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A RAPID DETERMINATION OF CHLORHEXIDINE DIGLUCONATE CONTENT IN ANTIMICROBIAL PREPARATION BY FIRST DERIVATIVE SPECTROPHOTOMETRY

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ABSTRACT A simple, direct and rapid spectrophotometric method has been developed to determine the chlorhexidine digluconate content in the antimicrobial formulation. The application of first derivative spectrophotometry has managed to eliminate the interferences from other ingredients used in the complex matrices. Measurement is made at λ = 276.1 nm in which Beer's law is adhered in the range of 0 – 50 μg/ml.

ABSTRAK Teknik analisis yang ringkas, cepat and secara langsung telah dipraktik untuk menentukan kandungan chlorhexidine digluconate dalam formulasi antimicrobial. Penggunaan spectroskopi derivatif pertama telah mampu menyingkirkan gangguan daripada ramuan lain yang digunakan dalam penyediaan formulasi antimicrobial. Analisis telah dibuat pada λ = 276.1 nm dimana hukum Beer's telah dipatuhi dalam julat 0 – 50 μg/ml.

(**Keywords** : First derivative spectrophotometry, chlorhexidine digluconate)

INTRODUCTION

Antimicrobial actives are used in pharmaceuticals, cosmetics, biological samples, food, wood and plastics products to kill or to inhibit the growth of micro-organisms. They are also used in sterile preparations such as eye drops and multi-dose injections to maintain sterility during use.

Other chemical compounds are commonly used with antimicrobial actives in commercial pharmaceutical products to prevent alteration and degradation of the product formulations. Therefore, analytical methodologies developed for the quantification of the active constituent in these matrices are usually designed to overcome the problems associated with interferences which originate from the other components.

Chlorhexidine is used either as the diacetate, digluconate or dihydrochloride salt. As an antiseptic and disinfectant agent, it is effective against a wide range of bacteria, some fungi and some viruses, and as an agent for the prevention of gingivitis.

Automated HPLC [1-4], flow-injection analysis [5-6] and polarographic methods [7-9] had been used in the determination of chlorhexidine and all are expensive and time-consuming.

The content of chlorhexidine gluconate in several pharmaceutical formulations had also been determined using UV-Vis spectrophtometry [10-12], the use of colorimetric methods in the analysis of chlorhexidine gluconate in pharmaceutical preparations was demonstrated by ion-pair-formation with acidic dyes [8,13]. But UV-Vis spectrophotometry is not particularly specific for chlorhexidine and is liable to be in error owing to other highly absorbing ingredients in the sample matrices [7,14].

Derivative spectrophotometry provides greater selectivity than common spectrophotometry and offers a powerful approach for resolution of band overlapping in quantitative analysis of multi-component mixtures.

Derivative spectra can be produced by processing the spectrophotometer output. The use of derivative spectra can increase the detection sensitivity of minor spectra features and reduce the error caused by the overlap of spectra bands of other species in the sample. It is possible to measure the absolute value of the derivative of the sum curve at an abscissa value (wavelength) corresponding to a zero-crossing of other components in the mixture. The zero-crossing derivative spectroscopic mode enhances the resolution of the single analyte by recording its derivative spectra at wavelength at which other components in the matrix exhibit no signals [15-16].

This paper reports a method for the rapid determination of chlorhexidine digluconate in an antimicrobial formulation in the presence of other ingredients such as, polymer dispersions, waxes and other additives. A first derivative spectrophotometric method at λ = 276.1 nm without involving any additional separation step is proposed. To further challenge the capability of the derivative spectroscopy, a series of dipped polymeric films was prepared by coating them with the antimicrobial formulation with and without chlorhexidine digluconate respectively.

MATERIALS AND METHODS

Instrumentation

All absorption spectra and derivatives were recorded with a Cary 50 UV-VIS spectrophotometer, equipped with a 1 cm path length quartz cell and interfaced to a compatible computer running the Cary WinUV software version 3.0.

Reagents

All the chemicals used were of analytical-reagent grade and all the solutions were prepared in a reagent alcohol and water (RA/W) solution. Type 3A reagent alcohol (RA) was prepared by mixing 90% ethanol 96%, 5% isopropanol and 5% methanol. RA/W solution was then prepared by diluting Type 3A reagent alcohol (RA) with distilled water in 1:1.

