

Quantification of Doxycycline Hyclate in Different Pharmaceutical Samples by UV Assay

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ABSTRACT

Objective: Doxycycline hyclate is a broad spectrum antibiotic with activity against a wide range of gram-positive and gram-negative bacteria and it is widely used as a pharmacological agent. A simple, selective, linear, precise and accurate ultraviolet detection (UV) method has been developed and applied for the determination of doxycycline hyclate in different pharmaceutical samples. **Methods:** Acid-base analysis and titrimetric method were utilized to determine the value of pH and moisture content of purchased pharmaceutical samples. A mixture of methanol and hydrochloric acid (0.01N Methanolic HCl) was used to determine the biochemical properties of doxycycline hyclate. UV detector set at 349 nm was used to monitor the effluent. The purified water was used as solvent. **Results:** In 1% aqueous solution of doxycycline, three samples (4th, 5th and 7th) showed lower pH values of 1.97, 1.98, and 1.99 respectively. Furthermore, the same samples indicated the additional moisture contents of 2.81%, 2.85% and 2.83% respectively while considering the acceptance level (1.4% to 2.8%). The method proved to be linear ($R^2=0.993$), precise ($RSD=0.79\%$ for inter-day precision), accurate ($Recovery=100.59\%$) and selective regarding possible impurities and excipients of the samples. The doxycycline content obtained in the sample analysis was within the range of 84.05% to 85.80%. **Conclusion:** The optimized and validated method may be successfully employed to perform routine quality control analyses. Investigation of the pH, moisture content and potency of doxycycline hyclate in different samples give a general view of local pharmacies trade and ensure that the method applied here was validated for this kind of analysis.

Keywords: Doxycycline hyclate; Methanolic HCl; Moisture content; Ultraviolet detection; Method validation.

INTRODUCTION

Doxycycline is a broad-spectrum antibiotic of the tetracycline class that is used in the treatment of infections caused by bacteria and protozoa¹. Like other agents of this class it kills bacteria and protozoa by inhibiting protein synthesis². Researchers show that tetracycline antibiotics are most extensively used antimicrobials worldwide³. Tetracyclines are one of the cheapest antibiotics, hence extensively used in countries with limited health care budgets⁴. In gram-negative bacteria, transportation of the doxycycline into the cell occurs dependent active transport system. The later system is either by passive diffusion or through an energy also believed to exist in gram-positive bacteria. Doxycycline is more lipophilic than the other tetracyclines, which allows it to pass easily through the lipid bilayer of bacteria. Doxycycline penetrates the bacterial cell and interferes with the protein biosynthesis, stopping the process of bacterial reproduction. It is the drug of choice in the treatment of sexually transmitted diseases⁵. It is preferred to other tetracyclines in the treatment of specific infections because of its fairly reliable absorption and its long half-life, which permits less frequent dosage.

Doxycycline presents itself in three forms: hyclate, monohydrate and hydrochloride. The molecular formulae of doxycycline hyclate is $C_{22}H_{24}N_2O_8 \cdot HCl \cdot \frac{1}{2}C_2H_6O \cdot \frac{1}{2}H_2O$. From doxycycline hyclate, it is possible to obtain other forms. The hyclate dissolved in water and neutralized with sodium hydroxide, then becomes doxycycline monohydrate which undergoes doxycycline hydrochloride with the addition of hydrochloric acid. Doxycycline hyclate is much more soluble than doxycycline monohydrate, which is one of the main reasons for its more frequent uses as antibacterial drug. Various methods for quantification of doxycycline *in vitro* and *in vivo* have been reported. These include *in vitro* experimental model⁶, fluorimetry⁷, TLC-fluorescence scanning densitometry⁸ and HPLC⁹⁻¹⁹ for the quantification of doxycycline in biological materials. HPLC was also applied for the determination of doxycycline in pharmaceutical formulations²⁰. Various chromatographic methods have also been reported for the determination of doxycycline in human tissues²¹ and foods^{22,23}. Sequential injection chromatography (SIC) for pharmaceutical preparations²⁴ and derivative spectrophotometry for the determination of doxycycline in pharmaceuticals, urine and honey have

