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Development of a simple UV Assay method for Artesunate in Pharmaceutical Formulations

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ABSTRACT

Artesunate (ART) is a readily available anti malarial in combination therapy. The assay method has posed a challenge because it does not have a readily recognisable absorption chromophore needed for UV spectroscopy. A simple and rapid assay method involving two reaction steps has been developed for assay of ART in pharmaceutical formulations. The method involves basic reaction of ethanolic solution of ART with 0.1N sodium hydroxide and then neutralisation and acidification of this reaction mixture with 0.1M solution of acetic acid in 20% ethanol. This gave a good UV spectrum for ART with λmax at 242nm. HPLC analysis of this mixture revealed the presence of two prominent peaks. The peaks were further identified with HPLC-mass spectrometer to possibly be those of glycal and furanose acetal. The method was validated for stability, linearity, accuracy, intra- and inter-day precision and was used to assay ART in commercial tablets. The method is simple and suitable for the assay of ART in pharmaceutical formulations.

Key words: Artesunate, Assay, Pharmaceutical Formulation, validation, commercial tablets.

INTRODUCTION

ART is the sodium salt of the hemisuccinate ester of artemisinin with molecular weight 384.4g (Figure 1). The chemical name is (3R,5aS,6R,8aS9R,10S,12R,12aR)- Decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10-ol, hydrogen succinate [1]. It is semi-synthetic derivative of artemisinin, synthesized by the esterification of dihydroartemisinin [2]. Of all available artemisinin derivatives, it has the most favourable pharmacological profile for use as ACT partner for the treatment of uncomplicated malaria [3]. The presence of hemisuccinate group confers it with water solubility and high bioavailability [3].

ART being an artemisinin derivative is a sesquiterpene lactone with an unusual peroxide bridge is difficult to detect and identify by standard spectrophotometric methods, since it absorbs light only at low wavelengths, has a relatively low molar extinction coefficient, and has no distinct UV-Visible spectrum or fluorescent properties [4, 5]. Therefore for the purpose of pharmacokinetic studies, ART has been detected by mass spectroscopy [6], electrochemical detector [7], evaporation light scattering detector [4], pre-column derivatisation [8], and post-column degradation reaction [9]. In pharmaceutical formulations ART has been assayed by HPLC with UV at low wavelength (≤ 220nm) [10, 11], HPLC with mass spectrometric detector [19], titrimetric method [1], and colorimetric method [5, 12, 13].

Since analysis is an important development of any dosage form, it necessary to have a simple, precise, accurate and sensitive method for assay of any drug product both as a bulk and in its formulation [14]. Simple UV method has become necessary for the assay of this drug because, UV unlike HPLC is simple, rapid and readily available in malaria endemic areas of the world. This will also help to checkmate influx of fake and adulterated products into the drug market and reduce the burden of malaria [15]. In order to assay ART by UV method, it is necessary to involve it in a reaction process that would break the endo peroxide ring and introduce a least one double bond in the molecule. Esimone *et al*., developed an assay method for ART involving reaction of the compound with simulated intestinal fluid (SIF) and analysed it at 287nm [15]. Due to the reactivity of this compound and possible its reaction product, any assay method for it should include stability study of the reaction product and possibly elucidation of the structure of the products responsible for the UV activity. This study aimed at the development of a simple and rapid UV- assay method for ART, identification of compounds that might be responsible for the UV absorption and validation of the method.

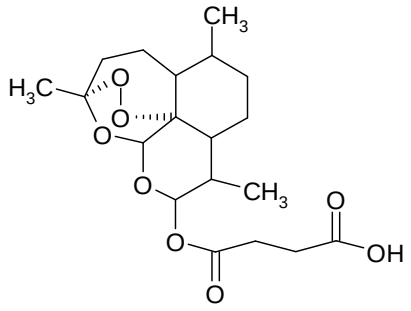

Figure 1: Artesunate

EXPERIMENTAL SECTION

ART was purchased from Mangalam Drugs through ERICA India, ammonium acetate and sodium hydroxide pellets were purchased from Sigma Aldrich, UK. Absolute Ethanol, acetonitrile and acetic acid HPLC grades were purchased from Fisher Scientific UK. Glacial acetic acid, Potassium dihydrogen orthophosphate and Orthophoshoric acid were purchased from BDH, UK.

