SPECTROPHOTOMETRIC DETERMINATION OF AMPICILLIN AND CLOXACILLIN IN PURE AND FIXED DOSAGE FORMS THROUGH CHARGE TRANSFER COMPLEXATION

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Keywords: ampicillin, cloxacillin, charge transfer, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)

Simple, precise and accurate visible spectrophotometric procedure was developed for the quantitative determination of two antibacterials, ampicillin and cloxacillin in a fixed dose combination. The method exploits the formation of charge transfer complexes between the antibacterials and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to determine the components of the drugs in a dosage form. The effect of different variables on the development and stability of the complexes were studied and optimized. Beer's law was obeyed. The method was successfully applied to the determination of the target analytes in different brands of the fixed dose combination.

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Introduction

Ampicillin (2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1 - azabicyclo-[3.2.0]-heptane-2-carboxylic acid (Fig. 1.) and cloxacillin (2S,5R,6R)-6-{[3-(2-chlorophenyl)-5-methyl-oxazole-4-carbonyl]amino}-3,3–dimethyl–7–oxo–4–thia – 1 –azabicyclo-[3.2.0]heptane-2-carboxylic acid (Fig. 1) belonging to β-lactam antibiotic of penicillin class, are used individually and in fixed dose combination as antibacterial agents.[1-2]

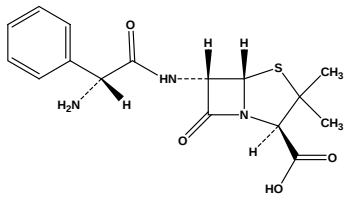

Figure 1a. Structure of Ampicillin

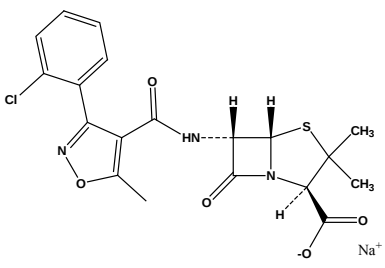

Figure 1b. Structure of Cloxacillin

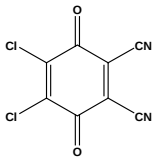

Figure 1c. 2,3-dichloro-5,6-dicyano-1,4 benzoquinone (DDQ)

Fixed dose combinations of antibacterial agents is an important innovation in pharmacy which is used to combat resistance to antibacterial agents by microbes. Treatment of infections caused by β-lactamase producing bacteria is effectively achieved using a penicillin which is susceptible to inactivation by β-lactamase, an enzyme that hydrolyses the β-lactam rings of penicillin and a β-lactamase resistant antibiotics.[3] The Nigerian market is currently flooded with several generics of ampicillin and cloxacillin combinations. This situation becomes more worrisome where several of these generics have varying prices wide enough to suggest compromise of standard. It is therefore necessary to develop a reliable, simple, affordable and efficient method for routine analysis of ampicillin and cloxacillin dosage form combinations.

At the present, many methods are available for the determination of ampicillin and cloxacillin individually, but only few of these methods could be used to analyze them combination with each other or other drugs. The use of High performance liquid chromatography (HPLC),[4-6] polarography,[7] ultraviolet (UV) spectrophotometry[8] have been reported for the assay of ampicillin and cloxacillin but some of these techniques require complex and expensive procedures and instrumentation.[9]

From our literature search, no visible spectrophotometric method using charge transfer has been reported so far for the determination of ampicillin and cloxacillin in a fixed dose combination. We report in this study, the development of a visible spectrophotometric method using charge transfer technique for the determination of ampicillin and cloxacillin

in a fixed dose combination. Charge transfer technique is carried out by monitoring the the reaction between 2,3-dichloro-5,6-dicyano-1,4 benzoquinone (DDQ) and the analyte using a spectrophotometric method.

Experimental

Reagents and Chemicals

Spectrophotometric measurements were carried out using a spectrophotometer (Jenway 6505, UK). Ampicillin trihydrate pure powder (Fidson Healthcare Ltd, Lagos Nigeria), cloxacillin sodium pure powder (Juhel Pharmaceuticals Ltd, Nigeria), chloranilic acid (Sigma-Aldrich Chemie, Germany), DDQ 98% (Sigma-Aldrich Chemie, Germany), acetone analytical grade (Sigma-Aldrich, Germany) were used for the development of the assay.

