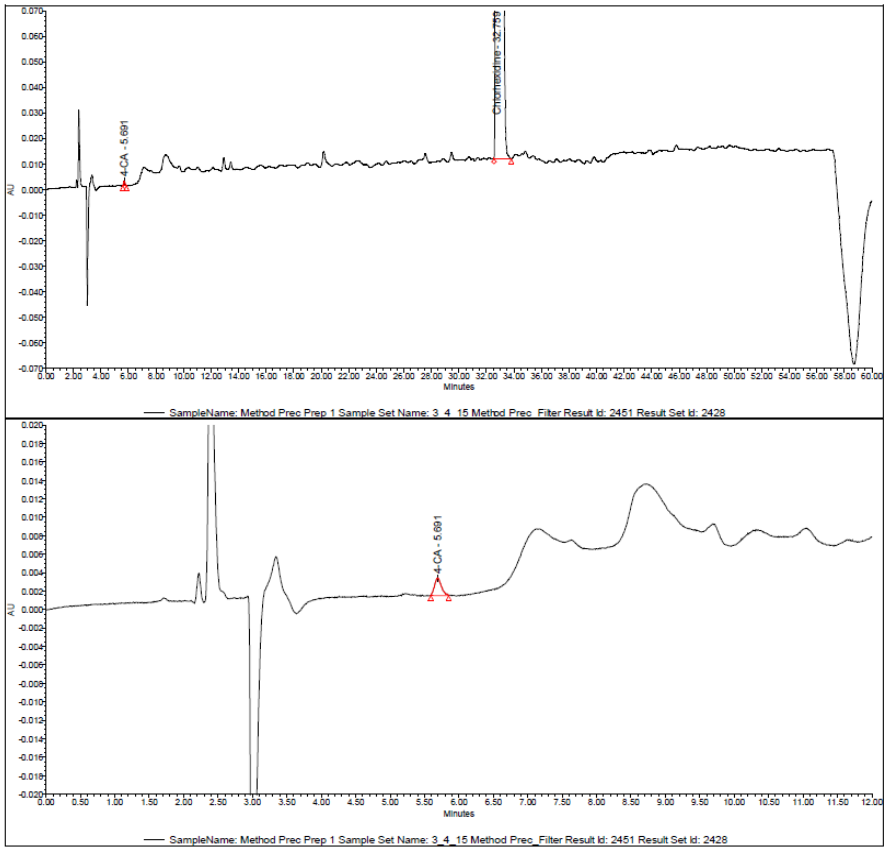
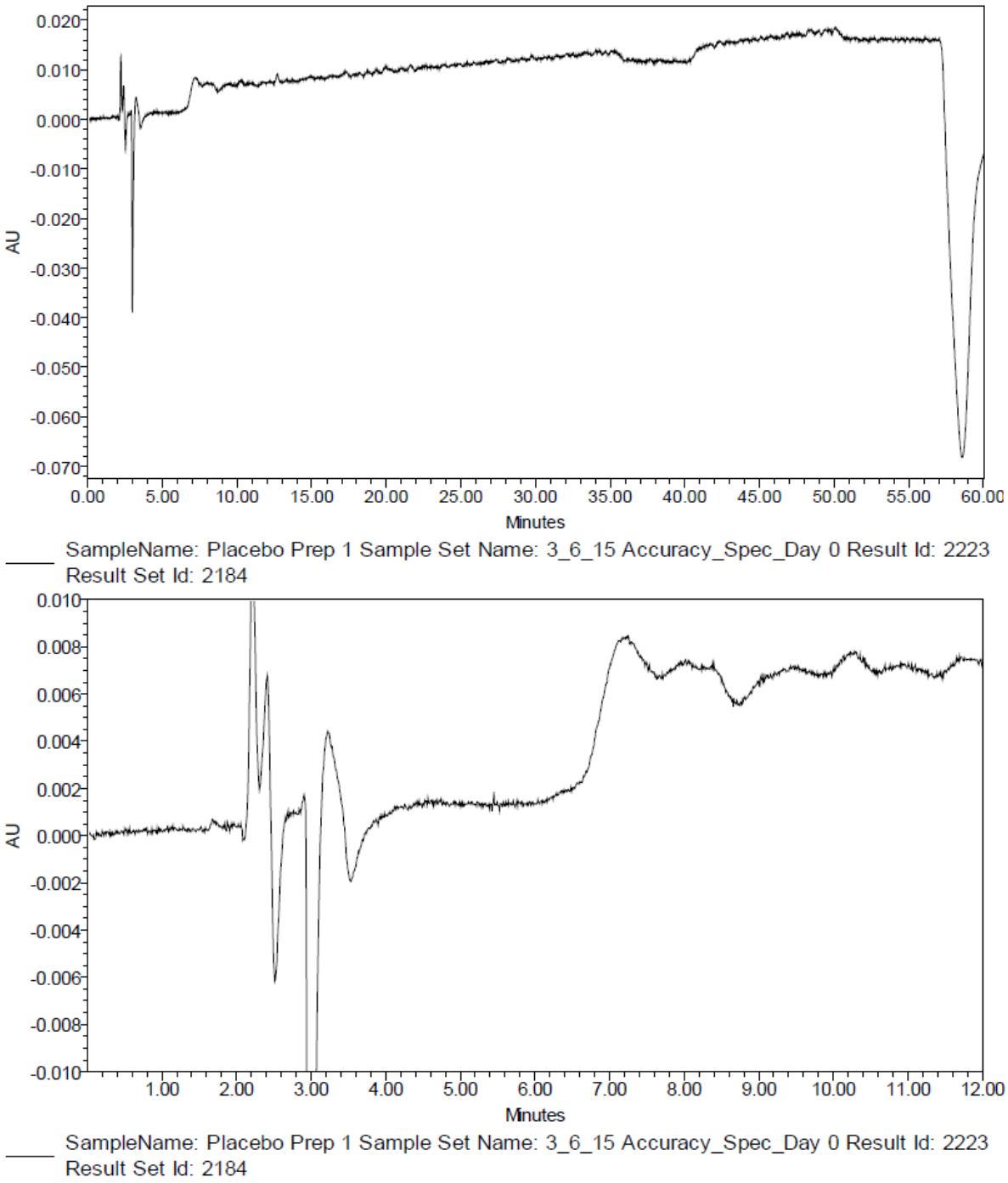


