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Development and Validation of Reverse Phase High Performance Liquid Chromatographic Method for Estimation of Chlorhexidine Gluconate In Mouthwash

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ABSTRACT

This research manuscript describes simple, sensitive, accurate, precise and repeatable reverse phase high performance liquid chromatography method for the estimation of Chlorhexidine gluconate in mouthwash. The sample was analyzed by reverse phase ACE 5 C18 column (150 mm × 4.6 mm i.d, 5 µm particle size) as stationary phase; acetonitrile : methanol : triethyl Amine (0.1 %) PH 3.0 (22: 49: 29, v/v/v) as a mobile phase at a flow rate of 0.8 ml/min. Quantification was achieved with Photo Diode Array detector at 258 nm. The retention time for chlorhexidine gluconate was found to be 2.477 min. The linearity was obtained in the concentration range of 10 -80 µg/ml for chlorhexidine gluconate. The method was successfully applied to mouthwash because no chromatographic interferences from formulation excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Chlorhexidine gluconate, RP-HPLC, Method validation.

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INTRODUCTION

Chlorhexidine gluconate is a Disinfectant and Topical antiseptic agent [1]. Chemically it is 1,1 – (hexane – 1,6 – diyl) bis (5-(4 – chlorophenyl)biguanide] di d – gluconate (figure1)[2]. Chlorhexidine gluconate is a official in Indian pharmacopeia (IP)[3], British pharmacopeia (BP)[4], United states Pharmacopeia (USP)[5] , Japanese Pharmacopeia(JP)[6],European Pharmacopeia [EP][7]. IP and BP describe UV, HPLC , titration methods for its estimation. USP describe GC and HPLC methods for its estimation. JP describes titration method for its estimation. EP describe HPLC method for its estimation. Literature survey reveals the HPLC [8-10] methods for estimation of chlorhexidine gluconate in whole blood by solid phase extraction and toxicological analysis in human serum. Literature survey also reveals HPLC [11-12] and UV[13] methods for estimation of chlorhexidine gluconate with other drug combination. In literature survey there is no any UV and HPLC methods for estimation of Chlorhexidine gluconate alone. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on first order derivative for estimation of chlorhexidine gluconate in mouthwash.

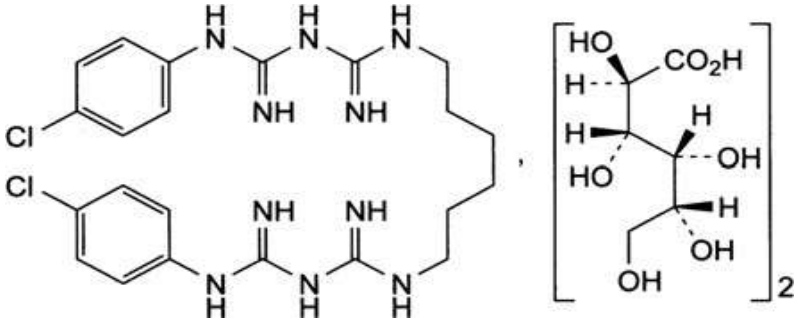

Figure 1 – Chemical Structure of Chlorhexidine gluconate

MATERIALS AND METHODS

Apparatus

The chromatography was performed on a Shimadzu (Japan) RP-HPLC instrument (LC-2010C_{HT}) equipped with Photo Diode Array (PDA) detector and LC-solution software, ACE C_{18} column (150 mm × 4.6 mm id, 5µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) were used in the study.

Reagents and materials

Chlorhexidine gluconate was kindly supplied as a gift samples from Shehat Pharmaceuticals Ltd. Himmatnagar, Gujarat, India. Chlorhexidine Gluconate mouthwash (0.2 %) was procured from

the local pharmacy. Acetonitrile, Methanol, triple distilled water (Finar Chemicals Ltd., Mumbai, India) used were of HPLC grade. Ortho-phosphoric acid (S.D Fine Chemicals Ltd., Mumbai, India) used were of AR grade. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Preparation of triethyl Amine (0.1%)

Triethyl Amine pH 3 was prepared by accurately adding 0.1ml of Triethyl Amine in 100 ml HPLC-grade water and the pH adjusted to 3.0 by diluted ortho-phosphoric acid.