The standard chlorhexidine digluconate solution 200 μg/ml was prepared from a chlorhexidine digluconate 20% solution and was further diluted with RA/W to prepare working standards of appropriate concentrations (2.5 – 50 μg/ml). All solutions were kept and protected from the light.

Procedure for the Pharmaceutical Formulations

The antimicrobial formulations which contained 0%, 1%, 2% and 4% chlorhexidine digluconate were analyzed directly and diluted with RA/W so that their concentrations fall in the Beer's range of 0 – 50 μg/ml of chlorhexidine digluconate.

Procedure for Dipped Polymeric Films

A series of dipped polymeric films were prepared by coating with the antimicrobial formulation which contained 0%, 1%, 2% and 4% chlorhexidine digluconate respectively. The prepared dipped polymeric films were extracted in 150 ml RA/W at room temperature for 1 hour on an orbital shaker at 200 rpm. The extracts were filtered with a Whatman 0.45 μm syringe filter and diluted with RA/W so that their concentrations fall in the Beer's range of 0 – 50 μg/ml of chlorhexidine digluconate.

Sensitivity

Sensitivity was determined for the Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD is defined as 3.3 s/k and LOQ is defined as 10 s/k, where s is the standard deviation of replicate determinations of *y*-intercept values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, the slope of the calibration curve.

Accuracy and Precision

To establish the reliability of the proposed method, the pharmaceutical formulations and the prepared dipped polymeric films were analyzed as discussed above in triplicate. The precision was determined for the repeatability within one day by calculating their respective relative standard deviation, RSD. The accuracy

of the method was measured for their respective recovery coefficients.

Spectrophotometric Measurements

Zero-order spectra of standard solutions of chlorhexidine digluconate (0 – 50 μg/ml) *versus* their solvent blank (RA/W) were recorded in the range of 200 – 400 nm. The first-order derivative spectra of standard solutions were obtained in the same wavelength range against their solvent blanks.

The absorbance values for chlorhexidine digluconate were measured at λ = 276.1 nm (zero-crossing of the other ingredients in the antimicrobial formulation and dipped polymeric films' extracts). The calibration curve for the derivative spectrophotometry was constructed by plotting the concentrations of chlorhexidine digluconate *versus* the absorbance values of the first derivative spectrum at λ = 276.1 nm.

RESULTS AND DISCUSSION

Derivative Spectrophotometric Method

The zero-order absorption spectra of the investigated matrix are shown in **Figure 1**. The peak maxima for standard chlorhexidine digluconate at zero-order spectrum was observed at λ = 259.9 nm but since the spectra overlap quite clear, determination of chlorhexidine digluconate could not be performed. In addition, the zero-order absorption spectra were not specific to the antimicrobial formulation with 0% chlorhexidine digluconate and dipped polymeric film's (with 0% chlorhexidine digluconate) extract in which absorbance values were recorded while both samples contained no chlorhexidine digluconate.

Derivative spectrophotometry based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem.

In this investigation the spectrophotometic parameters were optimized through the derivative spectra of chlorhexidine digluconate at different orders. The first-order derivative spectra are shown in **Figure 2**. The zero-crossing points were recorded at λ = 276.1 nm with evidently useful characteristics from the analytical view point.

The selection of this optimum wavelength by applying the quantitative zero-crossing method [16] was based on the fact that the absolute value of the total derivative spectrum at these wavelengths had the best linear response to chlorhexidine digluconate with an intercept very close to zero and least interferences from the othercomponents in the antimicrobial formulation and dipped polymeric films' extract.

Calibration Curves and Statistical Analysis

Under the optimized conditions, the absorbances of the standard solutions of chlorhexidine digluconate were measured at λ = 276.1 nm. The calibration curve was constructed by plotting the absorbance values against chlorhexidine digluconate over the concentration range of 0 – 50 μg/ml (**Figure 3**.). The statistical data are summarized in **Table 1**. The linearity of the calibration curve and conformity of the proposed method to Beer's law are validated by the high values of correlation coefficient (R^2 = 0.9999) of the regression equation and value of the intercept on ordinate which was zero.