additionally been developed²⁵. Many researchers reported that there are a few methods available till date for the determination of doxycycline hyclate in pharmaceutical samples²⁶⁻²⁸. However, the procedure provided by British Pharmacopoeia is widely used for the determination of doxycycline hyclate in pharmaceutical samples till date²⁹. The aim of the present study was to establish a standard procedure to quantify doxycycline hyclate in pharmaceutical samples and determine the biochemical properties of doxycycline hyclate. In addition, investigation of the pH, moisture content, purities and potency of this active ingredient in different samples collected from local pharmacies in Bangladesh have been performed. Furthermore, analytical method validation was done to check whether this method could pass the performance characteristics such as specificity, linearity, repeatability, intermediate precision and accuracy. Due to the extensive market value and clinical applications of this drug, product quality, shelf life, other active ingredients and excipients should be analyzed and justified for different brands.

MATERIALS AND METHODS

Reagents and Materials

Chemicals such as hydrochloric acid (HCl), Karl Fischer reagent and methanol were purchased from Merck (Darmstadt, Germany). Doxycycline hyclate reference standard (PromoChem, Teddington, United Kingdom) and HPLC grade solvent were used here for all analytical purposes. Ten doxycycline hyclate capsules in concentration with 100 mg/ml were collected from local pharmacies.

Instrumentation and Methodologies

Balance Sartorius LE225D, pH meter BT-600, Shimadzu UV-1650 visible spectrophotometer, KF Titrand were utilized. UV spectrophotometric method was applied here for the determination of doxycycline hyclate. Several performance characteristics were also analyzed such as pH, water content and impurities. To perform this analysis, following methods were applied:

pH analysis

Doxycycline hyclate is considered as soluble³⁷ or freely soluble³⁸ in water. Aqueous solution of doxycycline hyclate, containing 1% doxycycline, has a pH of 2-3^{39,40}. For pH analysis, 1.0g test sample transferred in a 100ml volumetric flask and dissolved⁴¹. Then volume with water up to the mark and shaken well. Rinsed and clean the electrode with deionized water. Wiped the electrode with tissue paper and immersed it into the sample solution. When the reading stabilized, pH value recorded.

Water content determination

Titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions³⁴. The water content should be not less than 1.4% and not more than 2.80%^{34,41}. For this analysis, weighed about 35 to 40 mg of the test sample and transferred it into a Karl Fischer's vassal. Afterwards KF Titrand automatically showed the results on the print out in percentage.

Light Absorbing Impurities Detection

Dissolved 0.25g in a mixture of 1 volume of 1M HCl and 99 volumes of methanol and diluted to 25.0ml with the same mixture of solvents⁴³. The absorbance of the filtrate determined at 490 nm is not greater than 0.07 (anhydrous and ethanol-free substance) and carried out the measurement within 1 hour of preparing the solution⁴³.

UV Assay

Standard preparation A (System Suitability) and B (Assay Calculation)

According to the Code of Federal Regulations (Title 21, Food and Drugs), 50mg of doxycycline hyclate was taken in 5ml volumetric flask and bring to the volume with 0.05N methanolic hydrochloric acid⁴¹. Here, 50mg of doxycycline hyclate was transferred in a 50ml volumetric flask dissolved with 0.01N methanolic HCl and volume up to the mark and sonicated. Then 1ml of this solution added to the 100ml volumetric flask, volume up to the mark with 0.01N methanolic HCl.

Sample preparation

Same as *Standard preparation*.

Procedure

Concomitantly measured the absorbance of the standard and sample solution in a 1cm quartz cell at the wavelength of 349 nanometers (nm)⁴¹ with a suitable spectrophotometer using 0.01N methanolic HCl as blank.