Preparation of standard stock solutions

An accurately take 20 % chlorhexidine gluconate liquid (0.1 mL) and transferred to 100 mL separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution having concentration 200 µg /mL for drug.

Preparation of working standard solutions

An aliquot of stock solution 2.5 mL was transferred in 10 mL volumetric flask and adjusted up to mark with methanol having concentration (50 µg/mL)

Preparation of Sample Solution

The chlorhexidine mouthwash liquid was transferred to 100 mL volumetric flask containing 40ml of methanol. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution (2.5 mL) was taken in to a 10 mL volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of chlorhexidine gluconate (50 µg/mL).

Chromatographic Condition

Stationary phase: C_{18} column (150 mm x 4.6 mm id., 5 µm).

Mobile phase: Acetonitrile: Methanol: Triethyl Amine pH 3.0

(22: 49: 29, v/v/v)

Flow rate: 0.8 ml/min

Injection volume: 20 µL

Temperature: 40 °C

Detection: At 258 nm using PDA detector.

Method development

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Chlorhexidine gluconate was obtained with a mobile phase Acetonitrile: Methanol: Triethyl Amine [22:49:29, v/v/v] at a flow rate of 0.8

mL/min to get better reproducibility and repeatability. Quantification was carried out at 258 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure. 2). System suitability test parameters for Chlorhexidine gluconate for the proposed method are reported in Table 1

Method Validation

The method was validated in compliance with ICH guidelines[6]

Calibration Curve (Linearity)

Calibration curves was constructed by plotting peak areas Vs concentrations of Chlorhexidine gluconate and the regression equations were calculated. The calibration curves were plotted over the concentration range 10-80 µg/mL of Chlorhexidine gluconate. Accurately measured mix Standard working solutions of Chlorhexidine gluconate (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µl) of each solution were injected under the Chromatographic conditions described above.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Chlorhexidine gluconate by the standard addition method. Known amounts of standard solution of Chlorhexidine gluconate was added at 50 %, 100 % and 150 % levels to pre quantified sample solutions of Chlorhexidine gluconate.

Method precision

Precision of the method was determined by performing interday variation and intraday variation (% RSD). Intra- day precision (% RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (% RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of sample solutions of chlorhexidine gluconate (40, 50, and 60 µg /mL) . The results were reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH)[14] guidelines.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where σ = the standard deviation of the response S = Slope of calibration curve.

ANALYSIS OF CHLORHEXIDINE GLUCONATE IN MOUTHWASH

The response of the sample solution was measured at 258 nm under the chromatographic condition mentioned above for the quantification of Chlorhexidine gluconate. The amounts of Chlorhexidine gluconate present in sample solution were determined by applying values of the peak area to the regression equations of the calibration curve.

RESULTS AND DISCUSSION

Linearity

Linear correlation was obtained between peak area Vs concentrations of Chlorhexidine Gluconate in the concentration range of 10-80 µg/ml. Regression parameters are mentioned in Table and the calibration curves of this drug at 258 nm are shown in Figure 4 .