Figure 24. Chromatogram of Active Free Product (Placebo Gel)



Demonstration of the linearity of response for 4CA and chlorhexidine in the method for determining 4CA in CHX gel

- Linearity for chlorhexidine

  The linearity of the response was performed for chlorhexidine over the range 2% to 150% of the original proposed specification limit for 4CA (0.78% w/w). Solutions of chlorhexidine diacetate standard were prepared at 0.02–1.7 µg/mL concentrations (equivalent of 0.03–2.4 µg/mL of chlorhexidine digluconate concentration), and the responses due to chlorhexidine should be recorded.

- Linearity for 4CA

  The linearity of the response was performed for 4CA over the range from Limit of Quantification (LOQ) to 150% of the specification level (0.78% w/w). Current proposed specification is 0.40% w/w for the 4CA. Solutions of 4CA standard at the appropriate concentrations were prepared, and the responses due to 4CA were measured.

  The relative response factor (RRF) for the 4CA was calculated with respect to chlorhexidine digluconate. The calculated RRF values were confirmed by quantifying the precision and intermediate precision data against external standard and using the RRF.

Demonstration of the range of the method for determining 4CA in CHX gel

The method for determining 4CA content in CHX gel was demonstrated to be suitable over the range from LOQ to 150% of the specification limit (0.78% w/w), based on the results for linearity, accuracy, and precision.

