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Long-term stability study of chlorhexidine gluconate mouth wash in experimental formula

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Abstract

Chlorhexidine is a bisbiguanide antiseptic and disinfectant that is bactericidal or bacteriostatic against a wide range of Gram-positive and Gram-negative bacteria. Stability study is the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods. Physical, chemical data are generated as a function of time and storage conditions. The purpose of this study is designed to evaluation the stability life of chlorhexidine gluconate mouth wash in experimental formula. Stability information from long-term testing was designed according to the European agency for evaluation of medicinal products storage conditions of high relative temperature and humidity. The prepared samples were analyzed according to united state pharmacopoeia -27. Assays were performed by high performance liquid chromatography analysis. Ascorbic acid, sodium citrate with organic solvents and antitoxins has been used to provide efficient, simple mixing method for chlorhexidine gluconate mouth wash. The assays and level of related substances predicated stable formula under long term stability study conditions. These results had shown non-significant changes ($p>0.05$). This formula provided successful distribution and stability of chlorhexidine gluconate in mouth wash solution and this formula is also suitable for mass production and stable products for more than 3 years**.**

Key word: stability study–long term – chlorhexidine gluconate

Introduction

Chlorhexidine gluconate is recognized as being an effective oral antimicrobial agent and is routinely used in periodontal therapy and for caries prevention [(1)]. Chlorhexidine has been found to have broad-spectrum antimicrobial action [(2-3)], and a relative absence of toxicity [(4)]. Chlorhexidine, in the form of a salt, has been used as an oral antiseptic in mouthwash, toothpaste, and chewing gum [(5)]. The treatment of chronic periodonitis focus on stopping destruction of periodontal support elimination of pathogenic bacteria in periodontal pocket [(6)]. This led to use of antimicrobial agents, among the antimicrobial agents chlorohexidine has been used in sub-gingival irrigation [(7)].

Stability is defined as the capacity of a drug substance or a drug product to remain within specifications established to ensure its identity, strength, quality, and purity throughout the retest period or expiration dating period, as appropriate [(8)]. According to the long duration of room-temperature shelf lives (may range up to several years), stability tests are often performed under stressed conditions

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(e.g. elevated temperatures) to accelerate the degradation process [9].

In a rational design and evaluation of dosage forms for drugs, the stability of the active components considered as the major criterion in determining their suitability [10]. Several forms of instability can occur. First, there may be chemical degradation of the drug, leading to substantial lowering of the quantity of the therapeutic agent in the dosage form. Second, although the degradation of the active drug may not be that extensive, a toxic degrading may be formed in the decomposition process. [11, 12]. An example of a product of degradation that is significantly more toxic such as the conversion of chlorohexidine gluconate to P-chloroaniline [13]. Third, instability of a drug product can lead to a decrease in its bioavailability, rather than to loss of drug or the formation of toxic degradation products (P-chloroaniline from Chlorhexidine gluconate degradation) [14, 15]. Fourth, there may be substantial changes in the physical appearance of the dosage forms [16]. Since most drugs are organic molecules, it is important to recognize that many pharmaceutical pathways are, in principle, similar to reactions described for organic compound [17]. The major difference that has to be considered is that most pharmaceutical reactions occur due to or are governed by water, oxygen, or light, rather than other active ingredients [18]. Thus, the most common routes of decomposition are: hydrolysis, oxidation, photolysis, racemization, and decarboxylation [19, 20]. The aim of this study is the evaluation of the physico-chemical stability for suggested formula on long storage period to be considered for large scale manufacturing.

Material and method

Chemicals:

All chemical and reagents used in this study were with high purity for analytical purposes. They were supplied from either Fluka or BDH companies.

Samples:

The mouth wash were evaluated for stability according the European Agency for the Evaluation of Medicinal Products. Mouth wash were packed in close glass bottle (three batches) to be close from the real conditions of storage in pharmacies dentist clinics (21, 22).