Preparation of solutions

A stock solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ 98%) (4.40529 x 10^{-3} M) was prepared by dissolving accurately weighed 100 mg of DDQ in acetone in 100 ml standard flask and the volume made up to 100 ml with acetone. Also 100 mg each of ampicillin and cloxacillin was weighed into 100 ml volumetric flasks respectively. The samples were then dissolved in enough acetone before making up to mark with acetone to obtain 2.479 x 10^{-3} M and 2.101 x 10^{-3} M solutions of ampicillin and cloxacillin respectively.

Determination of λ_{max} of the complexes

Absorption Spectrum of DDQ

A 4 ml of DDQ solution was introduced into a 10 ml flask and acetone added to make up to the mark. Measurement of the absorbance (under standard condition) of the solution between 350-800 nm wavelengths using acetone as blank was carried out.

Absorption Spectrum of Drug-DDQ Complex

A 2 ml solution each of the drug samples and 2 ml of DDQ solution was pipetted into two different 10 ml volumetric flask respectively and made up to mark with acetone. Absorbance readings were taken by scanning the solutions between 350 – 800 nm using acetone as blank.

Effect of Time on the Formation of the complexes

The absorbances of a mixture of 2 ml each of ampicillin and cloxacillin solutions and 2 ml of 4.405 × 10^{-3} M of DDQ solution respectively was determined at various time intervals from 5 s to 120 min at 524 nm ($\lambda_{max\ ampicillin}$) and 410 nm($\lambda_{max\ cloxacillin}$) at room temperature against acetone blank.

Stoichoimetric Determination of the Complexes

The stoichiometry of the reaction was determined with equimolar solutions of ampicillin (2.5 x 10^{-3} M), cloxacillin (2.1 x10^{-3} M) and DDQ respectively using Job's method [10].

Effect of pH on the complexes

Equimolar solutions of drug samples and DDQ, ampicillin (2.5 × 10^{-3} M), cloxacillin (2.101 x $10^{-3)}$ M were respectively mixed at the ratio of 2:2 and 6 ml of buffer solutions was added in each case to make up the volume to 10 ml. The same treatment was carried out with buffer pH ranging from 1 – 13 (preparations not shown) in different test tubes for colour development and kept for 60 minutes before determining the absorbances at 524 nm and 410 nm respectively against the blank of acetone, buffer and the reagent.

Determination of Beer's Calibration Plot for the Complexes

Serial concentrations (0.5, 1.0,…, 5.0 ml) of the standard solution of ampicillin (2.5 x 10^{-3} M) and cloxacillin (2.101 x 10^{-3} M) were transferred to different test tubes respectively. A constant volume of 5 ml of DDQ solution in acetone were added to each of the test tubes according to the stoichiometry determined. Sufficient volumes of acetone were also added to bring the volumes to 10 ml in each of the test tubes. The contents were mixed and left at room temperature for 60 mins after which the absorbance of each of the samples was determined at a wavelength of 524 nm and 410 nm respectively against acetone and DDQ blank. The absorbance values were plotted against the concentration to obtain the Beer's calibration curves for each drug complex.

Assay procedure for capsule dosage forms

The contents of 20 capsules were mixed and accurately weighed amount of the contents equivalent to 100 mg of Ampicillin and Cloxacillin was transferred into a 100 ml volumetric flask. About 50 ml of acetone was added and the mixture was sonicated and extracted for 20 minutes. The volume was brought to 100 ml with acetone and final solution was filtered. Aliquots of filtrate were studied as described under the preparation of Beer's plot procedure. The nominal contents of the capsules were calculated using the corresponding regression equation. The developed method was used to assay Ampiclox$^{(R)}$, Compiclox$^{(R)}$, Emzoclox$^{(R)}$ and Vitaclox$^{(R)}$ which are different brands of ampicillin/cloxacillin combination.

Results and discussion

A visible spectrophotometric method was developed for the determination of ampicillin and cloxacillin in bulk and fixed dosage forms. The developed method is based on the reaction of ampicillin and cloxacillin as n-electron donors (D) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as π-acceptor to give highly coloured complex species. π - acceptors are known to yield charge transfer complexes and

radical anions with a variety of electron donors.[11] Different solvents including, acetonitrile, acetone, ethanol, 1, 4–dioxane, and methanol were investigated in order to select the most suitable solvent to be used. Acetone is considered to be an ideal solvent for the colour reaction as it offers solvent capacity and gave the highest yield of the radical as indicated by high ε values.