Demonstration of accuracy of the method for determining 4CA in CHX gel

The accuracy of the analytical method was investigated using known quantities of 4CA, equivalent to 3%, 6%, 50%, 100%, and 120% of specification limit (0.78% w/w). The recovery results over the range of 90%–108% of the nominal concentration was averaged at 99.1% to demonstrate 4CA is quantitatively recovered by the method.

Demonstration of precision of the method for determining 4CA in CHX gel

- Repeatability

  The repeatability of the preparation of sample solutions was demonstrated by preparing and analyzing six sample solutions at 100% nominal concentration of a CHX gel. The mean measurement and RSD for the 4CA content results was then calculated. The quantification of 4CA was performed using an external reference standard of 4CA and with the calculated RRF value.

- Intermediate precision

  The intermediate precision of the analytical procedure is to be determined by preparing six sample solutions at 100% nominal concentration of a CHX gel on different equipment and by another analyst on a different day. The quantification of 4CA is to be performed using an external reference standard of 4CA and with the calculated RRF values.

Determination of the quantitation limit for 4CA in the method for determining 4CA in CHX gel

The quantitation limit for 4CA was determined using the signal-to-noise ratio. Known concentrations of 4CA were prepared, and the signal-to-noise ratio was calculated at each concentration of the analyte. The limit of quantitation was determined from the results of the lowest concentration that had a signal-to-noise ratio of ≥10.

Determination of the detection limit for 4CA in the method for determining 4CA in CHX gel

The detection limit for 4CA was determined using the signal-to-noise ratio. Known concentrations of 4CA were prepared, and the signal-to-noise ratio was calculated at each concentration of the analyte. The limit of detection was determined from the results of lowest concentration with a signal-to-noise ratio of ≥3.

Demonstration of the robustness of the method for determining 4CA in CHX gel

The robustness of the analytical method was investigated using deliberate method variations (e.g., column oven temperature, organic content, flow rate) of the chromatographic parameters. Standard sensitivity solutions and sample solutions were then analyzed with each of the modified methods. The variation factors tested to the method were qualified over the ranges.

Demonstration of stability of solutions prepared for the method for determining 4CA in CHX gel

Standard and sample solutions are to be prepared, and the 4CA content is to be determined using the defined analytical method. After storage at a refrigerated temperature (2°C–8°C) protected from light for 5 days, the 4CA content must be redetermined using freshly prepared standard solutions.

Validation Summary for Method for the Determination of Chlorhexidine Digluconate 4CA Impurity Content in CHX Gel by HPLC

To demonstrate the suitability of the HPLC assay method for determining chlorhexidine digluconate content in CHX gel by HPLC, the experiments conducted are summarized in the table below using the defined procedure.

Table 13. Summary of Validation Experiments for the Determination of Chlorhexidine Digluconate Content of in CHX Gel by HPLC

Experiment	Acceptance Criteria	Result
Specificity of the chromatographic system	None of the known related substance peaks or any excipients peak co-elute with chlorhexidine peak. The resolution between closely eluting peak pair must be > 1.5.	Resolution was demonstrated for chlorhexidine from its known potential impurities of synthesis and degradation and excipients in the formulation. Acceptable specificity was demonstrated with less than 1.0% label claim response from excipients of the formulation with chlorhexidine digluconate at 254 nm.

Experiment	Acceptance Criteria	Result
Linearity of detector Response to chlorhexidine diacetate (range equivalent to 70%–130% of nominal column loading concentration	Correlation coefficient (R) > 0.995 y-intercept of the linear regression must be < 2.0% of nominal column loading/concentration.	Linearity was demonstrated for the response due to chlorhexidine. Determined Coefficient (R^2) = 0.9996 Intercept = 5.3% of nominal column loading/ concentration
Range	Report results	Chromatographic linearity, accuracy, and precision demonstrated from 80% to 120% of the nominal concentration employed during the analytical procedure.
Accuracy	Mean recovery at each level should be 98.0%–102.0%. The % RSD of the % recovery at each level must be < 2.0%.	Accuracy was demonstrated by recovery from spiked active-free gel formulation.