Method

Instrumentation

A Hewlett-Packard 8453E Spectrophotometer equipped with 1.0cm quartz cell controlled using the Agilent ChemStation software revision A 10.02. 1757 and a Hewlett-Packard Deskjet 3745 printer was used for all absorbance measurements. The spectra of the solution were recorded over the range 200-400nm.

The HPLC consisted of a Hewlett Packard 1050 system, fitted with auto sampler, quaternary pump and variable wavelength detector.

The HPLC-Mass Spectrometer consisted of Waters 2690 Series fitted with auto sampler, quaternary pump and connected to a UV detector and Micromass ZMD Mass Spectrometer.

Preparation of working solutions

1) 0.1N Sodium hydroxide: 0.42g of sodium hydroxide was weighed and dissolved in water and made up to 100ml.

3) 0.1M Acetic acid in 20% Ethanol: 1.144ml of glacial acetic acid was measured and diluted to 200ml with 20% ethanol.

Decomposition reaction: Basic decomposition followed by acid reaction

The method applied in this study is the modification of the method used by Zhao in HPLC assay of artemisinin from plasma and saliva [16]; 10mg of ART was weighed, dissolved in absolute ethanol and made up to 10ml to give a concentration of 1.0mg/ml. One millilitre of the solution was transferred to a 10ml volumetric flask containing 4ml of 0.1N sodium hydroxide. The flask was placed in water bath at 50 ± 0.1 °C for 60 min. The solution was allowed to cool and made up to volume with 0.1M acetic acid in 20% ethanol. The stock solution was diluted to the following concentrations with 0.1M acetic acid in 20% ethanol: 10, 20, 30, 40 and 50µg/ml. Each concentration was prepared in quadruplicate**.** The UV spectra of the solutions were recorded over the range 200-400nm; the blank solution was 0.1M acetic acid in 20% ethanol. The results obtained were used to establish the linearity of the method.

Stability

Due to the limited stability of ART in solution and possibly that of the degradation product there is need to evaluate its stability in different solvents to establish how long the prepared solution must stay without significant degradation before analysis. Freshly prepared samples were diluted and assayed at 0, 0.5, 1, 2, 3, 24 and 48hours with solution adequately protected from light to prevent photo-degradation.

Accuracy

The accuracy of the method was established at four (4) concentration levels: 20, 30, 40, 50µg/ml and four repetitions at each level.

Precision

Precision is evaluated in terms of the relative standard deviation (% RSD) determined in a series of measurements [17]. The intra-day precision was determined at three-concentration levels (covering low, medium and high levels) and three different samples were analyzed the same day. The inter-day precision was determined at the same concentration levels as intra-day precision at two other days representing intermediate precision.

Investigation into the possible compound responsible for the UV absorption

HPLC Conditions

The mobile phase consists of acetonitrile: 10mM Phosphate buffer, pH 3.0 (50:50) and filtered with 0.4µm nylon filter assisted with suction pump and degassed in an ultrasonic bath for 15minutes, flowing through a Waters C_{18} (ODS2) Spherisorb® 250mm x 4.6 i.d. (5µm) column at 1ml/min; 20µl of the sample solution was injected into the HPLC system and analysed at the wavelength of 240nm.

HPLC-Mass Spectrometer

The mobile phase consists of equal volume of a 10mM ammonium acetate buffer (pH 3.2) and acetonitrile, also filtered with 0.4µm nylon and degassed; 50µl of each sample was injected into the system and the components were resolved by passing through Column-Waters C_{18} (ODS2) Spherisorb® 250mm x 4.6mm i.d. (5µm). The components were detected by the UV-detector after which it passed through a flow split which allowed about 20% of the mobile phase/elute into the mass spectrometer, where the liquid was vaporised by the presence of an electrospray ion source creating charged ions either by addition or removal of a proton. The ions move to the mass analyser of the mass spectrometer. The detector monitors the ion current, amplifies it and the signal is transmitted to the data system where it is recorded in form of mass spectra.