Sensitivity

The Limit of Detection (LOD) was calculated as 0 μg/ml and Limit of Quantitation (LOQ) was calculated as 0 μg/ml for first-derivative spectrophotometric method. (Table 1).

Accuracy and Precision

The results on accuracy and precision were tabulated in Table 2. The mean recovery and relative standard deviation were found to be 94 – 100% and not more than 2% for the first-derivative UV spectrophotometric method, indicating very good accuracy and precision.

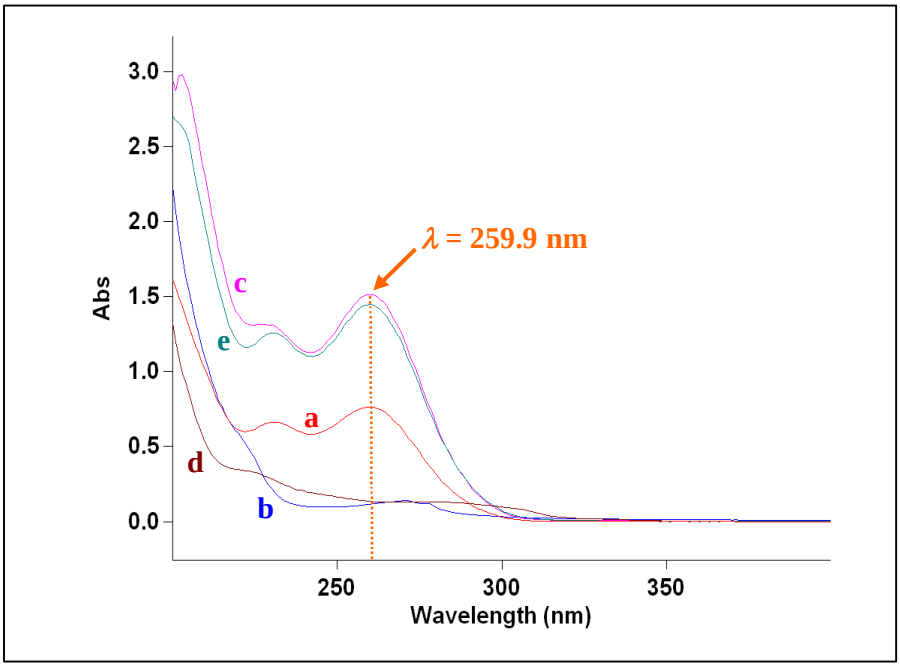

Figure 1. Zero-order spectra of (a) standard chlorhexidine digluconate solution, antimicrobial formulation (b) without chlorhexidine digluconate and (c) with chlorhexidine digluconate, dipped polymeric films' extracts (d) without chlorhexidine digluconate and (e) with chlorhexidine digluconate