Accuracy Level (% of nominal concentration)	**Mean accuracy** (%)
80	101.0
100	101.1
120	101.0

Precision: repeatability Preparation of sample solutions	The % RSD must be < 2.0% for the six assay results.	RSD = 1.8% (n=6) Repeatability was demonstrated for six replicate preparations of the above solutions.
Intermediate precision	The % RSD must be< 2.0% for the six assay results. The % RSD of the 12 assay results must be < 2.0%	Intermediate precision was demonstrated for different analysts using different equipment to analyze one batch of CHX gel on different days.
Robustness	% difference from initial assay should not be more than 2.0% System suitability criteria for resolution should meet at each robustness condition	Robustness was demonstrated by experiments that focused on amendments to chromatographic parameters, including percentage of organic component in mobile phase, amount of mobile phase modifier, column temperature, and flow rate.

Validation of The Method for Determination of Drug-Related Impurities in CHX Gel By HPLC

Demonstration of the specificity of the method for determining chlorhexidine digluconate in CHX gel

To demonstrate the specificity of the method and to demonstrate that the method is stability indicating, the known potential drug-related impurities of synthesis and degradation were chromatographed using the defined method. A sample of the relative retention times obtained are given in the table below. The specificity of the analytical procedure was demonstrated by the absence of interference with the peak due to chlorhexidine from the other potential impurities listed.

Table 14. Specificity of the Method

Compound name	Relative retention time*	RRF
Chlorhexidine	1.00	1.00
4CA (d)	0.15	27.71**
4-chlorophenyl urea (d)	0.50	1.34
1-(4-chlorophenyl) biguanidine (d)	0.22	1.12
1-phenylbiguanidine (s)	0.10	1.18

** Relative retention time of the peak relative to the peak due to chlorhexidine; (s) Impurity of synthesis; (d) degradation product.*

*** 4CA will be quantified with external standard, and RRF will not be used for any calculations.*

To demonstrate the absence of interference from the formulation excipients, the chromatograms were obtained using the defined method for the determination of chlorhexidine content and related impurities content for the following sample solutions of:

- Chlorhexidine for system suitability CRS
- Freshly prepared CHX gel
- Active-free product (placebo gel)
- Aged/stressed active-free product (aged/stressed placebo gel)
- Aged/stressed CHX gel

Inspection of the chromatograms was used to demonstrate sufficient selectivity between chlorhexidine and potential synthetic impurities/degradation products. This can also be additionally be confirmed by diode array spectroscopy. LC-MS data can also confirm the absence of co-eluting impurities.

Demonstration of the linearity of response for chlorhexidine and related drug impurities in the method for determining chlorhexidine digluconate in CHX gel

The linearity of the response obtained for chlorhexidine was investigated over the range 70%–130% of the nominal level recommended in the analytical method. Solutions of chlorhexidine standard were prepared at the appropriate concentrations, and the responses due to chlorhexidine was measured.

- Linearity for 4CA

  The linearity of the response was performed for 4CA over the range 50%–167% of the specification level (0.05% w/w) recommended in the analytical method. Solutions of 4CA standard were prepared at the appropriate concentrations, and the responses due to 4CA were measured.

- Linearity for 4-chlorophenyl urea

  The linearity of the response obtained for 4-chlorophenyl urea was investigated, over the range 10%–167% of the specification level (0.25% w/w) recommended in the analytical method. Solutions of 4-chlorophenyl urea standard were prepared at the appropriate concentrations, and the responses due to 4-chlorophenyl urea were measured.

