ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Development and Validation of a new UFLC method for the estimation of Chlorhexidine in bulk drug

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ABSTRACT

A simple, sensitive and specific UFLC method was developed to estimate Chlorhexidine in bulk drug. Acetonitrile and Water were used in 60:40 v/v ratio as mobile phase. The flow rate of eluent was fixed at 0.8 mL/min. Absorbance was monitored at λmax of 235 nm. A reverse phase column C18, (250mm x 4.6mm i.d., 5µm) was used as stationary phase. The retention time was found to be 2.99 minutes. The linearity range of Chlorhexidine was found to be 1-6 µg/ml at 235nm wavelength.

KEY WORDS: UFLC, Chlorhexidine, Retention time, Linearity.

1. INTRODUCTION

Chlorhexidine is a biguanide antiseptic. Its chemical name is N, N^1-1, 6-Hexanediylbis [N^1-(4-chlorophenyl) imidodicarbonimidic diamide] and its molecular formula is $C_{22}H_{30}Cl_2N_{10}$ (Figure.1.) (Jeffery, 1989). It has a broad spectrum of activity against different microorganisms. Hence it is widely used in dentistry, human and veterinary medicine (Fiorentino, 2010). Antimicrobial effects of Chlorhexidine are associated with the attraction between the drugs and bacterial cells bearing negative charge, thus disrupting the cell membrane integrity.

Literature survey reveals that several reports have been published on the spectroscopic (UV) (Gurdeep, 1991; Paresh, 2014; Rushikesh, 2016; Tarig, 2017) or chromatographic (HPLC) (Bagdanovska, 2014; Liljana, 2014; Zhesu, 2013; Dave, 2012; Zhang, 2012; Soyseven, 2012; Marco, 2011; Beckett, 2002; Snyder, 1997; Skoog, 1980) estimation of chlorhexidine. However, majority of the reports on HPLC revealed the usage of mobile phase containing buffers and longer retention times. Hence, it is felt worthwhile to develop and validate a new, simple, faster UFLC method to estimate chlorhexidine.

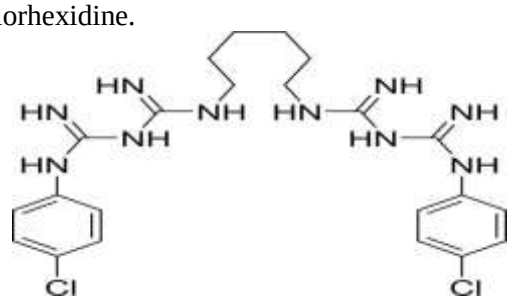

Figure.1. Chemical structure of Chlorohexidine

2. MATERIALS AND METHODS

Instrumentation: UFLC SPD-20A (SHIMADZU), UV-VIS spectrophotometer, UV-1800 (SHIMADZU), Analytical balance AY220 (SHIMADZU), pH meter MK V1 (DIGITAL), Ultra SonicatorPCi (BIOTECHNICS).

Chemicals and reagents: Analytically pure Chlorohexidine was gifted by MSN laboratories. HPLC grade Methanol (HIMEDIA), Acetonitrile (SIGMA ALDRICH), Triethylamine, Ortho phosphoric acid (FINAR) were purchased. Millipore water of HPLC grade was used.

Chromatographic conditions: Glassware used were thoroughly washed using chromic acid cleansing mixture, rinsed with water and dried. Acetonitrile and Water were used in ratio of 60:40 v/v as mobile phase. 0.8 mL/min was fixed as flow rate to deliver the eluent, the run time was 10 minutes and the injection volume was 20µL. Absorbance was monitored at λmax of 235 nm.

Preparation of mobile phase: A mixture of about 400 mL water and 600 mL Acetonitrile (HPLC grade) were mixed and degassed in an ultrasonicator for 5 min. 0.45 µ filter was used to filter the final solution under vacuum. The mobile phase thus prepared was also used as diluent.

Standard Solution Preparation: Standard stock solution of Chlorhexidine was obtained by dissolving 10mg of Chlorhexidine bulk drug in 10ml of methanol to give 1mg/ml of solution (Stock solution). Further dilutions were prepared from the standard stock solution to obtain 1, 2, 3, 4, 5, 6 µg/ml of the solutions.

3. RESULTS AND DISCUSSION

Determination of absorption maxima (λ_{max}): 10µg/ml standard solution of Chlorhexidine was prepared using methanol and scanned in UV Spectrophotometer from 200-400 nm.