Standard Concordance

$$\frac{\text{Average area of SS standard} \times \text{Weight of C standard} \times 100}{\text{Average area of C standard} \times \text{Weight of SS standard}} \\ \% \text{ of Concordance}$$

Assay

$$\frac{\text{Sample abs.} \times \text{Standard weight in mg} \times \text{potency of standard}(\%)}{\text{Standard abs.} \times \text{sample weight in mg}}$$

$$\frac{\text{Sample abs.} \times \text{Standard weight in mg} \times \text{potency of standard}(\%) \times 100}{\text{Standard abs.} \times \text{sample weight mg} \times (100 - \text{LOD})}$$

% of doxycycline hyclate on dried basis.

Here, SS standard = System suitability standard and C standard = Calculation standard

Method Validation

Specificity

The specificity of the method can be determined with the addition of impurities and degradation products, obtained experimentally or by inducing their formation⁴².

Standard preparation

Weighed and transferred accurately about 50 mg of doxycycline hyclate working standard into a 100 ml volumetric flask.

Placebo preparation

Placebo is a substance having no pharmacological effect but administered as a control in testing the efficacy of a biologically active preparation. Weighed and transferred accurately about 108.81 mg of placebo into a 100 ml volumetric flask. For both standard and placebo preparation, 60 ml of diluent added and sonicated for 15 minutes to dissolve. Cooled it and diluted it with same solvent. Diluted 2 ml of this solution to 50 ml with same solvent.

Linearity

Linearity was determined by linear regression analysis by the method of least squares. Health Canada (HC) states that

the coefficient of determination for active ingredients should be ≥ 0.997 , for impurities 0.98 and for biologics 0.95⁴⁵.

Preparation

Weighed and transferred accurately about 40 mg, 45 mg, 50 mg, 55 and 60 mg of doxycycline hyclate standard into five 100 ml volumetric flask. Added 60 ml of diluent into each 100 ml volumetric flask and sonicated to dissolve. Cooled it and dilute it with same solvent. Diluted 2 ml of this solution to 50 ml with same solvent. Plotted a graph of concentration versus absorbance (Abs).

Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. HC states that the RSD should be 1% for drug substances and 2% for drug products⁴⁶.

Preparation of standard

Weighed and transferred accurately about 50 mg of doxycycline hyclate standard into a 100 ml volumetric flask.

Preparation of sample

Weighed accurately about 50 mg of doxycycline hyclate standard and 108.81 mg placebo and transferred into a 100 ml volumetric flask. For both standard and sample preparation, same procedures were performed as mentioned above.

Accuracy

Samples were prepared at three concentrations levels over the range of 80 to 120% of the target concentration. Three individually prepared replicates at each concentration were analyzed. For the U. S. pharmaceutical industry, $100 \pm 2\%$ is typical for an assay of an active ingredient in a drug product over the range of 80 to 120% of the target concentration⁴⁴.

Preparation of standard

Weighed and transferred accurately about 50 mg of doxycycline hyclate working standard into a 100 ml volumetric flask.

Preparation of sample for 80 % recovery

Weighed accurately about 40 mg of doxycycline hyclate standard and 87.04 mg placebo and transferred into a 100 ml volumetric flask.

Preparation of sample for 100% recovery

Weighed accurately about 50 mg of doxycycline hyclate standard and 108.81mg placebo and transferred into a 100 ml volumetric flask.

Preparation of sample for 120% recovery

Weighed accurately about 60 mg of doxycycline hyclate standard and 130.57 mg placebo and transferred into a 100 ml volumetric flask. For both standard and sample preparation, same procedures were performed as other parameters analysis.

Intermediate Precision

Intermediate precision of an analytical procedure expresses intra-laboratory variations of the repeatability

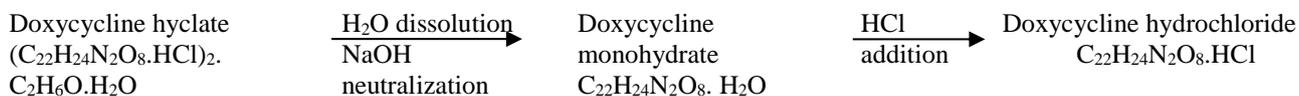


Figure 2: Conversion of doxycycline hyclate into doxycycline monohydrate and doxycycline hydrochloride.