Sample preparation:

Mouth wash were prepared by dissolving the chlorohexidine gluconate, ascorbic acid, and sodium citrate, sodium metabisulfite, in distilled water with stirring until the clear solution was obtained. Glycerin, paraben concentrate, color, and mint flavor then were added to the previous solution with stirring. The final volume was adjusted to 100 ml with distilled water.

The mouth wash ingredients are:

Ingredients	Gm / ml
Chlorhexidine (gm)	0.12
Ethanol 96% (ml)	3.50
Paraben (ml)	1.0
Glycerin (ml)	3.0
Citric acid (gm)	0.22
Sodium citrate (gm)	0.10
Sodium metabisulfite (gm)	0.15
Green color (gm)	0.001
Mint flavor (gm)	0.01
Total volume (ml)	100.00

Chemical analysis:

Analysis procedures were performed according to united state pharmacopoeia - 27 (23). Instruments used in the study were meeting the requirements of analysis.

Results

The initial evaluations of physcio–chemical properties for Chlorhexidine mouth wash are listed in Table-1. The initial evolution complies with USP requirements for chlorhexidine gluconate in mouth wash.

The high performance liquid chromatography assays in the three batches at zero time were achieved according USP-27 procedures are listed in Table-2. Each result (peak area and % and assays) represents the average of three runs.

The evaluation of the physcio-chemical properties with storage periods (one year) according the European agency for the evaluation of medicinal products conditions are listed in Table 3. Non-significant changes were estimated of assays during storage period ($p>0.05$). The estimations of Chlorhexidine gluconate in mouth wash during one year storage are listed in Table -4. The assays were ranges from (103.261% - 102.580 %). Each run represent the average of the three batches. Table-5 represents the determination of the related substance (p-chloroaniline) in mouth wash during storage period. The related substances concentration was evaluated by using HPLC. Figure-1 represent the changes of assays at three different conditions with storage time (Series 1: represent assays at 40 ºC ±2ºC, 45 % RH ± 5%, Series 2: represent assays at 50 ºC ±2ºC, 65 % RH ±5%, Series 3: represent assays at 60 ºC ±2ºC, 75 % RH ±5%, and Series 4: represent assays at 70 ºC ± 2ºC, 75 % RH ± 5%).

Discussion:

Samples were prepared according to the formula that listed in sample preparation. The mixing procedure were depends on two steps: first included dissolving the chlorhexidine with citric acid and sodium citrate in distilled water to avoid any decomposition of Chlorhexidine (24). All these steps were carried in temperature range of (25-27 °C). This could be related to avoid any decomposition of Chlorhexidine, especially chlorhexidine is very sensetive to heating and alkaline pH. [25,26]. The second step included the addition of other additive (glycerin, paraben, sodium metabisulfite, color and flavor) to Chlorhexidine gluconate solution. Paraben is designed in this

formula as preservative while the sodium metabisulfite considered as antioxidants to increase the stability of the chlorhexidine gluconate in the final solution [27]. The acidic pH (pH =5.5) of the mouth wash provided efficient mixing for the chlorhexidine gluconate in solution and prevent any side degradation during accelerated storage conditions and increasing the product live, however the pH of the mouth wash was in close range (5.55-5.50). The low changes in pH represent low formation of the related substance (alkaline p-chloroaniline) [28]. These methods have been repeated for three times to collect three different batches at the same conditions to avoid manufacturing or analysis errors [29, 30]. The chlorhexidine gluconate have been identified by HPLC analysis using direct compares with equivalents standard. The sample was first being filtered then injected to HPLC. The obtained chromatograms from the mouth wash were similar to standard preparations [23, 31]. The matching of chromatograms between the chlorhexidine gluconate in mouth wash and standard give strong evidence of the stability of chlorhexidine gluconate without any HPLC abnormal peaks (decomposition, or related substances of chlorhexidine gluconate). However, HPLC provided powerful information about the detection of chlorhexidine gluconate in manufactured mouth wash and uniformity of contain [23].

The assays of the three batches showed non-significant changes ($p>0.05$) in physical or chemical properties such as the color or the appearance, test, related substances or chlorhexidine gluconate concentration in the final products. However all obtain results indicated the stability of the formula with storage time, increasing temperature and relative humidity [31].