Assay of artesunate in Commercial Tablets

Four different brands of ART tablets which were registered by National Agency for Food Drug and Control (NAFDAC) were obtained from registered Pharmacies in Nigeria. Five tablets from each brand were weighed individually, their mean weights were calculated. Tablets were crushed and quantity equivalent to 10mg of the drug was weighed out and diluted with absolute ethanol, then made to undergo the decomposition reaction. Appropriate dilution was made and the absorption was obtained and the wavelength of 242nm. The average of five (5) repetitions was obtained.

RESULTS AND DISCUSSION

The UV-visible scan of the ART reaction mixture showed pronounced UV-absorption with the λmax at 242nm (figure 2). The linearity was established at the concentration range of 10 -50 µg/ml. The regression equation is y = 0.0084x - 0.0041 and the correlation coefficient (r) is 0.9999. The stability of the UV solution was established by plotting the log of absorbance against time (figure 3). The degradation appeared to follow first order kinetics giving T_{90} and T_{99} of 6.7 and 2.5 hours respectively. The degradation of the product from ART might be due to high rate of reactivity and instability associated with these groups of compounds as well as the presence of high acidic environment which might have catalysed further degradation. This infers that reliable result can only be obtained if it is analysed within 2.5 hours after the reaction.

The percentage accuracy results for the concentrations of 20, 30, 40 and 50µg/ml are 101.1, 101.8, 101.3 and 99.1 respectively. The results from the unpaired student t test show that there is no significant difference between the sample mean and the true value at 95% confidence level (p values are 0.51, 0.25, 0.35 and 0.70 respectively).

The intra-day precisions at concentrations of 20, 30 and 40 µg/ml are 2.41, 1.94 and 1.81 respectively. The inter-day precision at the same concentration are 2.00, 0.68 and 4.53 respectively.

Structures of the compounds responsible for UV activity of Artesunate

The chromatograms showed many peaks but the prominent among them were the peaks at 3.9 and 4.5minutes (Art1 and Art2; figure 4). Those two compounds with pronounced UV activity might be responsible for the increased UV absorbance and basis for the UV analysis of ART. Further investigation into the possible structure of these compounds by HPLC-mass spectrometer revealed peaks from UV response at 3.9, 4.4, 7.1 and 10.5 minutes while that of the mass spectrometer showed response at negative eletrospray at 3.8, 4.4 and 10.2 minutes (Figure 5). There was no response at positive eletrospray. The mass spectrometric analysis of the compounds eluted at 3.8 and 4.4 minutes both gave molecular weight of 266 for the peaks with

highest intensity (figures 6, 7). These weights corresponded with the molecular weights of two of the products obtained when ART was subjected to stress condition such as treatment with acid [18]. It was postulated that due to the fact that the products had strong UV response at 240nm, it is likely that they have double bonds in the ring structure. Therefore the compounds without such characteristics were eliminated. In addition, the furanose acetal presented in figure 7 seems to have a better UV absorption chromophore because the double bonds are conjugated. Furthermore, the compound furanose acetal appears to be in more abundance than glycal [18]. Therefore the compound eluted at 4.4 minutes consisting of stronger UV absorption indicated by bigger peak area might be furanose acetal while that eluted at 3.8 minutes might be glycal (figure 8). Better structural elucidation of the compound can be carried out by the use of Nuclear Magnetic Resonance (NMR).