- Linearity for 1-(4-chlorophenyl) biguanidine

  The linearity of the response obtained for 1-(4-chlorophenyl) biguanidine was investigated over the range 10%–167% of the specification level (0.5% w/w) recommended in the analytical method. Solutions of 1-(4-chlorophenyl) biguanidine standard were prepared at the appropriate concentrations, and the responses due to 1-(4-chlorophenyl) biguanidine were measured.

- Linearity for 1-phenylbiguanidine

  The linearity of the response obtained for 1-phenylbiguanidine was investigated over the range 10%–167% of the specification level (0.25% w/w) recommended in the analytical method. Solutions of 1-phenylbiguanidine standard were prepared at the appropriate concentrations, and the responses due to 1-phenylbiguanidine were measured.

Demonstration of the range of the method for determining chlorhexidine and drug-related impurities in CHX gel

The analytical method was demonstrated to be suitable over the range from 70% to 130% of the label claim of 7.1% w/w for chlorhexidine digluconate, based on the results for linearity, accuracy, and precision.

Also, the method was shown to be suitable over the range from LOQ to 167% of the specification for 4-chlorophenyl urea (0.5% w/w), 1-(4-chlorophenyl) biguanidine (0.5% w/w), and 1-phenyl biguanidine (0.25% w/w), based on the results for linearity, accuracy, and precision.

The method was shown to be suitable over the range from LOQ to 120% of the specification for 4CA, based on the results for linearity, accuracy, and precision.

Demonstration of accuracy of the method for determining chlorhexidine and drug-related impurities in CHX gel

The accuracy of the analytical method was investigated using known quantities of chlorhexidine diacetate reference standard, equivalent to 80%, 100%, and 120% of nominal concentration of chlorhexidine digluconate. The accuracy of the analytical method was further investigated using known quantities of 4-chlorophenyl urea, 1-(4-chlorophenyl) biguanidine, and 1-phenylbiguanidine equivalent to 50%, 100%, 120%, and 167% of each specification limit, spiked into the CHX gel sample.

Additionally, accuracy studies were carried out for 4CA at 26%, 32%, 38.5%, 45%, 52%, 100%, and 120% of 0.78% w/w the proposed developmental specification level by spiking known amounts of 4CA into the CHX gel sample.

Demonstration of precision of the method for determining chlorhexidine digluconate and drug-related impurities in CHX gel

- Repeatability of chlorhexidine digluconate content

  The repeatability of the chlorhexidine digluconate content in the sample solutions was determined by preparing six sample solutions at 100% of the nominal concentration.

- Intermediate precision

  Intermediate precision for chlorhexidine digluconate content

  The intermediate precision for the chlorhexidine digluconate content was performed by preparing and analyzing six samples for chlorhexidine digluconate in the same batch of CHX gel on different equipment, by two different analysts on different days.

  Intermediate precision for drug-related impurities content of chlorhexidine

  The intermediate precision for the drug-related impurities content was performed by preparing and analyzing six samples of CHX gel spiked with the known impurities at specification limits during the time of method validation. This experiment must be carried out on different equipment, by different analysts, on different days. Content levels of spiked known impurities and unknown impurities were determined.

Determination of the quantitation limit for chlorhexidine drug-related impurities in CHX gel

The quantitation limits for the known potential impurities of synthesis and degradation were determined using the signal-to-noise ratio. Known concentrations of specified known impurities of chlorhexidine were prepared, and the signal-to-noise ratio is to be calculated for each individual impurity. The limit of quantitation was determined from the result of lowest concentration with a signal-to-noise ratio of ≥10.

The quantitation limit should reflect that the sensitivity of the method for determining commonly occurring impurities is adequate for the quantitation of impurities at the reporting limit 0.015% w/w for 4CA, and maximum of 0.05% w/w for all other impurities.

Demonstration of the robustness of the method for determining chlorhexidine digluconate and related drug impurities in CHX gel

The robustness of the analytical method was investigated using deliberate variations of the chromatographic parameters, such as flow rate, column temperature, organic content, mobile phase modifiers, and gradient variations. Standard and sample spiked with known amount of specified impurities were analyzed with each modified method.

