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Comparative ultraviolet spectrophotometric assay and post-marketing potency evaluation of available brands of doxycycline hyclate capsules in Nigeria

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Abstract

Introduction: Doxycycline is a semi-synthetic tetracycline antibiotic in which a hydroxy group replaces the 5β-hydrogen, while the 6α-hydroxy group is replaced by hydrogen. It is used to inhibit bacterial protein synthesis and treat non-gonococcal urethritis and cervicitis, as well as exacerbations of bronchitis in patients with chronic obstructive pulmonary disease, and adult periodontitis. This study developed and validated an ultraviolet (UV) spectrophotometric method for the assay of doxycycline capsule formulations using 0.1N NaOH as the solvent and compared it with the previously developed and validated method, using 0.1N HCl. **Methods:** The UV methods were applied to evaluate the quality of marketed formulations by assessing identity, weight variation, and strength. **Results and Discussion:** Besides the detection wavelength of 265 nm versus 268 nm, the optical and validation parameters of the method using 0.1N NaOH as the assay solvent did not differ from the method using 0.1N HCl, as shown by the limits of detection [(µg/mL): 0.3200 versus 0.3219], limits of quantification [(µg/mL): 0.9698 versus 0.9755], and Sandell's sensitivity [(µg·cm^{-2}): 0.0311 versus 0.0255]. Of the six available brands of doxycycline tested, all passed the identity test, but only 66.67% met the USP specifications for dosage uniformity and doxycycline content. **Conclusion:** The new method proved to be simple, rapid, precise, accurate, and cost-effective, and compared favourably with the previously reported method using 0.1N HCl as the assay solvent. It was successfully used to assay the doxycycline content in capsule formulations.

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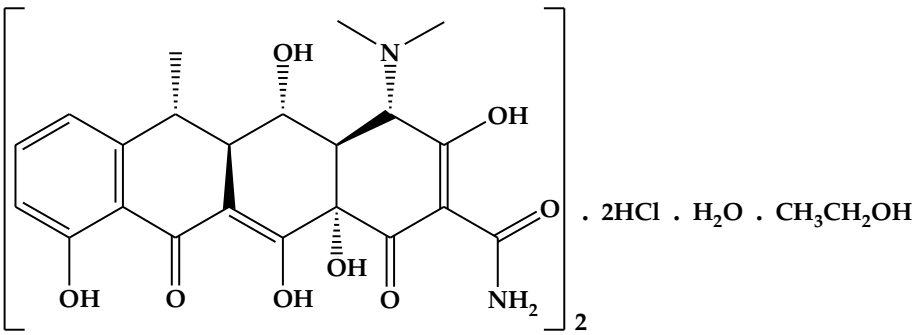
Introduction

Doxycycline is an isomer of tetracycline that differs only in the placement of one hydroxyl group, with the 5β-hydrogen replaced by a hydroxyl group and the 6α-hydroxy group replaced by hydrogen (Stephens *et al*., 1958). Therefore, doxycycline is chemically known as α-6-Deoxy-5-hydroxytetracycline, or α-6-Deoxyoxytetracycline, a broad-spectrum tetracycline antibiotic synthetically derived from oxytetracycline (McCormick *et al*., 1960; Petisi *et al*., 1962). It is a second-generation tetracycline known to have lower toxicity compared to first-generation tetracyclines (Stephens *et al*., 1958; Schach von Wittenau *et al*., 1962). The IUPAC name of doxycycline is (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (Pubchem, 2025a). The chemical structure of doxycycline (Fig. 1) features a four-ring core (naphthacene nucleus) with multiple functional groups, including hydroxyl, carbonyl, and amino groups, allowing it to bind to bacterial ribosomes and inhibit protein synthesis (Anonymous, 1972; Cunha *et al*., 1982).

Doxycycline appears in three forms: hyclate, monohydrate, and hydrochloride, with doxycycline hyclate and doxycycline monohydrate being the two most common (Jantratid *et al*., 2010; Parmar & Patel, 2025). From doxycycline hyclate, other forms can be obtained (Hasan *et al*., 2016). The hyclate, when dissolved in water and neutralised with sodium hydroxide, becomes doxycycline monohydrate, which is converted to doxycycline hydrochloride upon the addition of hydrochloric acid (Fig. 2).

Doxycycline hyclate is the hemiethanolate hemihydrate of doxycycline hydrochloride (Pubchem, 2025b). It is much more soluble than doxycycline monohydrate, which is a main reason for its frequent use as an antibacterial agent (Jantratid *et al*., 2010). It is a yellow, hygroscopic crystalline powder, freely soluble in water (up to 50 mg/mL), producing a clear, yellow-green solution, and somewhat soluble in methanol, sparingly soluble in ethanol (96%). It dissolves in alkali hydroxide and carbonate solutions. Doxycycline hyclate has a molecular weight of 512.94 g/mol and chars at 201°C without melting (Pubchem, 2025b). It reversibly binds to the 30S ribosomal subunit and possibly the 50S subunit, blocking the binding of aminoacyl-tRNA to the mRNA-ribosome complex, leading to inhibition of protein synthesis and resulting in a bacteriostatic effect (Cunha *et al*., 1982).