In conclusion chlorhexidine gluconate were stable in the designed formula, however, enhanced temperature and humidity have no effects on the stability of the product. The mouth wash could be stored for more than 3 years. The citric acid and anti oxidants in mixing with Chlorhexidine present as stability factors of the chlorhexidine gluconate in the formula. The HPLC methods used in this study reported efficient method for monitoring of Chlorhexidine gluconate and it's related in mouth wash products.

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Table- 1. The physical and manufacturing properties of chlorohexidine gluconate mouth wash in zero time storage:

Test	Result
% assay	103.243
Wt/ ml	1.00632
Color intensity	0.568
Color	Green
Identification	Positive
Microbial test	Negative
Viscosity (D type)	2.163 mm-2 S-2
pH	5.55
Test	mint test
Odor	Mint

Table -2. The HPLC peak area and percentages of assays at zero time for the three batches.

Bach No.	Peak area (mm2)	% assays
1	5653850.566	103.261
2	5658778.337	103.351
3	5646020.885	103.118

Standard Peak Area	5475301

Table -3 the physico-chemical properties in long stability study conditions:

Time (month)	Temperature °C	Wt\ml	color	Viscosity mm2S-2	pH	P-values
1st	40	1.00632	Green	2.163	5.55	0.112
	50	1.00632	Green	2.163	5.45	
	60	1.00701	Green	2.165	5.55	
	70	1.00701	Green	2.167	5.55	
2nd	40	1.00632	Green	2.163	5.55	0.121
	50	1.00701	Green	2.163	5.55	
	60	1.00702	Green	2.165	5.55	
	70	1.00704	Green	2.167	5.55	
3rd	40	1.00701	Green	2.163	5.55	0.114
	50	1.00708	Green	2.163	5.55	
	60	1.00710	Green	2.165	5.55	
	70	1.00712	Green	2.167	5.55	
4th	40	1.00701	Green	2.163	5.55	0.115
	50	1.00708	Green	2.163	5.55	
	60	1.00711	Green	2.165	5.55	
	70	1.00714	Green	2.167	5.55	
5th	40	1.00709	Green	2.163	5.55	0.177
	50	1.00711	Green	2.163	5.54	
	60	1.00711	Green	2.165	5.54	
	70	1.00712	Green	2.167	5.54	
6th	40	1.00712	Green	2.163	5.54	0.198
	50	1.00712	Green	2.164	5.54	
	60	1.00714	Green	2.165	5.54	
	70	1.00716	Green	2.168	5.54	
7th	40	1.00712	Green	2.163	5.54	0.199
	50	1.00712	Green	2.164	5.54	
	60	1.00714	Green	2.165	5.54	
	70	1.00716	Green	2.168	5.54	
8th	40	1.00712	Green	2.163	5.54	0.215
	50	1.00712	Green	2.164	5.54	
	60	1.00714	Green	2.165	5.54	
	70	1.00716	Green	2.168	5.54	
9th	40	1.00712	Green	2.163	5.54	0.215
	50	1.00712	Green	2.164	5.53	
	60	1.00714	Green	2.165	5.53	
	70	1.00716	Green	2.168	5.53	
10th	40	1.00712	Green	2.164	5.53	0.216
	50	1.00712	Green	2.164	5.53	
	60	1.00714	Green	2.164	5.53	
	70	1.00716	Green	2.170	5.52	
11th	40	1.00712	Green	2.164	5.52	0.215
	50	1.00712	Green	2.165	5.52	
	60	1.00714	Green	2.165	5.52	
	70	1.00716	Green	2.170	5.52	
12th	40	1.00712	Green	2.168	5.51	0.119
	50	1.00712	Green	2.168	5.51	
	60	1.00714	Green	2.169	5.51	
	70	1.00716	Green	2.171	5.50	

Table 4: long term stability data for chlorohexidine mouth wash:

