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Research Article

Development and Validation of HPLC Method for Chlorohexidine

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ABSTRACT

In HPLC method, Methanol and Water (75:25 % v/v) was used as mobile phase, at a flow rate of 1.0 ml/min, on HPLC system containing UV- detector with Openlab EZchrome software and Kro-masil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm. The method gave suitable retention time i.e. 3.60 min for chlorohexidine. The results of analysis in the method were validated in terms of Filter study, Solution stability, specificity, Linearity, accuracy, precision (Repeatability and intermediate precision), limit of detection, limit of quantification and robust-ness. A simple and precise method was developed for the assay of chlorohexidine in bulk drug and in tablet formulation. The method needs regular reagents for doing analysis and also less time consuming, it can be per-formed routinely in industry for routine analysis of bulk drug and marketed product of chlorohexidine.

INTRODUCTION

Chlorhexidine is a widely used antiseptic agent, known for its efficacy against a broad spectrum of microorganisms, making it a common choice in medical and dental applications. Due to its significance in both clinical and pharmaceutical settings, the accurate quantification of chlorhexidine is crucial for ensuring safety and efficacy in formulations.

In pharmaceutical industries, the validation of analytical method is used to demonstrate that the method is fitted for its purpose; it must follow a plan which includes scopes, Performance characteristics, and acceptance limits. Analytical methods need to be validated or revalidated prior to their introduction into routine analyses. Chromatography is an analytical technique based on the separation of molecules due to differences in their structure and/or composition. High-Performance liquid chromatography (HPLC) is types of liquid Chromatography used to separate and quantify compounds that have been dissolved in Solution. HPLC can be used to determine the amount of a specific compound in a solution.

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Chromatography

The analytical technique of high performance liquid chromatography is used extensively throughout the pharmaceutical industry. it is used to provide information on the composition of drug related samples. The information obtained may be qualitative, indicating what compound present in the sample or quantitative are providing the actual amounts of compound in the sample. HPLC is used routinely during drug manufacture. The aim of the analysis will depend on both the nature of the sample and stage of development HPLC is a chromatographic technique:

In HPLC a column is used. The Name given to liquid chromatography on a planner surface is thin layer chromatography (TLC).

I) Stationary phase

It is the combination of a suitable stationary phase and mobile phase that enables the separation of a mixture and thus the analysis of the components in the mixture

a) Normal phase

In mixture of components to be separated those analysts which are relatively more polar will be retained by the polar stationary phase longer than those analysts which are relatively less polar.

b) Reversed phase

In mixture of component to be separated those analysts which are less polar will be retained by the nonpolar stationary phase longer than those analytes which are relatively more polar.

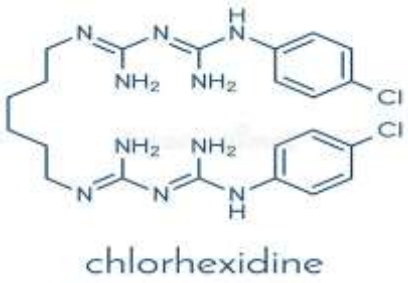


Fig. 1: Structure of Chlorohexidine

a) Mobile phase characteristics

Following points are considered for the selection of a mobile phase.

- Viscosity.
- Compressibility.
- Refractive index.
- UV cutoff
- Polarity.
- Vapour pressure

The viscosity generally increases with the number of carbons in the solvent. Straight chain alcohol shows a very proposed relationship of this nature. For example, to achieve 1ml/min flow rate in a 4.6 x 250 mm column packed with 5 micro meter Octadecyl silane material, a pressure of 1500 psi is required with methanol. Solvent of low viscosity are needed to be compatible with the limitations of the pump. Also, as viscosity increases, the efficiency of the system, as measured by the number of theoretical plates decreases. The sensitivity of the detection is related to the difference between the refractive index, i.e. the greater the difference, greater is the sensitivity. The UV cut-off is defined as the wavelength below which the solvent will absorb more than1.0 absorbance unit. The polarity of the solvent is a measure of the dielectric constant or the ability to elute particular types of compounds. The vapour pressure of a solvent plays an important role in mobile phase selection. solvent reservoir could easily change in composition due to the

evaporation of one of the more volatile constituents. The flammability of the mobile phase is a safety consideration. Careful attention should be paid to adequate ventilation and waste solvent disposition.

MATERIAL AND METHODS:

Drug: Chlorohexidine

Chemical Reagent: Methanol, Acetonitrile, water

Analytical Method

Preparation of standard stock solution for Chromatographic development:

In order to prepare stock solution, weighed accurately 20 mg Chlorhexidine Gluconate and transferred into 20 ml volumetric flask, added 15 ml of Water and sonicated to dis-solve the standard completely and diluted up to the mark with Water (1000 PPM).

Further diluted 2 ml of stock solution to 20 mL with mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.

Selection of analytical wavelength for HPLC method development: Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 210 nm.