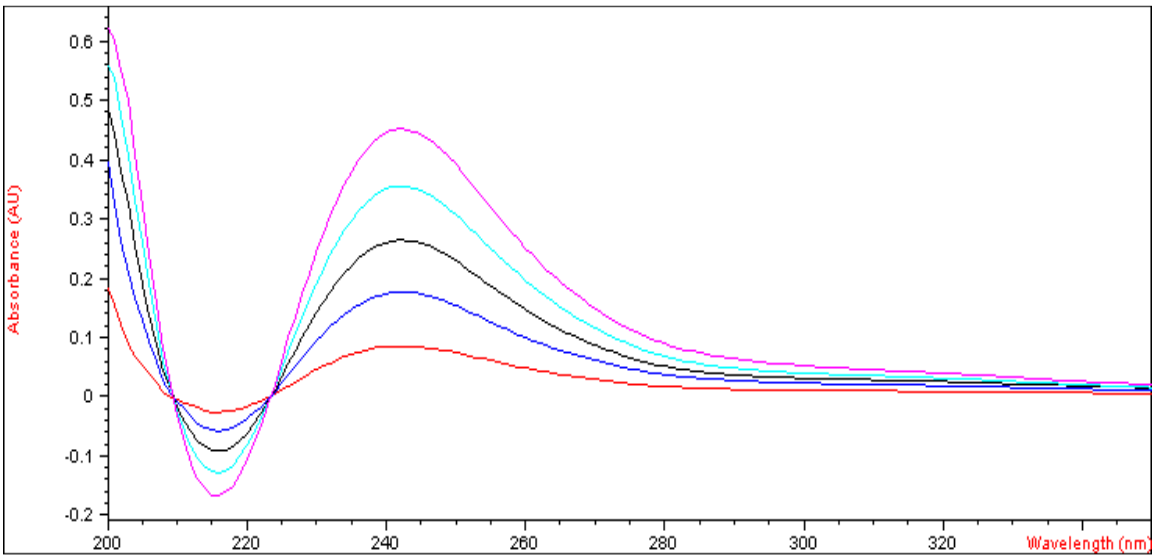

Figure 2: UV Scan of the reaction Mixture containing 10-50µg/ml of Artesunate

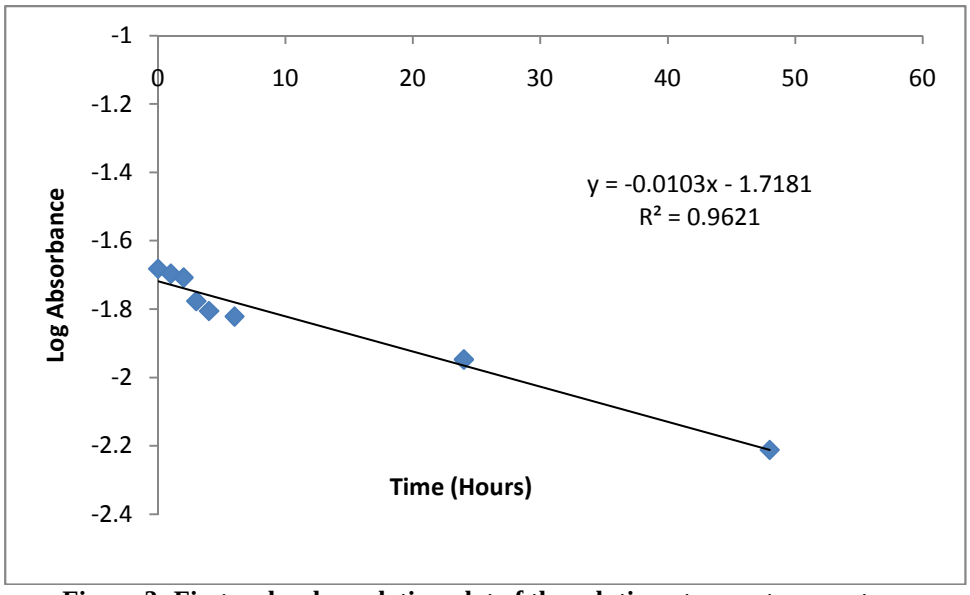

Figure 3: First order degradation plot of the solution at room temperature

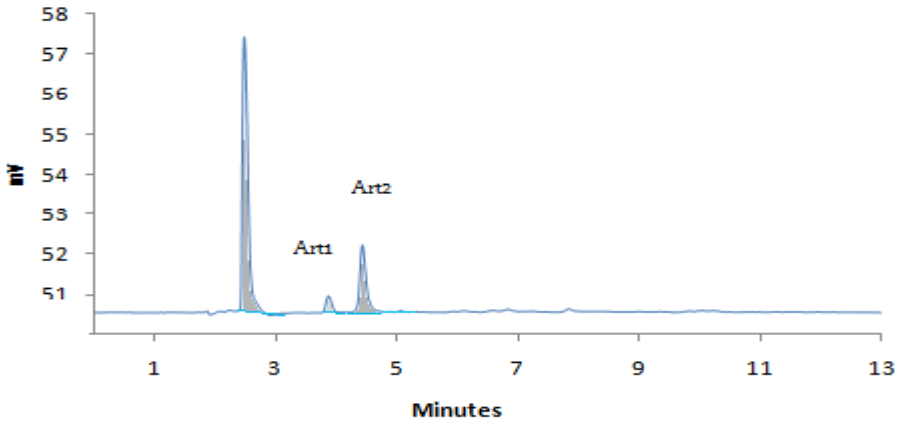

Figure 4: Chromatogram of the reaction mixture containing peaks of degradation products from 4µg/ml Artesunate