Doxycycline hyclate is often the preferred tetracycline due to its greater effectiveness and pharmacokinetic advantages, such as reliable absorption and a long half-life, which allow for less frequent dosing than other drugs in its class (Cunha *et al*., 1982; Parmar and Patel, 2025). It is commonly used in pharmaceutical forms like capsules, tablets, and injections. Doxycycline hyclate is effective in treating a wide range of bacterial infections, both Gram-positive and Gram-negative (Cunha *et al*., 1982). As an antibiotic, it inhibits the growth of



$(C_{22}H_{24}N_2O_8.HCl)_2.C_2H_6O.H_2O$ molecular weight of 1025.89

Fig. 1: Molecular structure and formulae of 2 units of doxycycline hyclate ($C_{22}H_{24}N_2O_8 \cdot HCl \cdot 0.5H_2O \cdot 0.5C_2H_6O$)

these bacteria. It also helps manage and treat conditions like acne, malaria (when combined with artesunate or quinine), skin infections, sexually transmitted infections (STIs) such as chlamydia, syphilis, gonorrhoea, pelvic inflammatory disease, and Lyme disease (Smilack, 1999; Chopra & Roberts, 2001; Henehan *et al*., 2017). Currently, doxycycline is the most widely used tetracycline and is regarded as an essential medicine by the World Health Organization (WHO, 2023).

A review of the literature on ultraviolet spectrophotometric methods for determining doxycycline content in pharmaceutical formulations revealed various studies that used both aqueous and organic solvents, highlighting these as simple and cost-effective techniques (Ramesh *et al*., 2010; Ramesh *et al*., 2011; Rakesh *et al*., 2013; Sreeram *et al*., 2015; Hasan *et al*., 2016; Patil *et al*., 2020; Sahoo *et al*., 2023; Bhardwaj *et al*., 2025). However, no previous report has established an ultraviolet spectrophotometric method for determining doxycycline using 0.1 N NaOH as the assay solvent. This study aimed to develop and validate an ultraviolet spectrophotometric method for measuring doxycycline in bulk and capsule dosage forms using 0.1 N NaOH as the assay solvent. The results were compared with those obtained using the previously reported but validated method of 0.1 N HCl. The developed method was applied to estimate the potency and strength of doxycycline capsules to determine whether the products currently marketed and used in Ilorin, North Central Nigeria, meet pharmacopeial standards regarding identity, weight variation, and potency, as part of the WHO's efforts to detect substandard and falsified pharmaceuticals already in the supply chain.

Materials and methods

Instrumentation

The photometric measurements were carried out using a PerkinElmer Lambda 35 UV/Vis Spectrophotometer (PerkinElmer, Singapore), which was equipped with 1 cm matched quartz cells for sample and reference solutions. The spectrophotometer had a spectral bandwidth of 1 nm, a wavelength range of 190-1100 nm, and an accuracy of ±0.1 nm. Sample scanning was performed at a speed of 50 nm/min with the slit width set to 1 nm. A Shimadzu electronic balance [(AUW220D) readability 0.0001 g], Shimadzu Corporation, Kyoto, Japan, was used for weighing. Precision measurements were achieved with micropipettes (P20: 2–20 μL) from Switzerland.

Reagents and materials

Doxycycline hyclate reference standard was procured from Toronto Research Chemicals Inc., Toronto, Canada. The sodium hydroxide (NaOH) pellets (500 g) and concentrated hydrochloric acid (HCl) (37%) used in the study were of analytical grade and required no further purification. Both assay solvents, at 0.1 N strength, were prepared by dissolving the appropriate amounts of NaOH and volumes of concentrated HCl in distilled water. Distilled water, produced in the laboratory using the water distiller, Pure-Hit Still (BASIC/PH4 model), was used throughout the experiments.

Sample collections

The available brands of doxycycline capsules were collected from local pharmacies using a simple random sampling method and labelled as DOX to conceal the brand names. All samples were visually inspected for the physical features of the hard capsules, packaging, and labelling details, and the descriptive characteristics of each were recorded.

Preparation of assay solvents

Hydrochloric acid solvent: About 8.4 mL of concentrated HCl was transferred into a 1L volumetric flask containing some distilled water, then filled to the mark with distilled water to produce approximately 0.1 N HCl.

Sodium hydroxide solvent: About 4.0 g of NaOH pellets was weighed, dissolved in distilled water, and diluted to volume with the solvent in a 1L volumetric flask to produce a 0.1 N NaOH solution.

Preparation of standard solutions

Calibrated volumetric flasks (50 mL and 100 mL) and pipettes (1 mL, 5 mL, and 10 mL) were used to prepare standard solutions. A standard stock solution (C_1: 1000 µg/mL) of doxycycline hyclate in 0.1 N HCl/NaOH was prepared in triplicate by accurately weighing and transferring 100 mg of the powder into a 100 mL volumetric flask. The powder was dissolved and brought up to volume with the appropriate solvent, and allowed to stand for at least 5 minutes to ensure complete dissolution. A working standard solution (C_2: 100 µg/mL) was prepared by pipetting 10 mL of the stock solution (C_1) into a 100 mL volumetric flask and diluting to volume with the appropriate solvent. A standard test solution (C_3: 20 µg/mL) was prepared by pipetting 10 mL of the working standard solution (C_2) and diluting it to the 50 mL mark in a volumetric flask with the appropriate solvent.

Qualitative analysis

For the spectrophotometric identity test, the reference standard and the test samples were prepared using identical procedures in 0.1 N HCl/NaOH and scanned from 410 nm to 210 nm. The spectra of doxycycline hyclate in the test sample solutions were compared to confirm that they exhibit absorption maxima only at the same detection wavelengths as those of the reference standard solutions. Additionally, the molar absorptivity values of the analyte at the detection wavelengths of the absorption maxima (λ_{max}) in 0.1 N HCl/NaOH were calculated and compared between the standard and test samples to ensure they do not differ by more than 2% (USP, 2023).