Resolution between the peaks and percent difference of the content values from initial results were calculated at each changed condition.

Demonstration of stability of solutions prepared for the method for determining chlorhexidine digluconate and drug-related impurities in CHX gel

Sample solutions were prepared, and the percent difference in the content of chlorhexidine digluconate and drug-related impurities from the initial values were determined using the defined analytical method. After storage at room temperature (15°C–25°C) protected from light, the concentration/impurity content were redetermined at predetermined intervals for 48 hours using freshly prepared standard solutions.

Validation Summary for the Determination of Drug-Related Impurities in CHX Gel by HPLC

In order to demonstrate the suitability of the HPLC method for the determination of drug-related impurities content of CHX Gel, the experiments are summarized in the table below that were conducted using the defined procedure.

For the known drug related impurities, the method validation studies were performed according to the specification limits proposed for validation. For 4CA, the method validation proposed specification levels are NGT 0.05% w/w and NGT 0.78% w/w, at the time of validation studies. The validations were conducted for the 4CA at the specification levels of NGT 0.08% w/w (release specification) and NGT 0.35% w/w (shelf life specification).

Table 15. Summary of Validation Experiments for the Determination of Drug-Related Impurity Content of CHX Gel by HPLC

Experiment	Acceptance criteria	Result	
Specificity of the chromatographic system	None of the known related substance peaks or any excipients peak co-elute with 4CA peak. The resolution between closely eluting peak pairs must be > 1.5. Report results.	Resolution demonstrated for chlorhexidine and known potential impurities of synthesis and degradation and excipients of the formulation.	
Response factors of potential drug-related impurities		RRF of all relevant drug-related impurities in chlorhexidine digluconate have been determined where suitably pure samples of the impurities are available.	
		Name	**RRF at 254 nm**
		Chlorhexidine	1.00
		4CA	27.71
		4-chlorophenyl urea	1.34
		1-(4-chlorophenyl) biguanidine	1.12
		Phenylbiguanidine	1.18

Experiment	Acceptance criteria	Result
Linearity of detector response for 4CA (Range: 50%–167% of 0.05% w/w)	Correlation coefficient(R) > 0.95 for all impurities. y-intercept of the linear regression must be < 10.0% of concentration at upper specification acceptance criterion for all impurities.	Linearity was demonstrated for the response due to 4CA. Determined coefficient (R2) = 0.9988 % y-intercept = 5.5% of concentration at upper specification acceptance criterion (0.05%)
Linearity of detector response for 4CA (Range: 10%–120% of 0.78% w/w)		Linearity was demonstrated for the response due to 4CA. Determined coefficient (R2) = 0.9994% y-intercept = 2.1% of concentration at upper specification acceptance criterion (0.78%)
Linearity of Detector Response to for 4-chlorophenyl urea (Range:10%–167% of 0.25% w/w)		Linearity demonstrated for the response due to 4-chlorophenyl urea. Determined coefficient (R2) = 0.9929 y-intercept = 5.08% of concentration at upper specification acceptance criterion (0.25%)
Linearity of Detector Response to for 1-(4-chlorophenyl) biguanidine (Range: 10%–167% of specification limit)		Linearity was demonstrated for the response due to 1-(4-chlorophenyl) biguanidine. Determined coefficient (R2) = 0.9942 y-intercept = 4.59% of concentration at upper specification acceptance criterion (0.50%)
Linearity of Detector Response to for 1-phenylbiguanidine (Range: 10%–167% of specification limit)		Linearity was demonstrated for the response due to 1-phenylbiguanidine. Determined coefficient (R2) = 0.9943 y-intercept = 2.75% of concentration at upper specification acceptance criterion (0.50%)
Range	Report results	4CA, 4-chlorophenyl urea, 1-(4-chlorophenyl biguanidine, 1-phenylbiguanidine were validated in the range from 50% to at least 120% of the appropriate upper specification limit.
Accuracy	The mean % recovery result must be 80%–120% RSD for all recovery values should not be more than 15.0%.	Accuracy was demonstrated by recovery from samples spiked with potential drug-related impurities (4CA, 4-chlorophenylurea, 1-(4-chlorophenyl biguanidine, 1-phenylbiguanidine).
Precision repeatability	The % RSD for the individual impurities greater than 0.1% from the six results should not be more than 10.0%	Repeatability was demonstrated by analysis of six replicate preparations of a sample solution.