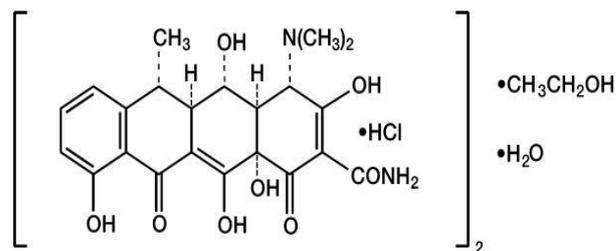


Figure 1: Structure of doxycycline hyclate.

test performed by a different analyst, on a different day. The assay results obtained by two operators on different days should have a statistical RSD $\leq 2\%$ ^{44,45}.

Preparation of standard

Weighed and transferred accurately about 50 mg of doxycycline hyclate standard into a 100 ml volumetric flask.

Preparation of sample

Weighed accurately about 50 mg of doxycycline hyclate standard and 108.81 mg placebo and transferred into a 100 ml volumetric flask. For both standard and sample preparation, same procedures were performed as it was done for specificity, linearity, repeatability and accuracy analysis.

RESULTS

Method optimization

According to the United States Pharmacopoeia (USP), Doxycycline hyclate has potency not less than 80% and not more than 92%³³ whereas it should be approximately 84.7% declared in British Pharmacopoeia (BP)³⁴. For UV assay of sample-1, Weight of SS standard = 0.05084g, Weight of C standard = 0.05085g, Sample weight = 0.05093g, Average area of SS standard = 0.668, Average area of C standard = 0.666, Sample absorbance = 0.664. By putting these values into the calculating formula, assay result for sample-1 was 85.80 % on dried basis and repeated the formula for rest of the samples.

Assay validation

Specificity

The specificity of the method can be determined with the addition of impurities and degradation products, obtained experimentally or by inducing their formation⁴². Here, the specificity of the chromatographic method was determined by the screening of a placebo solution and the assay solution. The placebo solution was prepared in the same manner as the investigated solution but without doxycycline hyclate. It would be investigated by injecting of the extracted placebo to demonstrate the absence of interference with the elution of analyte.

Linearity

The linearity of an assay method is its ability to elicit test results, which are directly proportional to the

Table 1: Summary report of pH, water content, impurities and UV assay.

| Sample | Water by KF 1.40%- 2.80% | pH at 1% aqueous 2.0-3.0 | Light absorbing Impurities ≥ 0.07 | Assay at 349nm 80.0%- 92.0% |
|--------|--------------------------------|--------------------------------|--|--------------------------------------|
| 1 | 2.41% | 2.15 | 0.018 | 85.80% |
| 2 | 2.16% | 2.32 | 0.020 | 85.24% |
| 3 | 2.08% | 2.39 | 0.019 | 85.53% |
| 4 | 2.81% | 1.97 | 0.022 | 84.31% |
| 5 | 2.85% | 1.98 | 0.017 | 84.03% |
| 6 | 2.11% | 2.31 | 0.022 | 85.76% |
| 7 | 2.83% | 1.99 | 0.019 | 84.52% |
| 8 | 2.07% | 2.02 | 0.016 | 84.71% |
| 9 | 2.19% | 2.29 | 0.017 | 84.95% |
| 10 | 2.05% | 2.25 | 0.020 | 84.05% |

Table 2: Placebo interference/Specificity study.

| S. No. | Weight of Placebo (mg) | Absorbance | Weight of Standard (mg) | Absorbance of Standard | % Placebo Interference |
|---------|------------------------|------------|-------------------------|------------------------|------------------------|
| 1. | 108.72 | 0.009 | 60.55 | 0.695 | 0.60 |
| 2. | 108.75 | 0.005 | | | 0.34 |
| 3. | 108.90 | 0.002 | | | 0.13 |
| Average | | | | | 0.36 |