Weight variation test

For hard capsules containing 25 mg or more of the active ingredient, which constitutes 25% or more by weight of the dosage unit according to the USP Stage 4 Harmonization ⟨905⟩ Uniformity of Dosage Units specifications, the test was performed by weighing 20 capsules individually (USP, 2007). The average weight was calculated, and each capsule's weight was compared to this average. The weight variation was expressed as a percentage.

$$\boldsymbol{weight\ variation} = \frac{(\boldsymbol{Individual\ weight} - \boldsymbol{Average\ weight})}{\boldsymbol{Average\ weight}} * \mathbf{100}\%$$

The test passes if none of the individual weights are less than 90% or more than 110% of the average. If test requirements are not met, the powder will be removed, and the net content of powder will be weighed individually and compared with the average net weight. The requirements are satisfied if no more than two individuals' differences exceed ± 10% of the average. In any case, the difference should not be more than or equal to 25% (USP, 2007).

Quantitative analysis

Generation of a standard calibration curve

From the 100 µg/mL working standard solutions (C_2), suitable sample volumes or aliquots (2.5, 5, 7.5, 10, 12.5, and 15 mL) were taken in 50 mL volumetric flasks and diluted to volume with the appropriate solvent to prepare a series of standard solutions (C_3) with strengths of 5, 10, 15, 20, 25, and 30 µg/mL, respectively. Each concentration was prepared in triplicate, and their absorbances in the ultraviolet region were measured immediately against the appropriate blank (0.1 N HCl/NaOH) at 268 and 265 nm, respectively. The concentration of each solution was determined from the corresponding standard calibration curve.

Validation

Method validation, including linearity, limit of detection (LOD), limit of quantification (LOQ), solution stability, repeatability, ruggedness, and accuracy, was conducted following the guidelines set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH, 2023).

Linearity: Calibration curves were created by plotting absorbance at the wavelength of maximum absorption (λ_{max}) for the molecule against the corresponding concentration using linear regression analysis for both methods.

Limit of Detection (LOD): Is the lowest possible concentration at which the method can detect but not quantify the analyte within the matrix with a certain level of confidence. This was calculated based on the standard deviation of the y-intercept and the slope of the calibration curve using the equation:

$$\text{LOQ} = \frac{3.3\sigma}{S}$$

Where σ is the standard deviation of the y-intercept, and S is the slope of the calibration curve (ICH, 2023).

Limit of Quantification (LOQ): Represents the lowest concentration of the analyte that can be reliably quantified by the method. Similarly, this was calculated based on the standard deviation of the y-intercept and the slope of the calibration curve using the equation (ICH, 2023):

$$\boldsymbol{LOQ = \frac{10\sigma}{S}}$$

Solution stability: Two separate 20 µg/mL standard and sample solutions in 0.1N HCl and NaOH were prepared in triplicate using the same procedure at room temperature (25 ± 2 °C). Their absorbances were measured at 0 hours (immediately after preparation) and at 1, 2, 4, 6, and 12 hours. The concentration and % change in absorbance or remaining (% R) were calculated from their absorbance values using:

$$\boldsymbol{\%\, R = \frac{At}{A0}\, x\, 100}$$

Where At = absorbance at time t and A0 = initial absorbance, with an acceptance limit of ≤ 2% change from the initial value (ICH, 2023).

Precision: The repeatability and ruggedness studies involved estimating the working concentration of doxycycline hyclate reference standard (i.e., 20 µg/mL) at three different intervals on the same day (intraday) to demonstrate the repeatability of assay results under the same operating conditions. The analyses of the test solutions were performed by two different analysts (I and II) to demonstrate the ruggedness of the method. A robustness test was also performed to assess the effect of deliberate, slight changes in spectrophotometric conditions on the determination of doxycycline hyclate in the capsules. Absorbance values were recorded for the variations of ± 1 nm in the detection wavelength of maximum absorbance. The absorbance, observed concentrations, and relative standard deviation (% RSD, i.e., the standard deviation expressed as a percentage of the mean) were calculated.

Accuracy (% Recovery): This assesses the specificity of the method when excipients are present under the spectrophotometric conditions used to analyse the doxycycline components of the capsules. It was assessed using the standard addition method at three different concentration levels—80%, 100%, and 120% of the 20 µg/mL test solution (i.e., 16, 20, and 24 µg/mL)—in triplicate (n = 3). Each pre-quantified concentration level of the samples was spiked with approximately 20 µL of the stock standard solutions, thoroughly mixed by vigorous shaking, and the absorbance re-measured at the detection wavelength of maximum absorption for both methods. The amount of added analyte was calculated, and the total amount of doxycycline hyclate was determined using the corresponding regression equations. The percentage recoveries at each level were calculated using the formula:

$$\text{Recovery} = \frac{Observed\ concentration}{Calculated\ concentration} x\ 100$$

Evaluation of the doxycycline hyclate content of capsules

The contents of 20 capsules from each of the six brands were emptied, triturated, and weighed together. The powder equivalent to 100 mg of doxycycline was calculated, weighed, and transferred to a 100 mL volumetric flask in triplicate using 0.1N HCl/NaOH to dissolve and fill to the mark. The resulting mixtures were allowed to stand for 5 minutes at room temperature and thoroughly shaken to ensure complete dissolution of the analyte. The 1000 µg/mL sample stock solutions (C_1) were filtered through Whatman filter paper Grade 42 (125 mm). Exactly 10 mL of each of the triplicate

solutions (C_1) was transferred into another 100 mL flask and diluted to volume with the appropriate solvent to prepare a 100 µg/mL working solution (C_2). Precisely a 10 mL aliquot of the working solution (C_2) was transferred into a 50 mL volumetric flask using a pipette, and the final volume was adjusted to mark to produce a 20 µg/mL sample solution (C_3), as shown in Fig. 3. The absorption spectra in both 0.1 N HCl/NaOH were scanned over the range of 210–410 nm, and absorbance values were recorded. The doxycycline hyclate concentrations were calculated from the regression equations derived from the standard calibration curves.