Optimization of HPLC method

We will develop a method until we get good chromatography. An acceptance criterion for good chromatography is as follows:

- Retention time: Optimum R.T.
- Asymmetry (Tailing factor): 0.8 to 2.0
- Theoretical plates: NLT 2000

Following trials are taken for estimation of Chlorhexidine Gluconate.

Principle: Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.

Preparation of standard stock solutions

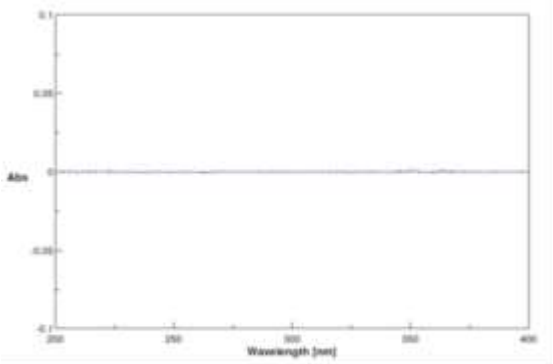
In order to prepare stock solution, weighed accurately 10 mg Chlorhexidine Gluconate and transferred into 20 ml volumetric flask, added 15 ml of Water and sonicated to dis-solve the standard completely and diluted up to the mark with Water (500 PPM).

Selection of solvent

Water was selected as the solvent for dis-solving Chlorhexidine Gluconate.

Selection of analytical wavelength

Water as a blank and Chlorhexidine Glu-conate standard solution (10 PPM) was scanned from 400 nm to 200 nm. Absorp-tion maxima was determined for drug. Chlorhexidine Gluconate showed maximum absorbance at 210 nm shown in results.



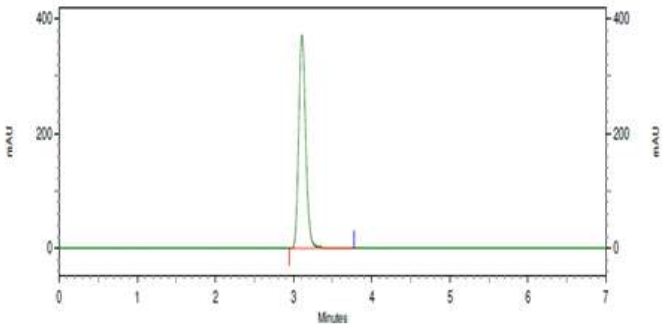
Blank spectra (water)

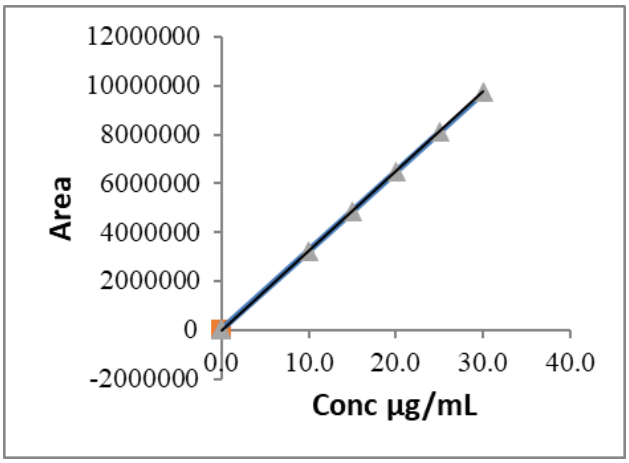
Chromatographic Conditions:

- Standard solution: Chlorhexidine gluconate 100 PPM

- Detector: U.V. Detector
- Column: Kromasil C18
- Column Dimension: (250 mm X 4.6 mm i.d.) 5µm
- Column Oven temperature: 40°C
- Injection Volume: 20µl
- Wavelength: 210 nm
- Mobile phase: Acetonitrile :
- Water (70:30% v/v)
- Flow Rate: 1.0 ml/min

Chromatogram





Linearity Graph of Chlorohexidine

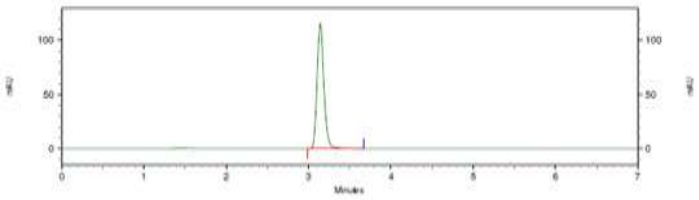
Analytical Method Validation:

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples.

Accuracy :

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Recovery studies were performed to validate the accuracy of developed method.

Sample Name: ACCURACY 150%_1



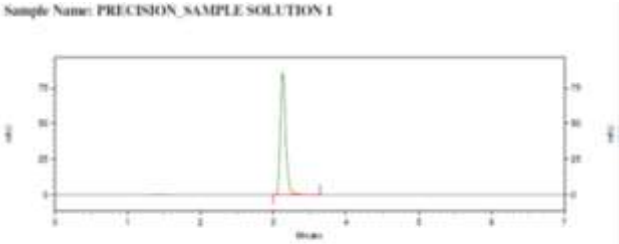
VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Chlorhexidine Gluconate	3.15	9701060	1.28	7314
Totals		9701060		

Precision:

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample.