Table 3: Linearity study.

| S. No. | Concentration % | Sample weight (mg) | Concentration (ppm) | Absorbance |
|--------|-----------------|--------------------|---------------------|------------|
| 1. | 80% | 47.94 | 16.06 | 0.566 |
| 2. | 90% | 54.04 | 18.10 | 0.635 |
| 3. | 100% | 60.55 | 20.28 | 0.697 |
| 4. | 110% | 65.99 | 22.10 | 0.784 |
| 5. | 120% | 71.53 | 23.95 | 0.848 |

concentrations of drug actives in samples in a given range. Proof of linearity justifies the use of single-point calibrations. The linearity was checked on samples of standard doxycycline hydrochloride at five different concentrations (16.06-23.95 ppm). A regression curve was constructed: $y = 0.036x - 0.016$, with $R^2 = 0.993$; where x represents concentration in ppm, y represents the peak area, and R is the correlation coefficient.

Accuracy

The accuracy of the method was checked by determining recovery values. Series of solution were made containing 80, 100 and 120 % of doxycycline hydrochloride. Recoveries for different concentrations ranged from 99.58 to 101.93% for the determination of doxycycline in bulk drug and in tablets³⁶.

Repeatability

The % RSD due to doxycycline hydrochloride concentration for the six samples was found to be less than 2.0%. Six separated sample preparations were analysed according to

the method of analysis. The % of RSD due to doxycycline hydrochloride concentration for the assay meets the requirements. The obtained results are given in Table 5, together with the calculated values of their standard deviation, SD , and relative standard deviation, RSD .

Intermediate precision

In line with ICH guidelines^{30,31} precision is subdivided into short term (within-run precision or intrabatch) and intermediate precisions (betweenrun or interbatch) which measure precision with time, and may involve different analysts, equipment and reagents. Intrabatch precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV). For interbatch precision, intrabatch experiments are repeated on four different days by different analysts. Precision from the four-day experiments is compared with the intrabatch precision.

DISCUSSION

In this study, there was no residual effect that could be harmful to humans. In 2001, Zarghi *et al* developed a method for determination of doxycycline in human plasma using HPLC assay based on a mbondapak C18 column and a lambda (347 nm) UV detector⁹. Reagents like acetonitrile, potassium dihydrogen phosphate, 24% perchloric acid aqueous solution were utilized there. In 2011, Ramesh *et al* developed and validated three cost-effective spectrophotometric methods for the determination of doxycycline in bulk drug and in tablets where HCl, NaOH, H₂SO₄ medium and iron(III) chemicals were used³⁶. In 2012, Kogawa *et al* developed and validated an accurate, sensitive, precise and rapid gradient reversed-phase high-performance liquid chromatographic method for the determination of doxycycline hydrochloride in bulk drug and tablets⁴⁷. CN Luna column at 360 nm was utilized as stationary phase and water, 0.1% TFA-acetonitrile and 0.1% TFA were used as the mobile phase at a flow rate of 1.0 mL/min. In 2015, Kogawa *et al* developed and validated an eco-friendly method of infrared spectroscopy for quantification of doxycycline in raw material⁴⁷. The proposed spectrophotometric methods do not require any expensive equipment and specialized technicians when compared alongside HPLC and bioassay. Besides, other characteristics of these methods are the short time and less space required for performance and handling respectively than other methods. Although there is not enough evidence of pH determination of antibiotics and drug products, here it was performed because doxycycline hydrochloride causes ulcer in stomach due to acid reflux and heartburn. For this reason, pH level should be within the range of acceptance. Here, sample 4th, 5th and 7th had slightly higher acidic pH levels that would be a concern of health safety issues. Besides, stability of doxycycline hydrochloride in aqueous solutions depends on the pH value. On the other hand, Karl Fischer titration method in analytical chemistry to determine trace amounts of water in a sample³². In the environmental science, it is used to test the percent (%) of