Experiment	Acceptance criteria	Result	
Intermediate precision	The % RSD for the individual impurities greater than 0.1% from the six results should not be more than 10.0%. The % RSD for the individual impurities from the 12 results (two analysts) should not be more than 15.0%.	Intermediate precision was demonstrated for different analysts using different equipment to analyze the same batch of CHX gel on different days.	
Quantitation limit	Signal-to-noise ratio for the impurities should be > 10.	**Quantitation Limit (QL)**	
		4CA	0.015%
		4-chlorophenyl urea	0.025%
		1-(4-chlorophenyl biguanidine)	0.05%
		phenylbiguanidine	0.05%
		Sensitivity Reported	
Robustness	% difference from initial impurity content should not be more than 10.0%. System suitability criteria for resolution should meet at each robustness condition.	Robustness was demonstrated by experiments that focused on amendments to chromatographic parameters, including percentage of organic component in mobile phase, amount of mobile phase modifier, column temperature, and flow rate.	
Stability of solutions of impurities	% difference in mean content for the individual impurities from the initial to the different time interval should not be more than 10.0%	Solutions of samples were stable for up to 12 hours when stored at ambient room temperature (15°C–25°C).	

Microbiological Limits Test

CHX gel samples should be tested for the routine microbiological tests in compliance with the current Ph. Eur./USP/JP harmonized method (e.g., Ph. Eur. 2.6.12 and Ph. Eur. 2.6.13). The criteria originate from the cutaneous use criteria described in the current harmonized Ph. Eur./USP/JP (e.g., Ph. Eur. 5.1.4/USP 1111 Microbial Quality of Non-Sterile Pharmaceutical Preparations) and are listed in table 16.

Table 16. Microbial Limit Test Criteria for CHX Gel

Test	Criteria	Justification of criteria
Total aerobic microbial count	NMT 102 CFU/g	Complies with Ph. Eur. 5.1.4 and USP <1111> for cutaneous application
Total yeast and mold count	NMT 101 CFU/g	
S. aureus	Absent/1 g	
P. aeruginosa	Absent/1 g	

Antimicrobial Effectiveness Test and Kill-Time Study Summary

Although CHX gel product is inherently preserved, antimicrobial effectiveness testing was performed by GSK as per the test method specified in USP <51> and Ph. Eur. 5.1.3. The CHX gel was challenged with approximately 105–106 cfu/mL of bacteria and fungi to include *S. aureus, P. aeruginosa, E. coli, C. albicans,* and *A. brasiliensis* (as a spore suspension). Surviving organisms in the inoculated samples were enumerated after 2, 7, 14, and 28 days storage at 25°C. The test criteria require a 2log(10) reduction of bacteria in 2 days followed by a 3log(10) reduction in 7 days, and no increase in 28 days. Additionally, a 2log(10) reduction of fungi in 14 days is required with no increase in 28 days. All result met the Category A acceptance criteria of Ph. Eur. Chapter 5.1.3 for preparations of cutaneous application. This study ensures stability of CHX gel against the range of vegetative and spore-forming challenge organisms.