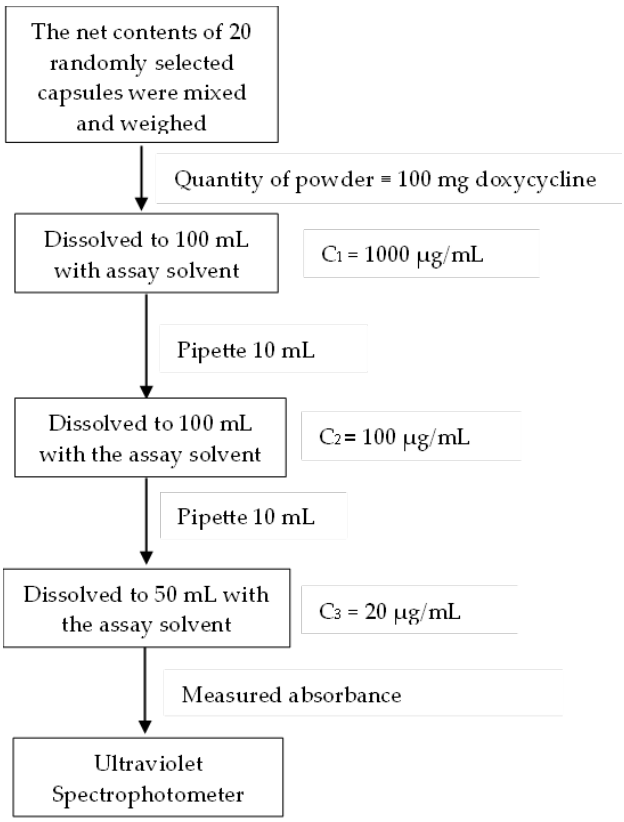


Fig. 3: Summary of the study design for preparation of the doxycycline hyclate test solutions

Statistical analysis

The recovery results are shown as means (% relative standard deviation of the mean) of triplicate measurements, and precision as the mean (% RSD). For the potency test of samples, doxycycline hyclate capsules contain between 90.0% and 120.0% of the labelled amount of doxycycline, which corresponds to a range (the difference between the highest and lowest values) of 30% (USP, 2022). Three quality categories were established based on the ratio of measured to expected doxycycline content in the sample. A ratio of 90 - 120% indicates good quality per USP standards. A ratio of 80 - 90% or 120 - 130% indicates low quality, while a ratio below 80% or above 130% suggests substandard or falsified products (Antignac *et al*., 2017). All statistical analyses were conducted using Excel 2021 and SPSS version 27.

Results and discussion

Descriptive characteristics of the analysed samples

A total of six brands were collected as samples from drug retail outlets located in Ilorin, a metropolitan area in North Central Nigeria. All the brands have batch numbers and are registered with the National Agency for Food and Drug Administration and Control (NAFDAC), with the majority (60%) of them manufactured in China. The shelf life of all the samples is approximately three years.

Identification tests

This test revealed that all samples contained doxycycline as the active ingredient per the label. The individual ultraviolet spectra of all test samples for all six brands compared favourably with the spectrum of the doxycycline hyclate analytical reference standard and exhibited maximum absorption at detection wavelength (λ_{max}) of 268 and 265 nm for 0.1N HCl and 0.1N NaOH, respectively (Fig. 1A). Additionally, a comparison of the computed molar absorptivity values of the test samples with those of the reference standards at the two detection wavelengths indicated that the percentage differences were less than 1.5%, which

Table 1: Comparison of the calculated absorptivity of the test samples with the reference standard.

Doxycycline	ε_{268} (Δ%)	ε_{265} (Δ%)
Standard	20,124	16,507
DOX-1	19,858 (-1.33)	16,300 (-1.26)
DOX-2	19,887 (-1.18)	16,318 (-1.15)
DOX-3	19,856 (-1.34)	16,295 (-1.29)
DOX-4	19,908 (-1.08)	16,338 (-1.03)
DOX-5	19,927 (-0.98)	16,352 (-0.94)
DOX-6	19,879 (-1.22)	16,316 (-1.16)

Abbreviations: ε: -molar absorptivity; Δ: - Difference

Table 2: Weight variation test

Brand	Average weight (g)	RSD (%)	Deviation (Range) %	Remarks
DOX-1	0.3023	2.08	7 (97 - 104)	Passed
DOX-2	0.2237	3.12	14 (92 - 106)	Passed
DOX-3	0.2396	7.69	25 (87 - 112)	Failed
DOX-4	0.2170	6.04	28 (83 - 111)	Failed
DOX-5	0.2247	3.46	13 (94 - 107)	Passed
DOX-6	0.3069	3.68	15 (93 - 108)	Passed

Abbreviations: RSD: relative standard deviation; n = 20; * (deviation should not exceed ± 25%)

is well within the USP specification of a ±2 % difference limit (Table 1).

Weight variation test

Two of the brands, DOX-3 and DOX-4, failed the test as both exhibited deviations of 25% and 28%, respectively. This result was corroborated by the relatively higher RSD values (7.69% and 6.04%, respectively) for the average weight determinations of the two brands compared to the other brands (Table 2).

Linearity and range

Calibration curves for the doxycycline hyclate analytical reference standard solutions were created by plotting absorbance against concentration at the two detection wavelengths. Linearity was observed within a concentration range of 5 - 30 µg/mL. The regression equations yielded coefficients of determination (R^2) of 0.9999 and 0.9998 at 268 and 265 nm, respectively, indicating excellent linearity. The RSD of absorbance in triplicate analyses was less than 2.0% (Table A1).

The concentrations derived from the calibration plots showed exceptional recovery, with a range of 2.43% at 268 nm and 1.93% at 265 nm, well within the 4% limit. The standard calibration curves for the reference doxycycline hyclate at both detection wavelengths are displayed in Fig. 4.