Table 4: Accuracy study.

| Sample No. | Level | Sample Weight (mg) | A (mg) | B (mg) | B/A×100 (% Recovered) | Mean Value % | %RSD |
|------------|-------|--------------------|--------|--------|-----------------------|--------------|------|
| 1. | 80% | 166.24 | 80.66 | 80.82 | 100.20 | 101.22 | 0.91 |
| 2. | | 166.24 | 80.66 | 82.27 | 102.00 | | |
| 3. | | 166.24 | 80.66 | 81.84 | 101.46 | | |
| 1. | 100% | 165.93 | 98.92 | 100.36 | 101.46 | 101.11 | 0.59 |
| 2. | | 165.93 | 98.92 | 99.34 | 100.42 | | |
| 3. | | 165.93 | 98.92 | 100.36 | 101.46 | | |
| 1. | 120% | 168.30 | 119.82 | 119.47 | 99.71 | 99.43 | 0.26 |
| 2. | | 168.30 | 119.82 | 119.04 | 99.35 | | |
| 3. | | 168.30 | 119.82 | 118.89 | 99.22 | | |

A=Theoretical concentration, B=Concentration recovered, RSD=Relative standard deviation

Table 5: Repeatability study.

| Injection No. | Level | Sample Weight (mg) | Absorbance | Assay (%) | Mean Assay (%) | Standard Deviation | %RSD |
|---------------|-------|--------------------|------------|-----------|----------------|--------------------|------|
| 1. | 100% | 166.36 | 0.705 | 98.70 | 99.80 | 0.72 | 0.65 |
| 2. | | 166.83 | 0.715 | 100.10 | | | |
| 3. | | 166.76 | 0.712 | 99.68 | | | |
| 4. | | 167.06 | 0.718 | 100.52 | | | |
| 5. | | 166.65 | 0.711 | 99.54 | | | |
| 6. | | 165.92 | 0.716 | 100.24 | | | |

Table 6: Intermediate precision study.

| S. No. | Weight of sample (mg) | Absorbance | Assay % | Weight of sample (mg) | Absorbance | Assay % | |
|---|-----------------------|------------|---------|-----------------------------------|------------|---------|--------|
| 1. | 166.36 | 0.705 | 98.70 | 167.80 | 0.705 | 100.55 | |
| 2. | 166.83 | 0.715 | 100.10 | 168.40 | 0.711 | 101.40 | |
| 3. | 166.76 | 0.712 | 99.68 | 168.21 | 0.707 | 100.83 | |
| 4. | 167.06 | 0.718 | 100.52 | 167.50 | 0.700 | 99.83 | |
| 5. | 166.65 | 0.711 | 99.54 | 168.60 | 0.712 | 101.55 | |
| 6. | 165.92 | 0.716 | 100.24 | 167.30 | 0.704 | 100.41 | |
| Mean assay (n=6) | | | 99.80 | Mean assay (n=6) | | | 100.76 |
| Standard deviation (n=6) | | | 0.72 | Standard deviation (n=6) | | | 0.43 |
| Relative standard deviation (n=6) | | | 0.65 | Relative standard deviation (n=6) | | | 0.63 |
| Mean assay (Analyst 1 & Analyst 2) n=12 | | | | | | | 100.28 |
| Standard deviation n=12 | | | | | | | 0.72 |
| Relative standard deviation n=12 | | | | | | | 0.79 |

Table 7: Summary report of method validation.

| S. No. | Analytical performance parameter | Acceptance limit | Result |
|--------|-------------------------------------|-------------------------------|--------|
| 1. | Specificity/Placebo interference | NMT 2% | 0.36 |
| 2. | Accuracy | 80% | 101.22 |
| | | 100% | 101.11 |
| | | 120% | 99.43 |
| 3. | Repeatability | % RSD NMT 2% | 0.65 |
| 4. | Linearity (Correlation Coefficient) | R ² Value NLT 0.99 | 0.99 |
| 5. | Intermediate Precision | % RSD NMT 2% | 0.79 |