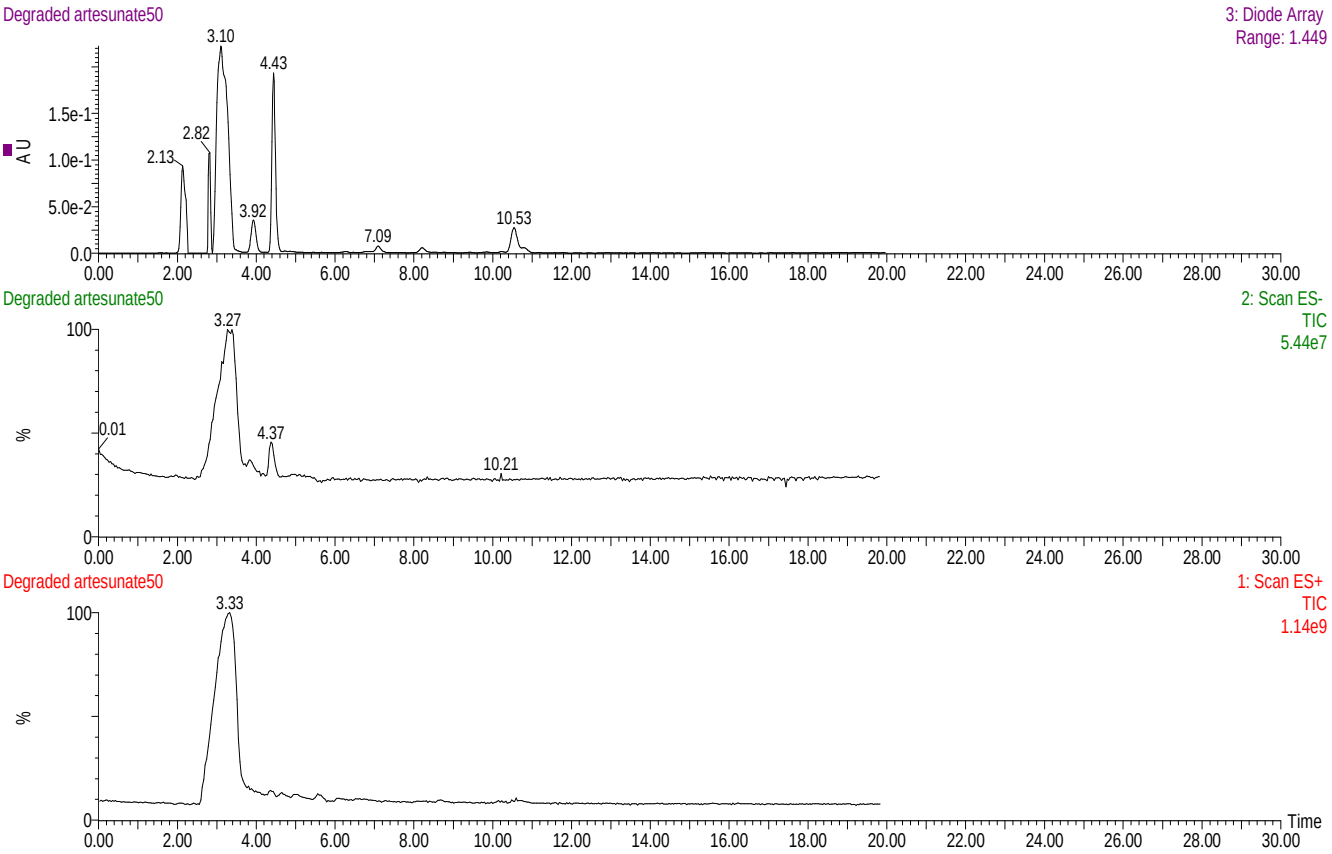

Figure 5: Chromatogram of degradation product from Artesunate; UV Response, MS Response at negative and positive electrospray.