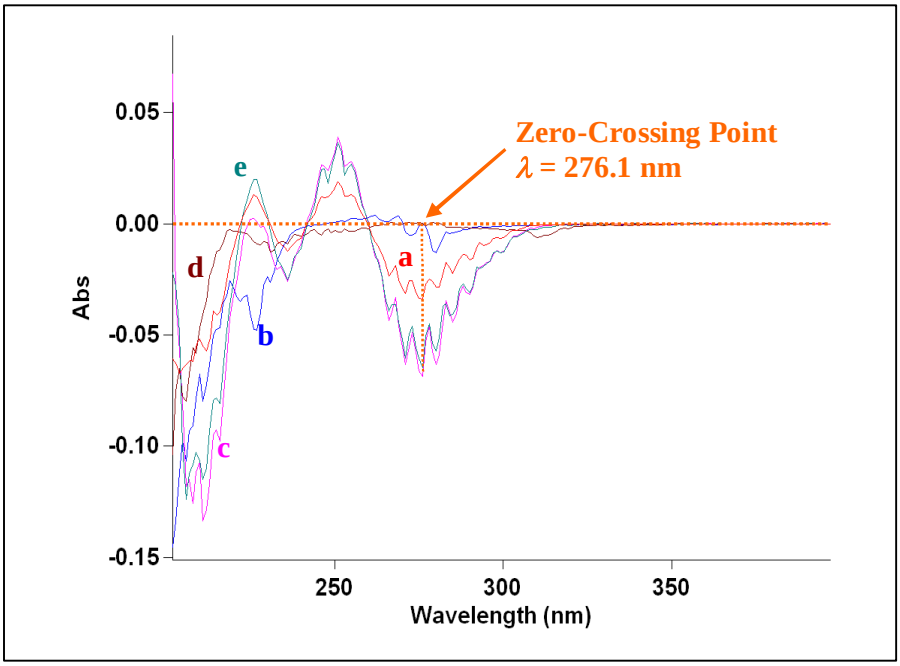

Figure 2. First-derivative spectra of (a) standard chlorhexidine digluconate solution, antimicrobial formulation (b) without chlorhexidine digluconate and (c) with chlorhexidine digluconate, dipped polymeric films' extracts (d) without chlorhexidine digluconate and (e) with chlorhexidine digluconate

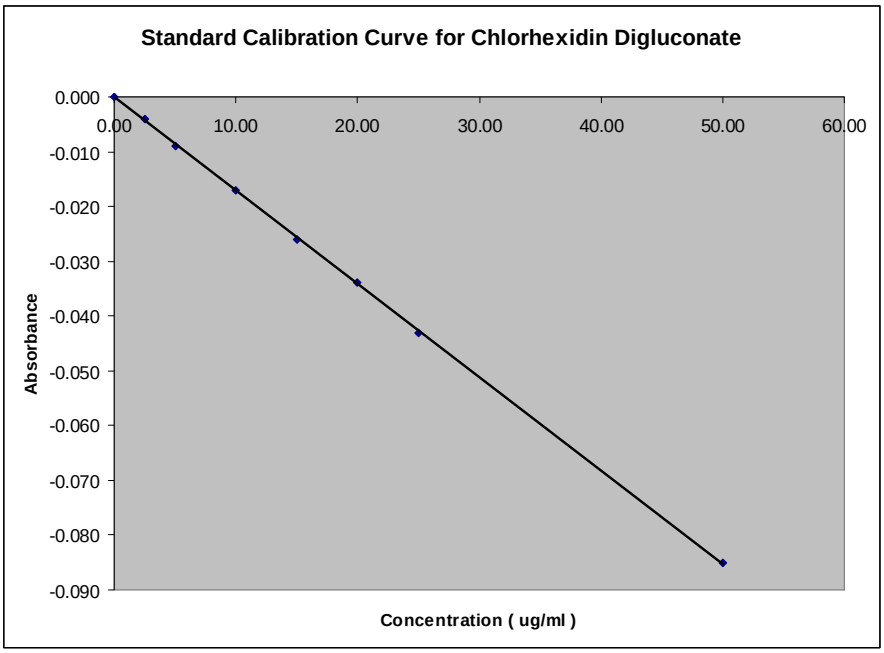

Figure 3. Standard calibration curve for chlorhexidine digluconate based on first-derivative spectrophotometry

Table 1. Optical characteristic of calibration curve for chlorhexidine digluconate using first-derivative spectra at λ = 276.1 nm

Parameter	**First Derivative Spectra**
Wavelength (nm)	276.1
Linearity (μg/ml)	0 – 50
Regression Equation	y = -0.0017x
Correlation Coefficient (R^2)	0.9999
Standard Deviation of Slope	0
Standard Deviation of y-Intercept	0
Limit of Detection (LOD) (μg/ml)	0
Limit of Quantitation (LOQ) (μg/ml)	0

Table 2. Result of accuracy and precision using first-derivative spectra at λ = 276.1 nm

Sample	**Spiked Value (%)**	**Found[a] (%)**	**RSD (%)**	**Recovery (%)**
Antimicrobial Formulation	1	0.99	0	98.53
	2	1.98	0.86	99.02
	4	4.00	1.47	100.00
Dipped Polymeric Film	1	0.97	1.76	96.57
	2	1.89	0.90	94.61
	4	3.84	0.88	96.08

[a]Mean of three determinations

CONCLUSION

A simple, reliable and inexpensive first derivative spectrophotometric method for determination of chlorhexidine digluconate content in an antimicrobial formulation has been successfully developed. No preliminary separation step is required and it allows the determination of an individual active constituent in complex matrices.

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