NMT=Not more than, NLT=Not less than

water content in soil and cosmetic industry to test the percent (%) of moisture content in cosmetics like soap, detergents, cream etc. After the analysis of ten different samples of doxycycline hyclate, sample no. 4th, 5th and 7th showed results those had some differences considering the specification limit. Due to the high moisture content value, these samples potency might be lost before the given

expiry date. A linear regression curve was constructed and the correlation coefficient (R²) and assessment value calculated. Here, a plot of concentration against peak area shows a straight line where R² value is 0.993. Precision determined the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to doxycycline hyclate concentration for the

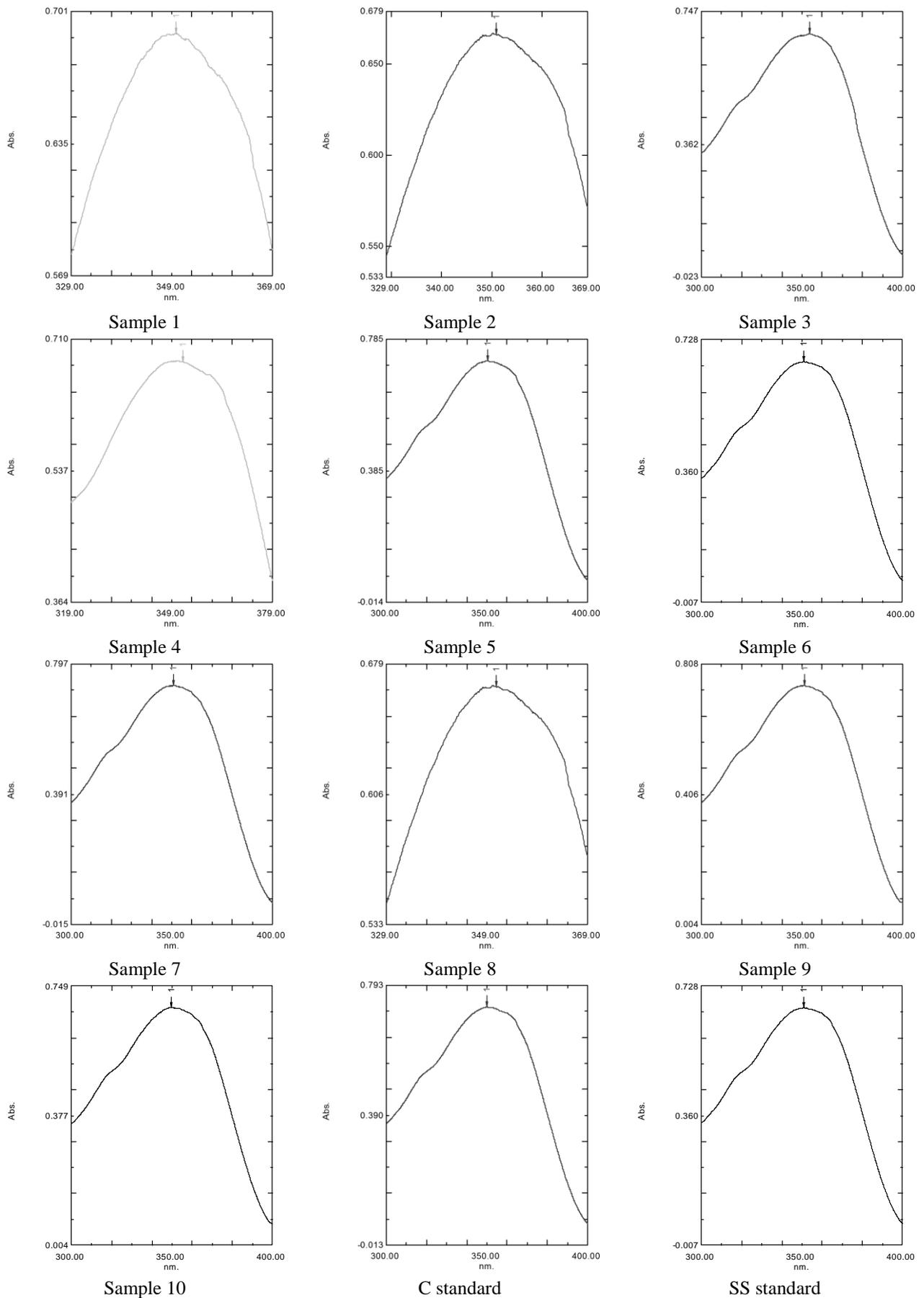


Figure 3: Ultraviolet spectrum of ten samples and standards.

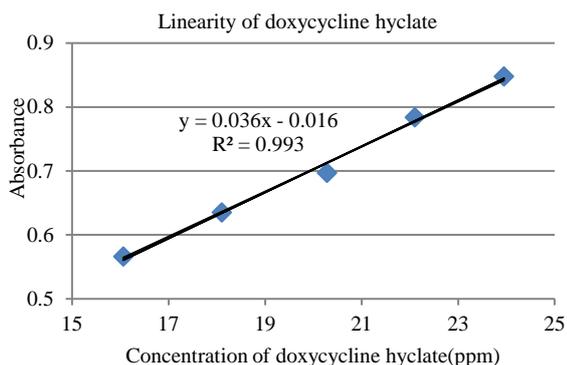


Figure 4: Linearity of doxycycline hyclate.

six samples was 0.65%. From the accuracy results, the percentage recovery values for doxycycline hyclate satisfy the acceptance criteria (98% - 102%) for accuracy across the range of 80-120%. A comparison of the proposed method with other methods is given in Table - 8. The results obtained by the proposed method have an RSD of 0.65 %, better than that reported by Šatínský et al.²⁴, Salinas et al.²⁵ and Lopez-Paz et al.²⁶. The first two methods^{24,25} have relatively high RSD values, higher than RSD_{max}. This indicates that the proposed method is more precise and accurate than some of the aforesaid published methods. Mahrous and Abdel-Khalek²⁷ described a long pre-treatment of the drug through mixing with acetic acid and sodium cobalt nitrite, then boiling the mixture, followed by cooling. In addition, the same author²⁸ described a determination