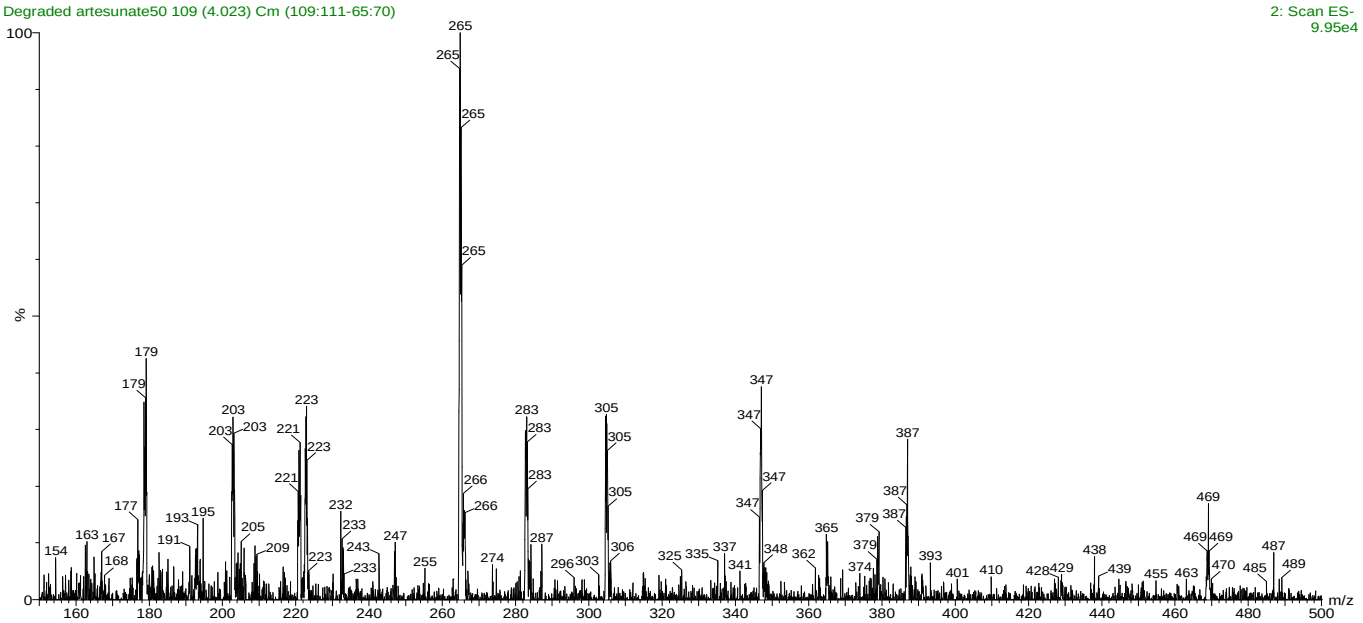

Figure 6: Mass Spectrum of elute from Artesunate degradation product at 3.8 minutes.