Solution stability

Results of the test to determine whether doxycycline hyclate remains chemically stable in 0.1N NaOH compared to 0.1N HCl confirmed better stability in 0.1N HCl (as indicated by the stable λmax of 268 nm and % R range of 100.00 – 100.73% versus the noticeable spectral shift λmax range of 265 – 270 nm as well as % R range of 100.00 - 118.60%) from 0 – 24 hours for the reference standard. Similar observation was noticed for the sample (i.e., a stable λmax of 268 nm and % R range of 100.00 - 101.28% versus the marked spectral shift λmax range of 265

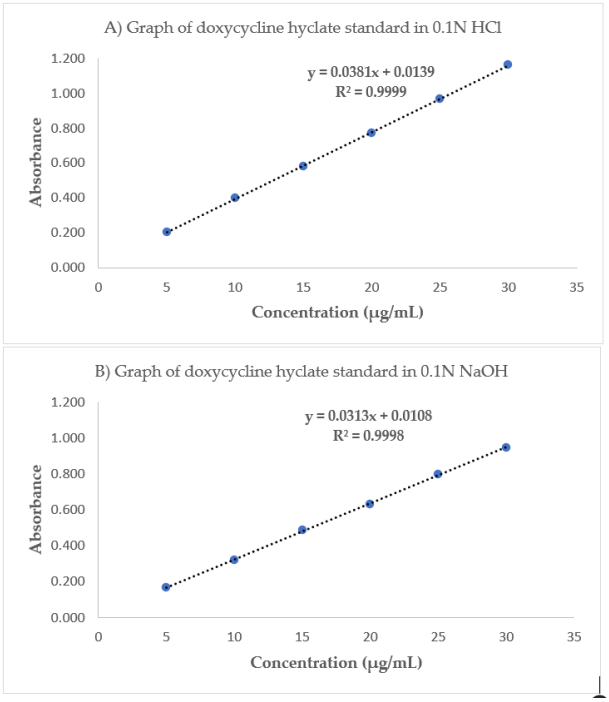


Fig. 4: Linearity graphs of doxycycline hyclate reference standard in A) 0.1 N HCl and B) NaOH at 268 and 265 nm, respectively.

– 270 nm as well as % R range of 100.00 - 117.92%). Corresponding changes were also observed in the concentrations of the reference (20.55 – 24.44 µg/mL, approximately 18.9%) and the sample (21.40 – 25.30 µg/mL, about 18.2%) in the alkaline solvent, versus no change in their concentrations in the acidic medium (19.87 – 20.02 µg/mL and 20.13 – 20.40 µg/mL, for the reference and sample, respectively), as shown in Table A2.

Precision [RSD (%)]

Precision measures the level of agreement among individual test results when the method is applied repeatedly to triplicate analyses of a homogeneous sample. Variability in the values of repeatability and ruggedness appeared to be relatively lower using 0.1 N HCl at 268 nm (range: 0.08 and 0.73) compared to 0.1 N NaOH at 265 nm (range: 0.50 and 1.51), as summarised in Table A3A.

Comparison of the robustness of the method as a result of the slight variations (±1) in the detection wavelengths demonstrated the reverse, i.e., a relatively higher precision with less variability using 0.1 N NaOH at 265 nm compared to 0.1 N HCl at 268 nm (average RSD of 0.81 versus 1.51%; and RSD range: 0.56 versus 0.74%, respectively) as presented in Table A3.

Accuracy [Recovery (%)]

The accuracy is expressed as the percentage of the true value. The obtained average recovery values at the detection wavelengths are well within a ± 2% limit, indicating good accuracy of the method. Comparison between the two methods indicated similarity in the accuracy exhibited by both (i.e., average recovery of 99.67%) although comparison of the precision and variability around it indicated higher RSD with less variability for the 0.1N NaOH method at 265 nm compared to 0.1N HCl method (i.e., an average RSD of 0.64 versus 1.90%; and RSD range of 0.51 versus 1.11%, respectively) as shown in Table 3.

Table 3: Recovery of the added amount in the samples

λmax (nm)	Before addition Concentration (µg/mL) Predicted	Observed	RSD (%)	After addition Concentration (µg/mL) Calculated	Observed	RSD (%)	Recovery (%)
268	16	17.57	3.11	17.97	17.86	2.52	99.38
	20	21.80	0.90	22.20	22.17	1.41	99.89
	24	26.42	1.94	26.82	26.75	1.77	99.75
265	16	18.63	0.45	19.03	18.94	0.42	99.52
	20	23.42	0.14	23.82	23.77	0.56	99.75
	24	27.79	0.67	28.19	28.12	0.93	99.75

Abbreviations: RSD: relative standard deviation

Table 4: Comparison of the optical characteristics and validation parameters

Parameter	Method	
Assay solvent	0.1 N HCl	0.1 N NaOH
Detection wavelength [(λ_{max}) nm]	268	265
Linearity range (µg/mL)	5 - 30	5 - 30
Regression equation	*y = 0.0381x + 0.0139*	*y = 0.0313x + 0.0108*
Coefficient of determination (R^2)	0.9999	0.9998
Limit of detection (µg/mL)	0.3219	0.3200
Limit of quantification (µg/mL)	0.9755	0.9698
Specific absorbance (dL/g/cm)	392	322
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	20,124	16,507
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0255	0.0311
Accuracy [Recovery (RSD) %]	99.67 (1.90)	99.67 (0.64)
Precision [RSD (range) %]		
Robustness	1.52 (0.74)	0.81 (0.56)
Repeatability	1.16 (0.08)	1.35 (0.50)
Ruggedness	1.17 (0.73)	0.90 (0.72)

Abbreviations: RSD:- relative standard deviation; λ_{max}:- wavelength of maximum absorption

Potency evaluation of the doxycycline capsule samples

The amounts of doxycycline hyclate salt in the formulations were converted to the base form using a factor of 0.8664 [i.e., the ratio of the molecular mass of doxycycline base (444.43 g/mol) to that of doxycycline hyclate (512.94 g/mol)]. The percentage contents of the samples were calculated and ranged from 80.56% to 99.33%. Two samples (DOX-4 and DOX-5), which represent about 33% of the analyzed brands, failed the test according to USP acceptance criteria due to insufficient doxycycline content in the capsules (Table 5).