Sample Name: PRECISION_SAMPLE SOLUTION 1

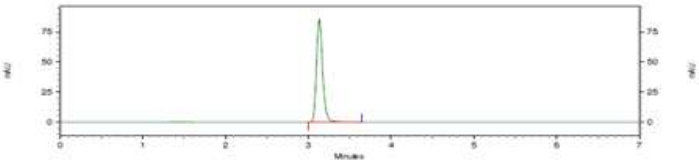


VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Chlorhexidine Gluconate	3.14	6350801	1.32	8018
Totals		6350801		

Fig. Typical chromatogram of Repeatability precision

Sample Name: INTER MEDIATE PRECISION_SAMPLE SOLUTION 1



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Chlorhexidine Gluconate	3.12	6323580	1.31	7930
Totals		6323580		

Fig. Typical chromatogram of Inter-day precision

Robustness:

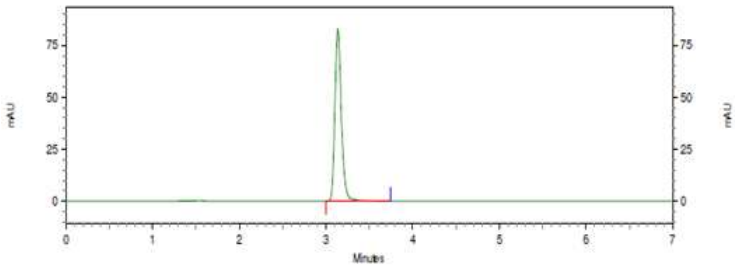
The robustness of an analytical method is a measure of its capacity to remain unaffected by

small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven tem-perature

Change in Wavelength by +3 NM:

Sample Name: ROBUSTNESS_STD SOLUTION +3 NM

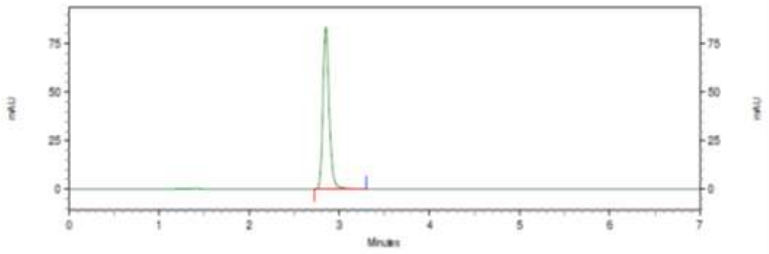


VWD: Signal A, 213 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Chlorhexidine Gluconate	3.15	6280253	1.34	8134
Totals		6280253		

Change in Flow rate by + 10% (1.1 mL/min)

Sample Name: ROBUSTNESS_STD SOLUTION +10% FLOW RATE

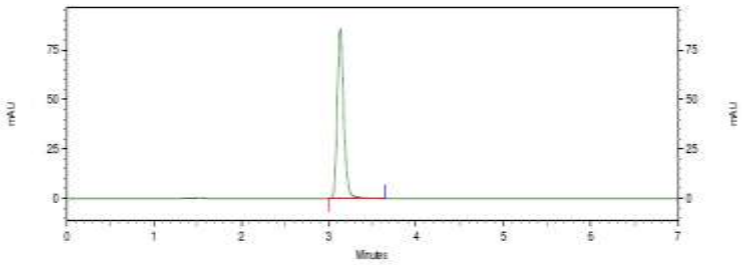


VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Chlorhexidine Gluconate	2.84	5760325	1.33	7489
Totals		5760325		

Change in Column Oven temperature by +2°C:

Sample Name: ROBUSTNESS_STD SOLUTION +2 COT



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Chlorhexidine Gluconate	3.14	6432036	1.30	7824
Totals		6432036		

Limit of detection & limit of quantification:

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 12320.40666 (Residual standard deviation of a regression line)
s = 326471.220 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S
LOD = 3.3 x 12320.40666 / 326471.220
LOD = 0.125 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S
LOQ = 10 x 12320.40666 / 326471.220
LOQ = 0.377 µg/mL

CONCLUSION:

The present work development of simple, accurate, precise and suitable HPLC meth-od. Literature survey revealed that several methods have been reported for determination of chlorohexidine in bulk drug or in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive and suitable reversed-phase high performance liquid chromatography method was developed and validated for the determination of chloro-hexidine in bulk drug and pharmaceutical dosage form. In developed HPLC method, the analyte was resolved by using isocratic program and mobile phase was used Meth-anol : Water (75:25 % v/v) at a flow rate of 1.0 ml/min, on HPLC system containing UV- visible detector with Openlab EZ-Chrome Software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm.

The results of analysis in the developed method were validated in terms of linearity, accuracy, precision, and robustness, limit of detection and limit of quantification. The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each ana-lyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2%

showing high degree of precision of the proposed method.

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