Month / Conditions	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
40 ºC ±2ºC, 45 % RH ± 5%	103.261	103.250	103.118	103.002	102.980	102.917	102.885	102.879	102.783	102.719	102.681	102.658
50 ºC ±2ºC, 65 % RH ±5%	103.253	103.244	103.112	102.980	102.920	102.910	102.810	102.790	102.785	102.710	102.675	102.630
60 ºC ±2ºC, 75 % RH ±5%	103.247	103.232	103.108	102.973	102.915	102.909	102.795	102.755	102.712	102.705	102.654	102.612
70 ºC ±2ºC, 75 % RH ± 5%	103.147	103.112	103.036	102.958	102.893	102.869	102.720	102.643	102.640	102.637	102.643	102.580

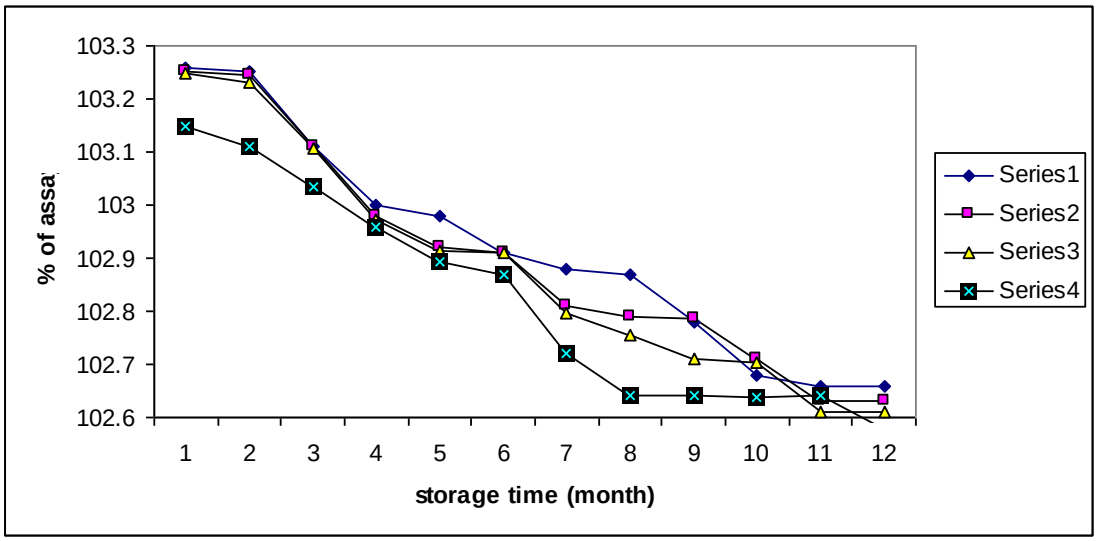
Where RH is the relative humidity.

Table - 5. Related substance (p-chloroaniline) concentration with storage.

Storage condition	Concentration of p-chloroaniline in µg/ml*											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
40 ºC±2ºC, 45 % RH ± 5%	1.64	1.64	1.65	1.65	1.65	1.66	1.67	1.68	1.70	1.77	1.83	1.88
50 ºC ±2ºC, 65 % RH ±5%	1.65	1.66	1.66	1.68	1.68	1.69	1.70	1.77	1.79	1.82	1.85	1.94
60 ºC ±2ºC, 75 % RH ±5%	1.66	1.68	1.69	1.72	1.77	1.78	1.79	1.80	1.84	1.88	1.89	1.94
70 ºC ±2ºC, 75 % RH ± 5%	1.66	1.69	1.73	1.78	1.84	1.88	1.93	1.96	1.97	2.10	2.18	2.26

* Note the USP limit no exceed 3 µg/ml

Figure-1. The changes of assay percentages chlorohexidine gluconate with time (month).



Where:
Series 1: represent assays at 40 ºC ±2ºC, 45 % RH ± 5%
Series 2: represent assays at 50 ºC ±2ºC, 65 % RH ±5%
Series 3: represent assays at 60 ºC ±2ºC, 75 % RH ±5%
Series 4: represent assays at 70 ºC ± 2ºC, 75 % RH ± 5%