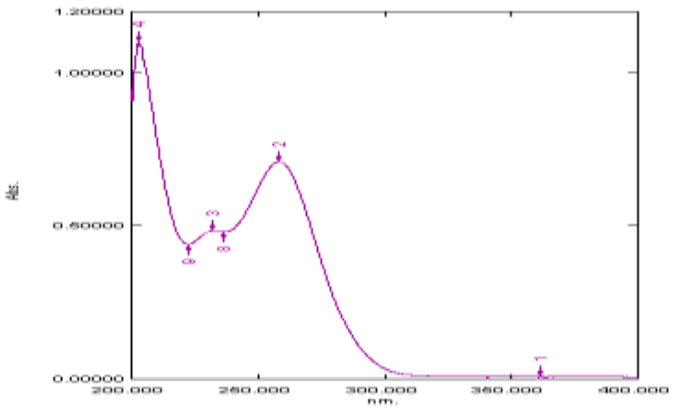

Figure: 3 UV spectrum of chlorhexidine gluconate at 258 nm

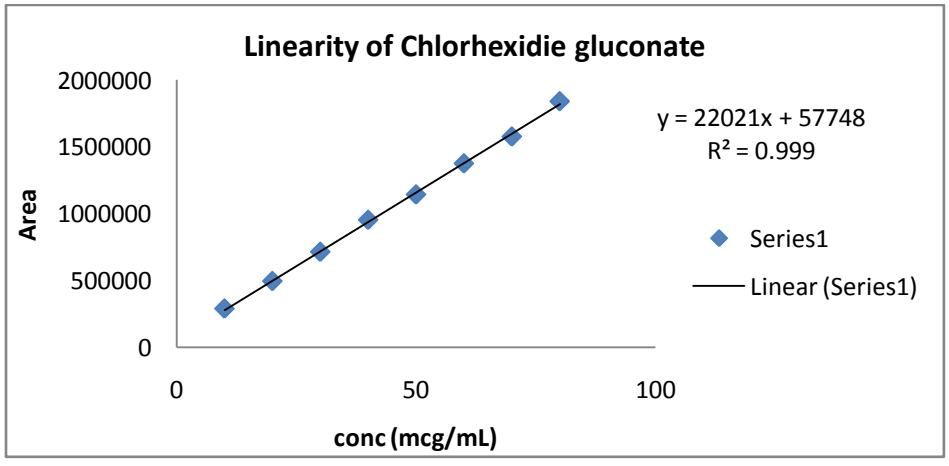

Figure: 4 Calibration curve of Chlorhexidine gluconate at 258 nm

Table 1: System Suitability Parameters of Chromatogram for chlorhexidine gluconate

Parameters	Chlorhexidine gluconate ± RSD (n = 6)
Retention time (min)	2.477 ± 0.1543
Tailing factor	1.439 ± 0.1728
Theoretical plates	3945 ± 0.5321

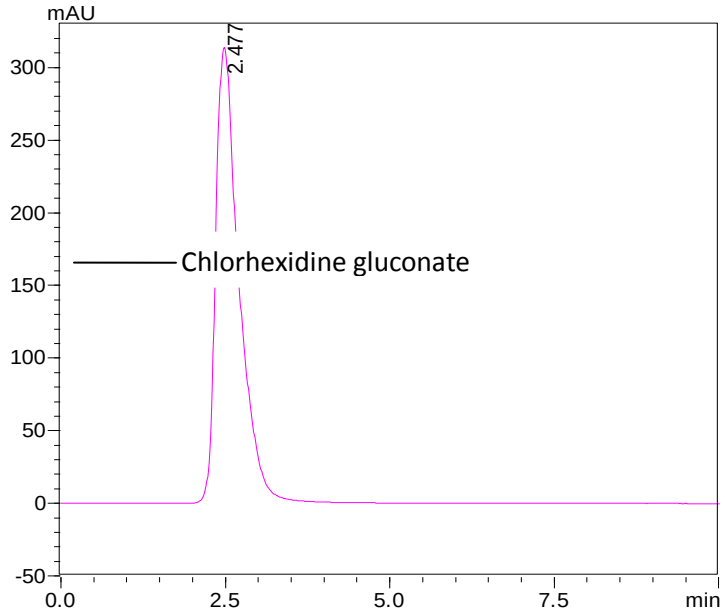

Figure.: 2 Chromatogram of Standard Solution of Chlorhexidine gluconate(50 µg/ml) at 258 nm

Method precision

The RSD value for chlorhexidine gluconate was found to be 1.34 % respectively. the RSD value was found to be < 2% , which indicates that the proposed method is repeatable.

Intermediate precision (Reproducibility)

The low RSD value of interday and intraday for chlorhexidine gluconate, respectively, reveal that the proposed method is precise.

LOD AND LOQ

LOD value for chlorhexidine gluconate was found to be 0.044 µg/ml and LOQ value for chlorhexidine gluconate was found to be 0.136 µg/ml (Table 4). These data show that the proposed method is sensitive for the determination of Chlorhexidine gluconate.