In acetone, complete electron transfer from ampicillin and cloxacillin designated as donors (D) to DDQ designated as acceptor (A) occur as shown in scheme 1.

$$D + A \rightleftharpoons [D \rightarrow A] \rightleftharpoons D^{\cdot+} + A^{\cdot-}$$

DA complex Radical ions

This results in the formation of intensely coloured radical ions with high molar absorptivities. The dissociation of the DA complex is promoted by the ionizing power of the polar solvent acetone as a result of its high dielectric constant.[12]

The solution of DDQ in acetone yellow in colour with an absorption maximum at 358 nm resulted in differently coloured chromogens on reacting with the colourless solutions of ampicillin and cloxacillin. The complexes, which were formed instantaneously, was scanned in the visible range of 300 - 800 nm, and showed maxima at 524 nm and 410 nm for ampicillin and cloxacillin respectively (Fig. 2).

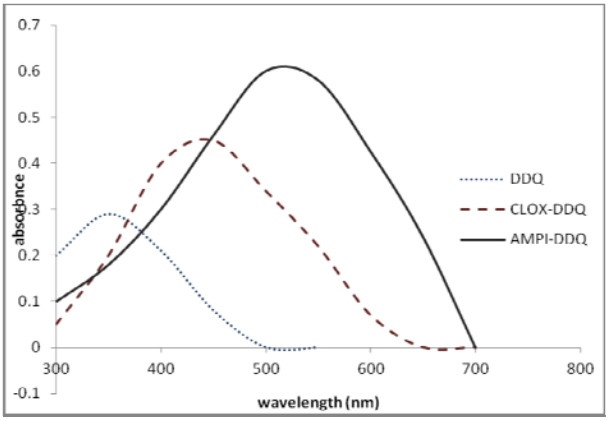

Figure 2. Absorbance spectra of DDQ, Cloxacillin- DDQ complex, and Ampicillin-DDQ complexes

In charge transfer complexation, the absorption maxima of the complexes formed are often shifted some nanometres to the longer wavelength side of the absorption band (bathochromic shift) of either of the components. This shift has been observed between DDQ and other drugs.[13] The difference in absorption maxima between ampicillin and cloxacillin complexes was exploited in their quantitative determination in fixed dosage form without the need for extraction.

Stoichiometric determination of the complexes

The stoichiometric ratio of the reactants was determined using the Job's continuous variation method (data not shown). A 1:1 ratio of charge- transfer complex was observed for the ampicillin- DDQ interaction and cloxacillin-DDQ interaction respectively. This indicated that one mole each of ampicillin and cloxacillin interacted with one mole of DDQ meaning that only one site on the penicillin basic structure interacts with DDQ.

Optimization of reaction conditions

The influence of different parameters on the colour development was also studied to determine optimum conditions. The effect of DDQ concentration on its reaction with the ampicillin and cloxacillin were investigated. When various concentrations of DDQ solution was added to a fixed concentration of ampicillin and cloxacillin, 0.88 *mol dm*$^{-3}$ of DDQ solution was found to be the minimum concentration required for quantitative determination of ampicillin and cloxacillin.

The absorbances of complexes were stable for over 2 h as was obtained by the plot of the absorbance against time (data not shown). The optimum reaction time required for complete colour formation monitored at room temperature (29 ± 2 ^{o}C) was obtained after 60 mins. The developed colours were stable up to 24 h at room temperature.

The absorbances and hence the stability of the ampicillin complex varied with the pH of the media. The stability of ampicillin-DDQ complex increased from pH 1 and peaked at pH 2 and remained stable up to pH 8 when the absorbance fell sharply indicating dissociation of the complex (Fig 3).

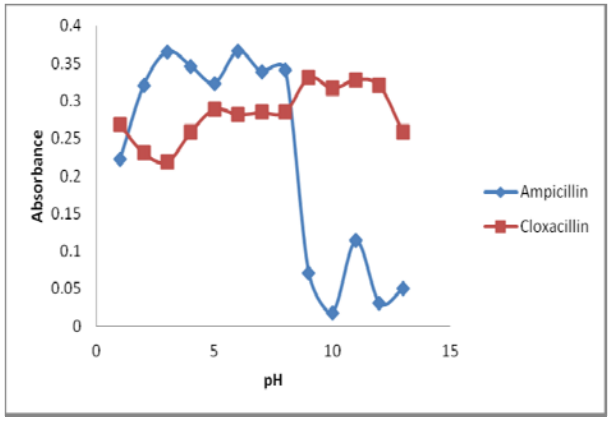

Figure 3. Effect of pH on the formation Ampicillin - DDQ and Cloxacillin-DDQ Complexes

On the other hand, the stability of cloxacillin showed less variation with pH which indicates that pH does not significantly affect the formation and stability of the cloxacillin-DDQ complex (Fig. 3). These suggest that acidic conditions are optimum for the ampicillin-DDQ complexation where as basic conditions are preferred for cloxacillin-DDQ complexation.