Discussion

The present study developed and validated an assay method for determining the doxycycline hyclate content in capsule dosage forms using 0.1 N NaOH as the solvent and compared it with the validated method using 0.1 N HCl. The technique was also applied to assess the potency of marketed doxycycline capsule brands. The results showed similarity in the limit of detection, limit of quantification, precision, and accuracy between the two methods. However, the 0.1 N NaOH method appeared slightly less sensitive than the 0.1 N HCl method, as evidenced by Sandell's sensitivity (0.0311 $\mu g.cm^{-2}$ vs. 0.0255 $\mu g.cm^{-2}$), specific absorbance (322 $dL.g^{-1}.cm^{-1}$ vs. 392 $dL.g^{-1}.cm^{-1}$), and molar absorptivity (16,507 $L.mol^{-1}.cm^{-1}$ vs. 20,124 $L.mol^{-1}.cm^{-1}$), indicating higher absorption at 268 nm with 0.1 N HCl compared to 265 nm with 0.1 N NaOH.

A review of existing literature on simple, rapid, and cost-effective ultraviolet spectrophotometric methods for determining doxycycline hyclate in pharmaceutical dosage forms reveals several approaches, especially in aqueous and non-aqueous solvents. These studies show conflicting results and lack data on key optical and validation parameters such as LOD, LOQ, Sandell's sensitivity, and molar absorptivity. Rakesh *et al*., Sreeram *et al*., and Patil *et al*. developed aqueous methods using distilled water as the assay solvent, but reported different λ_{max} values of 271 nm, 276 nm, and 260 nm, respectively; only Patil *et al*. reported LOD and LOQ. Similarly, Ramesh *et al*., Kogawa and Salgado, and Bhardwaj *et al*. used 0.1 M/0.01 M HCl but reported conflicting detection λ_{max} values of 240 nm, 268 nm, and 274 nm. Conflicting detection wavelengths of maximum absorption were also observed in non-aqueous

Table 5: Assay of selected brands of doxycycline 100 mg capsules

Brand	Amount (mg) [RSD (%)] 268 nm	Amount (mg) [RSD (%)] 265 nm	Difference (95% CI)	P-value	Average amount of salt (mg)	Base equivalent (mg)	Average content (%)	Remarks*
DOX-1	113.05 [1.63]	113.08 [0.95]	-0.03 (-7.38, 7.32)	0.988	113.07	97.96	97.96	passed
DOX-2	103.64 [1.12]	105.41 [0.91]	-1.77 (-5.31, 1.77)	0.165	104.53	90.56	90.56	passed
DOX-3	113.88 [1.24]	115.42 [1.91]	-1.54 (-10.61, 7.53)	0.541	114.65	99.33	99.33	passed
DOX-4	97.56 [0.90]	97.90 [0.52]	-0.34 (-1.80, 1.12)	0.423	97.73	84.67	84.67	failed
DOX-5	92.80 [0.50]	93.16 [0.34]	-0.36 (-1.59, 0.86)	0.331	92.98	80.56	80.56	failed
DOX-6	106.18 [1.75]	106.32 [1.55]	-1.30 (-5.57, 5.31)	0.928	106.25	92.06	92.06	passed

Abbreviations: SD: standard deviation; RSD: relative standard deviation; * USP Acceptance Criteria: 90.0 - 120.0%

methods. The detection wavelength of 268 nm for the validated 0.1 N HCl method in the present study supports the previously reported λ_{max} by Kogawa and Salgado. The detection λ_{max} of 265 nm for the developed method using 0.1 N NaOH is close to the 268 nm observed with 0.1 N HCl, as expected.

Doxycycline hyclate solutions prepared in 0.1 N HCl remained stable for at least 24 hours, maintaining a consistent λmax (268 nm), an acceptable percentage of remaining drug, and low %RSD (<1.3%). Conversely, solutions made in 0.1 N NaOH showed wavelength shifts (265–270 nm), increased absorbance, significant deviations in % remaining, and greater variability, indicating alkaline degradation and poor stability. The absorbance values of the analyte solution in 0.1 N NaOH were only within the acceptable limit of ≤ 2% for the first 1 hour after the sample preparation, compared to those in 0.1 N HCl, which appeared chemically stable for up to 24 hours. Colour change in the alkaline solution from light yellow to orange-brown was also observed after approximately 2 hours, and the colour darkened over time. These results corroborate a previous report of Sahoo *et al*. (2023) and indicate alkaline degradation of doxycycline, thereby making 0.1 N NaOH unsuitable as a stability-preserving assay solvent for prolonged analysis.