Accuracy

The recovery experiment was performed by the standard addition method. The recoverie obtained was 100.10 ± 0.480 % for Chlorhexidine gluconate (Table 2). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2.

Table 2: Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean% Recovery ± SD
Chlorhexidine	I	30	50 %	101.03 ± 0.573
Gluconate	II	30	100 %	99.55 ± 0.330
	III	30	150 %	99.74 ± 0.537
Mean% Recovery ± SD				100.10 ± 0.480

Analysis of chlorhexidine gluconate in mouthwash

The proposed validated method was successfully applied to determine chlorhexidine gluconate in mouthwash. The result obtained for Chlorhexidine gluconate was comparable with the corresponding labelled amounts (figure 5) (Table 3)

Table 3: Analysis of chlorhexidine gluconate mouthwash (0.2 %) by proposed method (n = 6)

Sample no.	Label claim (%)	Amount found (%)	% Label claim
1	0.2	0.199	99.5
2	0.2	0.201	100.5
3	0.2	0.198	99
4	0.2	0.200	100
5	0.2	0.201	100.5
6	0.2	0.200	100
Mean		0.199	99.91
SD		0.00116	0.5845
%RSD		0.5829	0.5850

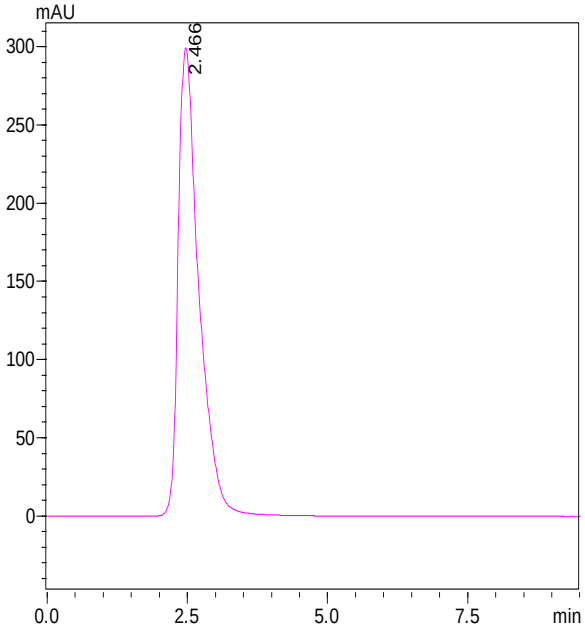

Figure: 5 Chromatogram of sample solution of Chlorhexidine gluconate(50 µg/mL) at 258 nm

Table 4: Regression Analysis Data and Summary of Validation Parameter for the proposed Method

Parameters		RP HPLC Method Chlorhexidine Gluconate
Detection wavelength(nm)		258 nm
Concentration range (µg/mL)		10-80
Regression equation Y= mx + c		Y = 22021x + 57747
Correlation coefficient		0.9992
Repeatability (% RSD[a], n = 6)		1.3407
Precision (%RSD)[a]	Intraday (n=3)	0.111 – 0.160
	Interday (n=3)	0.156 – 0.271
LOD[b] (µg/mL)		0.044
LOQ[c] (µg/mL)		0.136
% Recovery (Accuracy, n= 3)		100.10 ± 0.480
% Assay ± SD[d] (n = 6)		99.91 ± 0.58

RSD[a] = Relative standard deviation, LOD[b] = Limit of detection, LOQ[c] = Limit of quantification, S. D[d] = Standard deviation

CONCLUSION

A simple, sensitive, repeatable and specific RP-HPLC method has been developed for the estimation of chlorhexidine gluconate using a PDA detector. The method was validated for accuracy, precision, linearity, specificity, LOD & LOQ and robustness. In this proposed method the linearity is observed in the concentration range of 10-80 µg/ml for chlorhexidine gluconate with co-efficient of correlation, (R^2) = 0.9992. The result of the analysis of chlorhexidine gluconate mouthwash by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the chlorhexidine gluconate in pharmaceutical dosage form without any interference of excipients.

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