To evaluate the *in vitro* antimicrobial efficacy of chlorhexidine digluconate gel at a concentration of 5.68% w/w and 7.1% w/w, kill-time studies were conducted. This test evaluates *in vitro* antimicrobial efficacy by examining the rate at which concentrations of an antimicrobial agent kill a bacterial isolate. The formulation was able to exhibit significantly antimicrobial activity by demonstrating a greater than 4log(10) reduction (>99.99% kill) against the indicator organisms after a 30-second contact time.

Non-Specification Tests: Elemental Impurities

A risk assessment was conducted to evaluate the potential for elemental impurities present in CHX gel. This risk assessment was performed in accordance with ICH Q3D “Guideline for Elemental Impurities” and considered the following: the input API, excipients, processing aids (water), manufacturing equipment, and container closure system. ICH Q3D does not specifically list the topical administration route, so the guidance associated with the oral route was used for the risk assessment on CHX gel.

No ICH Q3D elements from Class 1, 2A, 2B, or 3 were deliberately used at any stage of the manufacturing of the components, packaging, or the finished product itself. The risk assessment therefore was focused upon the Class 1 elemental impurities (cadmium, lead, arsenic, and mercury) and the Class 2A elemental impurities (cobalt, vanadium, and nickel). Class 2B and Class 3 were discounted from the rest of the assessment as recommended in the ICH Q3D guidance. The likelihood of Class 1 and 2A elements reaching the final dose delivered to the patient were deemed to be low, considering:

- Information from suppliers on how components of the drug product formulation were made.
- Secondary manufacturing process that converted components into the finished product.
- Primary packaging into an aluminum foil sachet.

Due consideration was given to the fact that the suppliers had complied with earlier regulations and pharmacopoeia standards rather than the more extensive range in ICH Q3D. Nevertheless, different batches of drug product over the course of the product development were tested for the relevant elemental impurities, using an ICP-AES method. The testing proved that only very low levels of the relevant elements were detected, viz <10% of the PDE limit. These data provide support that potential elemental impurities in CHX gel arising from individual components of the dosage unit are controlled to within safe levels by the components’ specifications.

Based on the actual data of testing conducted by GSK on different drug product batches, the cumulative effect of the material specifications, in combination with adherence to the overall control strategy, provides assurance to control elemental impurities in the product to within safe levels. These impurity levels are well below the proposed ICH Q3D permitted daily exposures without the need to include in the product specification.

6. Clinical and Nonclinical Studies

Chlorhexidine digluconate salt is soluble in water to at least 50% w/v and is a substantive disinfectant effective against a wide range of bacteria and some fungi. The CHX gel contains 7.1% w/w chlorhexidine digluconate, which translates into 4% w/w level of chlorhexidine as specified by the April 2013 World Health Organization Guidance on Postnatal Care of the mother and newborn (World Health Organization, 2013). Chlorhexidine is also regularly used as a disinfectant in oral products and in surgical settings.

Efficacy is supported by three published community-based randomized controlled trials of chlorhexidine digluconate 7.1% solution in South Asia and a further non-inferiority study of chlorhexidine gel versus solution for antimicrobial efficacy supports a bridge of efficacy from solution to the gel formulation (El-Arifeen S et al. 2012; Hodgins S, 2010; Mullany L et al. 2006; Soofi S et al. 2012). These data were supplemented by literature reviews of clinical and nonclinical safety information.

Three *in vitro* tests were performed with the gel formulation: antibacterial equivalency (kill time and substantivity, described above) and a skin-irritancy study, to bridge efficacy and safety data from the published studies of chlorhexidine solution to the gel product

The *in vitro* assessment of skin irritancy was conducted comparing the chlorhexidine solution formulation with the final drug product of CHX gel and showed there was no difference in skin irritancy. It was reasonable to presume that this would be the case on human newborn baby skin. It was subsequently demonstrated that the final drug product of CHX gel produced little or no irritation on human newborn baby skin, as was the case in Hodgins' study.

A summary of the product characteristics including clinical and non-clinical information are available on the EMA website (https://www.ema.europa.eu/en/umbipro-h-w-3799)

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