The observed spectral changes in 0.1N NaOH solutions of the analytes or the reference standard indicate a chemical transformation of doxycycline rather than a true increase in drug concentration. Under alkaline conditions, doxycycline undergoes rapid deprotonation of phenolic and enolic functional groups within the tetracyclic β-diketone system (Fig. 1), which alters the electronic distribution of the chromophore and produces shifts in UV absorption maxima (Bolobajev *et al*. 2016; Valcavi *et al*. 1981). In addition to ionization effects, alkaline media promote base-catalysed epimerization at the C-4 dimethylamino centre and intramolecular rearrangement to iso-doxycycline, as well as oxidative and dehydration reactions leading to anhydro-derivatives (Foye *et al*., 2008; Blaschke *et al*., 1979). The developed UV spectrophotometric method using 0.1N NaOH as the analytical solvent demonstrated satisfactory analytical performance for the determination of doxycycline hyclate. Solution stability studies showed that both the reference standard and sample solutions remained stable for at least 1 h after preparation. However, prolonged standing in alkaline medium resulted in progressive spectral shifts and significant deviations in remaining concentration due to base-catalysed degradation of doxycycline. Consequently, the proposed method is suitable for the assay of doxycycline hyclate capsules, provided that measurements are performed within 1 h of solution preparation, and freshly prepared solutions are used for all measurements. The present study was unable to establish whether storage of analytical solutions at a lower temperature than room temperature and protection from light through the use of an amber volumetric flask or foil wrapping can

improve the chemical stability and analysis time beyond 1 h.

Applying the developed method to analyse doxycycline capsules shows that it is simple, fast, precise, and accurate. Comparing this new method with the previously validated method using 0.1N HCl revealed no significant difference in the doxycycline hyclate content of the capsules, as confirmed by the 95% confidence intervals for the differences in the measured amounts of the active pharmaceutical ingredient, which include zero (Table 5). Quality assessment of six commercially available brands of doxycycline capsules indicated that all brands passed the identity test, but only about 67% passed the weight variation and potency tests. One brand (i.e., DOX-4) failed both tests, while DOX-3 and DOX-5 failed only the weight variation and potency tests, respectively. The DOX-4 brand showed the highest (28%) deviation in weight variation among all the studied brands, and it also failed the potency test with 84.67% doxycycline content, below the labelled claim. The DOX-5 brand, which passed the weight variation test, had the lowest (80.56%) percentage of the labelled claim among all the brands, illustrating that passing the weight variation test is necessary but not sufficient for passing the potency test. Both weight variation and potency tests are key quality control parameters for pharmaceutical dosage forms, as they evaluate different aspects of quality and are related but distinct tests.

Out-of-specification or substandard medicines, as defined by WHO, are authorized medicines that failed to meet their quality standards and/or specifications. They differ from falsified medicinal products, which are products that are intentionally mislabelled to misrepresent their composition or source. They also differ from unregistered or unlicensed medicines or products, which are substances that lack approval and/or necessary evaluation by the regional or national regulatory authority for the market where they are distributed, sold, or used (WHO, 2024).

Conclusion

The developed method proved to be simple, rapid, precise, accurate, and cost-effective, and it compared favourably with the previously reported method. It was successfully used to assay the doxycycline content of capsule dosage forms. However, both the regulatory authority and the manufacturer need to continuously strengthen regulatory oversight and quality control to reduce the prevalence of substandard and falsified products in the country.

Authors contributions

STA conceptualized, and BOL conducted the experiments. STA analysed the data. AS and OIE contributed to the supervision of the project. STA and ODB drafted the manuscript. AOS, KMS, and JOS revised the manuscript. All authors read and approved the final manuscript.

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Ethical approval statement

Not applicable.

Informed consent statement

Not applicable.

Conflict of interest

The authors declare that they have no competing interests. No funds, grants, or other support were received for the study.

Declaration of generative AI and AI-assisted technologies in the writing process

Grammarly was used to improve readability and language during the preparation of the manuscript. The authors reviewed and edited the content as needed after use and take full responsibility for the

content of the publication.