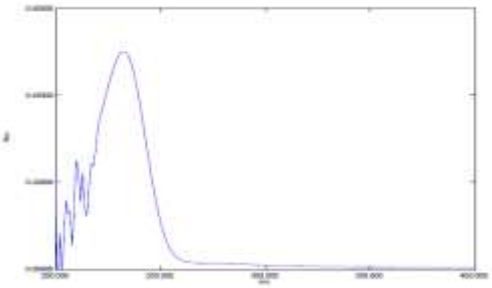

Figure.2. Overlay spectra of Chlorhexidine in methanol

System suitability: Standard solutions of 1-6µg/ml of Chlorhexidine was prepared from the 1mg/ml standard stock solution and injected six times into the HPLC system. The system suitability parameters were studied from standard chromatograms. The results are presented in Table.1.

Table.1. Results for System suitability of Chlorhexidine

S.No	Retention time	Theoretical plate number	Tailing factor
1	2.987	2954	1.14
2	2.930	2945	1.16
3	2.999	2967	1.76
4	2.994	2985	1.56
5	2.923	2967	1.78
6	2.978	2984	1.43
Mean	2.968409	2967	1.47

From this study, it was recognised that all the system suitability parameters were within the limits. Hence it has been concluded that instrumentation and methodology were suitable.

Method development and Optimization: After numerous trails with various solvents, the mobile phase containing HPLC grade acetonitrile and water in the proportion of 60:40 v/v respectively was selected to estimate and validate Chorhexidine in bulk form by UFLC. Maximum resolution for Chlorhexidine was obtained with this mobile phase at the detection wavelength of 235nm. Mobile phase at a flow rate of 0.8 ml/min produced optimum separation with good resolution. A reverse phase Column C18, (250mmL. x 4.6mm i.d., 5µm) was used as stationary phase. The retention time of Chlorhexidine was found to be 2.99 minutes (Figure.3.)

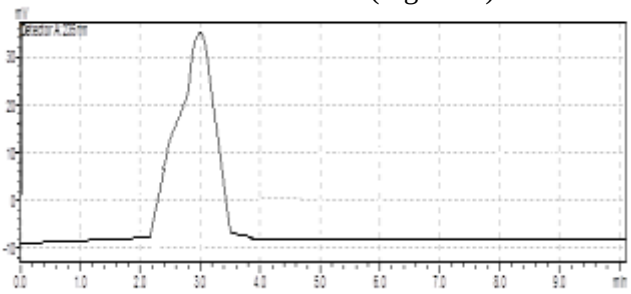

Figure.3. Chromatogram of Optimized method

Method Validation parameters: ICH guidelines were taken as criteria for validating the linearity, accuracy, precision, specificity, ruggedness and robustness, LOD and LOQ values for the developed method.

Linearity: The detector linearity response for Chlorhexidine was demonstrated by preparing solutions of chlorhexidine standard in a range of 1-6µg/ml concentration. These solutions were spiked and the area of the response of the same was recorded. A graph of concentration versus peak area (Figure.4.) was plotted and correlation coefficient between concentration and area was evaluated (Table.2.). The calibration curve was found to be linear in 1-6µg/ml concentration range.

Table.2. Linearity data of Chlorhexidine

S.NO.	Linearity (µg/ml)	Peak area
1	1	217013
2	2	368231
3	3	574542
4	4	763853
5	5	939362
6	6	1104321

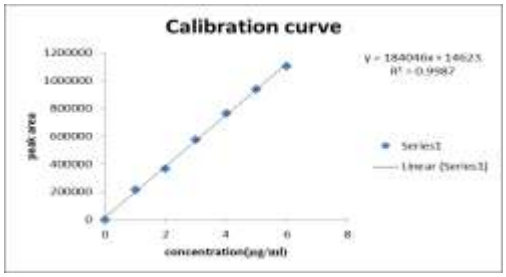

Figure.4. Calibration curve of Chlorohexidine

Accuracy: The accuracy of the method was studied by determining the recovery of Chlorhexidine at three levels of concentrations. It was performed in three different levels for at 50%, 100%, 150% (Figures.5, 6 and 7). Samples were analyzed at each level in triplicate. From the results, % recovery was calculated (Table.3).