Table 1. Assay parameters and regression analysis

Drug	Regression equation	LR, mol dm^{-3}
Amp	y =0.0364x + 0.1568	1.25x10^{-3} – 12.5x10^{-3}
Clox	y = 0.0677x +0.1888	1.05x10^{-3} – 10.5x10^{-3}

Amp= ampicillin sodium, clox=cloxacillin sodium, LR= linear range

The preference for an acidic medium for ampicillin-DDQ complexation suggests that the free amino group with a pKa of 7.3 influences the reaction. As a result the tests were carried out at pH 6 and pH 9 for ampicillin and cloxacillin respectively. The protonation of the nonbonding electrons on the oxygen atom of a carbonyl or hydroxyl group is an important first step in the reactions under acidic conditions of compounds such as amides and carboxylic acids because their conjugate acids are more reactive14. Since cloxacillin-DDQ reaction was optimum at basic condition, it then suggests that neither the carboxylic acid group nor the amide group was involved in the complexation of cloxacillin to DDQ.

Beer's Calibration Plots for the Complexes

A standard calibration plot for ampicillin and cloxacillin was constructed by plotting absorbance versus concentration of the drug in mol dm^{-3} of the ampicillin standard solution under optimum condition. A straight line was obtained for the complexed drug, indicating that spectrophotometric analysis of electron donor- acceptor complex formation can be used for quantitative analysis of the drug (Table 1).

Conformity with Beer's law was oberved in the concentration range of $1.25x10^{-3}$ – $12.5x10^{-3}$ mol dm^{-3} of ampicillin and $1.05x10^{-3}$ – $10.5x10^{-3}$ mol dm^{-3} cloxacillin. Molar absorptivity value of DDQ method was found as 2.5 x 10^5 mol dm^{-3} of ampicillin-DDQ and 4.6 x 10^5 mol dm^{-3}. of cloxacillin-DDQ L $mol^{-1}cm^{-1}$, respectively. The limits of detection (LOD) and limits of quantification (LOQ) were determined using the formula: LOD or LOQ = κSDa/b, where κ= 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept and b is the slope.

The LOD was determined to find out the lowest amount of Ampicillin and Cloxacillin that can be detected and it was found to be 0.47 mol dm^{-3} and 0.17 mol dm^{-3} for Ampicillin-DDQ and Cloxacillin-DDQ respectively. The LOQ was determined to find the lowest amount of Ampicillin and Cloxacillin that can be quantified and it was found to be 1.59 mol dm^{-3} and 0.59 mol dm^{-3} for Ampicillin- DDQ and Cloxacillin-DDQ respectively.

Table 2. Analysis of some pharmaceutical preparations

Formulations	Amount, mg		% Recovery
	Labelled	Found	(Mean ± SEM)
Ampicillin			
Ampiclox	250	257.8	103.1±3.1
Compiclox	250	264.3	105.7± 2.4
Emzoclox	250	246.8	98.7 ± 4.1
Vitaclox	250	269.0	107.6 ± 2.0
Cloxacillin			
Ampiclox	250	237.5	95.4±1.4
Compiclox	250	243.0	97.2 ±1.4
Emzoclox	250	232.8	93.1±1.3
Vitaclox	250	240.0	96.0±1.6

The recovery experiments carried out on dosage form showed high quantitative recoveries with low standard deviations as shown in Table 2**.** These indicated a high accuracy of the method of analysis.

Conclusions

A charge-transfer complexation between ampicillin and cloxacillin with DDQ occurred with a 1:1 stoichiometry with maximum wavelength of absorption of 524 nm and 410 nm for ampicillin-DDQ complex and cloxacillin- DDQ complex respectively. The reactions were favoured at acidic medium according to pH determinations except for cloxacillin-DDQ complex which is favoured by basic medium. Thermodynamically, the complexes were found to be very stable at room temperature. The method was used to assay the drugs in pure form and in dosage form with good precision and accuracy and can therefore be used in rapid qualitative and quantitative determination of ampicillin and cloxacillin in both pure form and in dosage form.

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Received: 19.08.2013.
Accepted: 26.08.2013.