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Appendix A

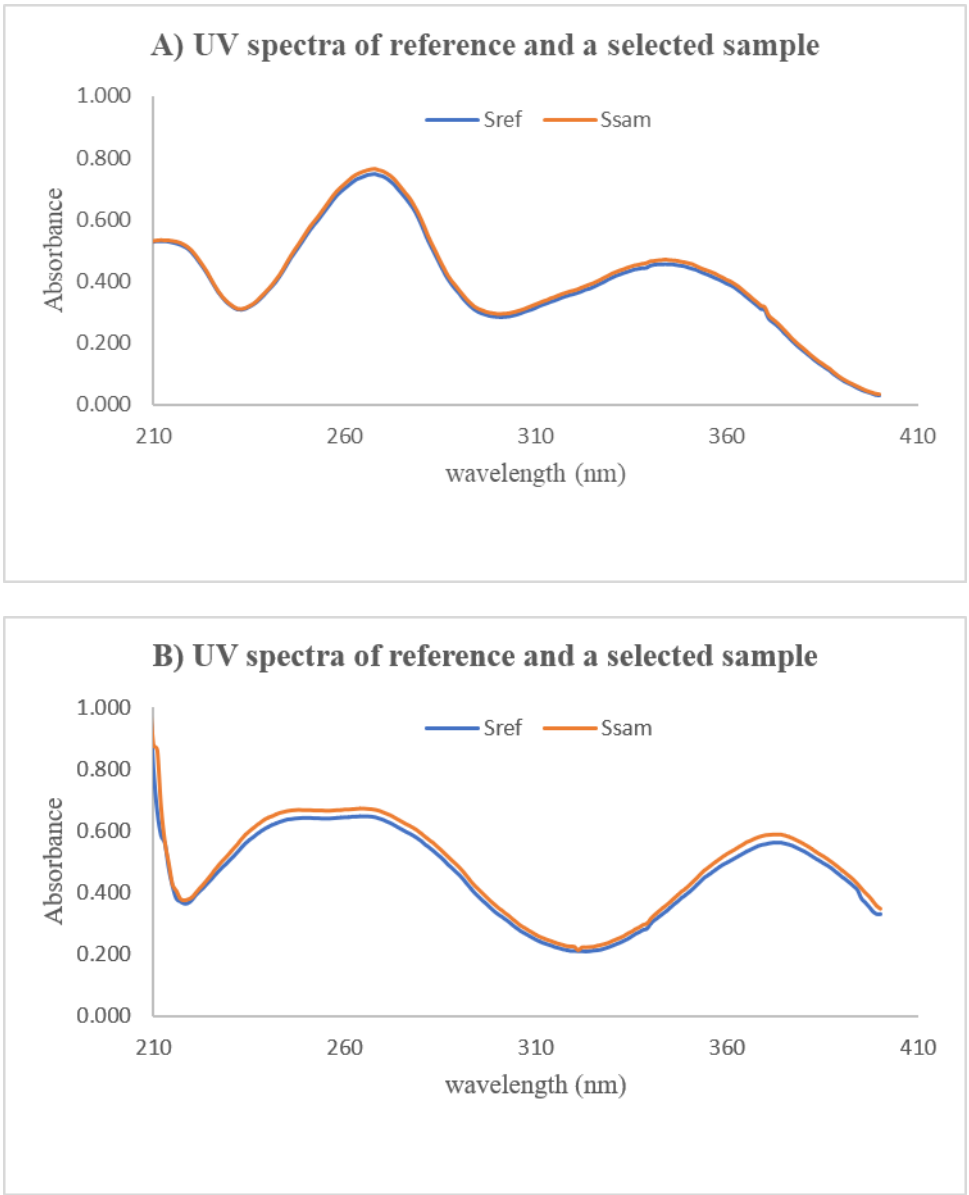

Fig. 1A: Comparisons of the UV absorption spectra of a selected sample (Ssam) with the spectrum of the analytical reference standard (Sref) in A) 0.1N HCl and B) 0.1N NaOH as assay solvents.

Table A1: Calibration curve plots for wavelength of maximum absorbance (λ_{max}) at different aliquots of the two assay solvents

S/No.	Conc (µg/mL)	Absorbance (%RSD)	Observed conc. (µg/mL)	Recovery (%)	Absorbance (%RSD)	Observed conc. (µg/mL)	Recovery (%)
		0.1N HCl (λ_{max} of 268 nm)			0.1N NaOH (λ_{max} of 265 nm)		
1	5	0.205 (1.49)	5.02	100.49	0.168 (1.19)	5.02	100.45
2	10	0.400 (1.04)	10.13	101.25	0.321 (0.95)	9.90	99.00
3	15	0.579 (1.34)	14.82	98.82	0.485 (1.26)	15.14	100.93
4	20	0.773 (1.09)	19.92	99.58	0.632 (0.78)	19.86	99.29
5	25	0.966 (0.45)	24.99	99.96	0.799 (1.01)	25.19	100.77
6	30	1.160 (0.47)	30.07	100.24	0.947 (0.48)	29.91	99.70

Abbreviations: Conc: concentration; RSD: relative standard deviation

Table A2: Comparison of the solution stability studies of the reference standard and test sample in both assay solvents

Time points (h)	λmax (nm) in	Reference standard				Sample			
	0.1N HCl	A	% RSD	% R	C (µg/mL)	A	% RSD	% R	C (µg/mL)
0	268	0.771	0.47	100.00	19.87	0.781	0.97	100.00	20.13
1	268	0.771	0.46	100.04	19.88	0.774	1.37	99.06	19.94
2	268	0.774	0.71	100.35	19.94	0.786	0.60	100.68	20.27
4	268	0.761	0.47	98.70	19.61	0.774	0.22	99.10	19.95
6	268	0.770	1.30	99.83	19.84	0.787	0.66	100.77	20.29
24	268	0.777	0.39	100.73	20.02	0.791	0.63	101.28a	20.40
	0.1N NaOH	A	% RSD	% R	C (µg/mL)	A	% RSD	% R	C (µg/mL)
0	265	0.654	0.85	100.00	20.55	0.681	1.19	100.00	21.40
1	265	0.649	0.53	99.24	20.39	0.675	0.99	99.12	21.21
2	267	0.623	1.40	95.21	19.55	0.647	1.79	95.00	20.32
4	270	0.632	1.28	96.59	19.84	0.660	1.14	96.96	20.74
6	269	0.682	0.98	104.33	21.45	0.703	1.36	103.28	22.12
24	270	0.776	1.35	118.60	24.44	0.803	2.74	117.92	25.30

Abbreviations: A: average absorbance; RSD: relative standard deviation; % R: % remaining or change in absorbance; C: concentration; λ_{max}: wavelength of maximum absorption

Table A3: Methods' Precision

Parameter	Time (h)	Assay solvent	Concentration (μg/mL)	RSD (%)	Recovery (%)
Repeatability	0	HCl	19.92	1.21	99.62
	6		19.85	1.14	99.27
	12		19.85	1.13	99.27
	0	NaOH	20.13	1.38	100.67
	0.5		19.97	1.58	99.87
	1		19.74	1.08	98.70
Ruggedness (analyst & spectrophotometer)	IA	HCl	19.77	0.74	98.83
	IIA		19.73	1.41	98.66
	IA	NaOH	20.22	0.80	101.10
	IIA		20.16	0.48	100.78
	IS	HCl	19.95	1.05	99.75
	IIS		19.66	1.47	98.31
	IS	NaOH	20.05	1.12	100.24
	IIS		20.12	1.20	100.62
Robustness λ_{max} (± 1)	267	HCl	19.85	1.70	99.27
	268		19.95	1.05	99.75
	269		19.71	1.79	98.57
	264	NaOH	20.03	0.56	100.14
	265		20.05	1.12	100.24
	266		19.78	0.74	98.91

Abbreviations: SD: standard deviation; RSD: relative standard deviation; λ_{max}: wavelength of maximum absorbance