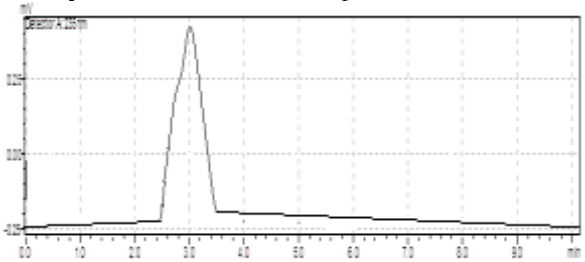

Figure.5. Chromatogram of Accuracy (level-50%)

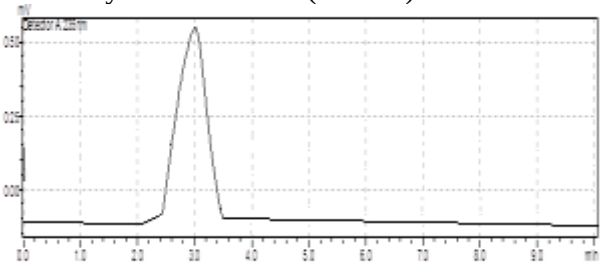

Figure.6. Chromatogram of Accuracy (level-100%)

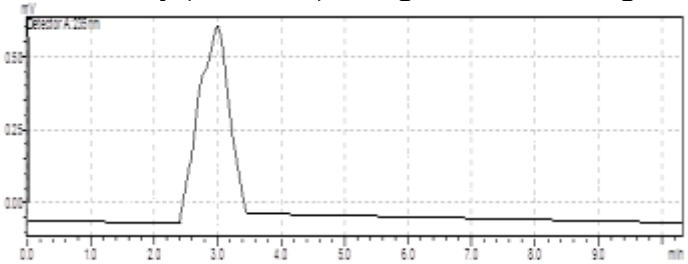

Figure.7. Chromatogram of Accuracy (level-150%)

Table.3. Accuracy data of Chlorhexidine

Concentration (%)	Area	Amount taken	Amount found	%Recovery	Mean Recovery
50%	969865	2.5	2.45	98%	
100%	1176543	5	4.95	99%	
150%	1453276	7.5	7.4	98.6%	98.5%

From the mentioned results, it is evident that the recovery was within the acceptable limits (98- 102%). Hence the developed method was found to be accurate.

Precision: Precision of the methods was studied as intra-day, inter-day. Intra-day precision studies were performed by analyzing three different concentrations of drug at three different times in a day. Inter-day precision was performed by spiking three different concentrations of the drug on three different days within a week (Table.4).

Table.4. Precision data of Chlorhexidine

Injection	Intra-day	Inter-day
Injection-1	574542	763853
Injection-2	575432	789557
Injection-3	578932	785432
Average	576302	779614
SD	2320.7	13804.3
%RSD	0.4	1.7

From the observed results, it was concluded that the %RSD was within the acceptable limits (<2%), thus the system was suitable for present work.

LOD and LOQ: The Limit of detection (LOD) and Limit of quantification (LOQ) for the developed method were determined based on the response and slope of the regression equation. A linear correlation between the peak area and applied concentration was found in the concentration range of 1-6 µg/ml under the described experimental conditions. The peak area (*y*) is proportional to the concentration of Chlorhexidine following the regression equation y=184046x+14623The experimentally derived LOD and LOQ values were found to be 0.2µg/ml and 0.7µg/ml, respectively.

Robustness: The robustness of the proposed method was analyzed by collecting aliquots from homogeneous lots by differing parameters like flow rate, mobile phase composition, wavelength (Table.5).

Table.5. Results of Robustness

Robust conditions	Retention time (min)	Remarks
Flow rate-0.8ml/min	2.99	At higher flow rate the retention time was decreased.
Flow rate-0.5ml/min	4.01	At lower flow rate the retention time was increased.

Ruggedness: Ruggedness is a measure of reproducibility of standard results under the changes in conditions normally expected from laboratory to laboratory and from analyst to analyst (Table.6).

Table.6. Results of Ruggedness of Different Analyst

S.NO.	Chlorhexidine	%Recovery
1	789556	99.7
2	785432	98.3
Average	787494	99

4. CONCLUSION

From the above results, it can be concluded that the developed UFLC method represented an excellent technique for determination of Chlorhexidine with good sensitivity, precision and reproducibility. It is simple, faster, specific, sensitive, and economic and can be used for estimation of Chlorhexidine in bulk drug for routine analysis.

5. ACKNOWLEDGEMENTS

The authors are highly thankful to the Principal and Management of Vaagdevi College of Pharmacy (affiliated to Kakatiya University), Warangal, Telangana, India, for providing all the necessary facilities to carry out this work.

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