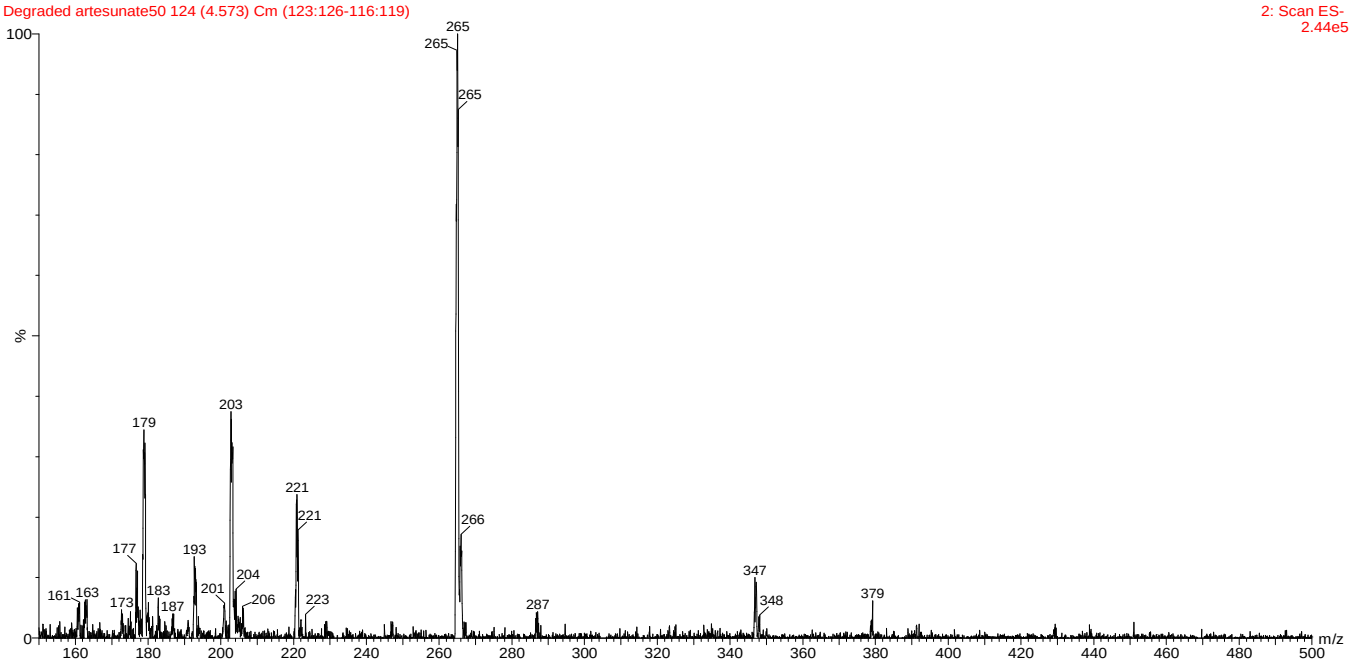

Figure 7: Mass Spectrum of the elute from Artesunate degradation product at 4.4minutes

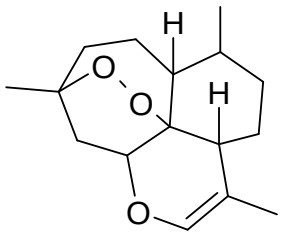

Figure 8: Glycal with molecular weight 264.37 [18]

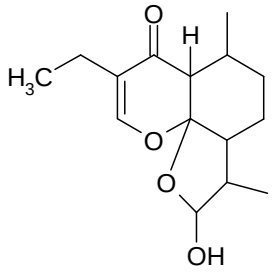

Figure 9: Furanose acetal with molecular weight 266.34 [18]

Assay of Artesunate Tablets

All the brands of ART tablets analysed give good relationships between the label claim and amount of the active drug found using this assay method. The reliability of the method was confirmed by the accuracy experiment.

Brand Code	Label Claim (mg)	Amount Found	% Content
ART1	50	49.71 ±1.18	99.41
ART2	50	49.02 ±1.63	98.04
ART3	50	49.62 ±1.74	99.23
ART4	50	49.18±1.62	98.35

CONCLUSION

A simple, inexpensive and accurate method for the assay of ART has been developed, validated and used to analyse the drug from commercial tablets in Nigerian market. There was no interference from the tablet excipients. This method can be applied for the UV assay of ART both in bulk and formulations.

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