employing ammonium vanadate but the later has less recovery percentage than the earlier one. In comparison of both methods^{27,28}, this proposed method have the higher recovery rate (100.59%). Here, the validated methods yielded good results and suggest their application in the quality control laboratories where the modern and expensive instruments are not available.

CONCLUSION

In this work, an analytical UV method was developed for the quantitative determination of doxycycline hyclate in samples. Its advantages over other existing method include its simplicity, speed and low cost. The proposed method not only provides a linear relation between absorbance and concentration in 349nm wavelength, but also ensures a simple, sensitive, accurate, and repeatable determination of doxycycline hyclate in pharmaceutical samples. Doxycycline was shown to be stable during all the procedure. Thus, the result parameters demonstrated that the spectrophotometric method could be applied for the analysis of the pharmaceutical formulations assuring the

quality and efficacy of the doxycycline hyclate under investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Table 8: Comparison of the proposed method with other methods.

| Reference | Concentration (ppm) | RSD (%) | Recovery (%) | Conditions |
|------------|---------------------|---------|------------------|-------------------------------------|
| This paper | 15.0-25.0 | 0.65 | 100.59 | UV detection |
| [24] | 2.0-100.0 | 5.05 | 99.3 | Sequential injection chromatography |
| [25] | - | 5.0 | 95 | Derivative spectrophotometry |
| [26] | 10.0-80.0 | 1.40 | 2.3 ^a | FIA-spectrophotometry |
| [27] | 10.0-30.0 | - | 100.3 | Spectrophotometry |
| [28] | 20.0-100.0 | - | 99.6 | Spectrophotometry |

^a = Relative error

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