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# Stability Indicating UV Spectrophotometric Method Development and Validation for Estimation of Doxycycline Hyclate in Bulk and Pharmaceutical Formulation

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**Keywords:** validation, Doxycycline Hyclate, UV Spectrophotometry, forced degradation, spectrum.

**ABSTRACT**

Doxycycline Hyclate, its IUPAC name is (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride hemiethanolate hemihydrates. Doxycycline Hyclate is salt forms of Doxycycline. It is obtained from oxytetracycline or methacycline or any other means. Doxycycline has a high degree of lipid solubility and a low affinity for calcium binding. It is highly stable in normal human serum. It is used to treat many kinds of infection like skin, dental, urinary tract infection and respiratory infection. It is also useful for the treatment of malaria, acne and certain sexually transmitted diseases. A simple, rapid, accurate, specific method was developed and validated for the estimation of Doxycycline Hyclate in pharmaceutical dosage form. The absorption maxima were found to be 274.4nm. Water was used as a solvent for the experiment. Doxycycline Hyclate shows linear response between 14.4 to 33.6 µg/ml. and correlation coefficient were found to be 0.99 with linear equation y=0.0313x + 0.011.the accuracy of doxycycline hyclate at 80%,10% and 120% were found to be 102%,99.6% and 99.5% respectively. The % RSD of system precision and method precision was found to be 0.045 and 0.094 respectively. A further forced degradation study of drug was carried under the acidic, alkaline, thermal and UV degradation.

## INTRODUCTION

Doxycycline Hyclate, its IUPAC name is (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride hemiethanolate hemihydrates[1]

Doxycycline Hyclate is freely soluble in water and in methanol, sparingly soluble in ethanol (96 percent). It also dissolves in solution of alkali hydroxide and carbonates. [4]

Doxycycline Hyclate is a salt form of Doxycycline. It is a tetracycline antibiotic use to treat many kinds of infection like skin, dental, urinary tract infection and respiratory infection. It is also useful for the treatment of malaria, acne and certain sexually transmitted diseases.

It's a synthetic broad spectrum antibiotic, it binds reversibly to the 30S ribosomal subunit as well as 50S subunit and blocks the binding of aminoacyl-tRNA to the mRNA-Ribosome complex. And inhibits the protein synthesis, in addition, doxycycline also inhibits the collagenase activity. [5]

A simple, rapid, accurate, specific method was developed and validated for the estimation of Doxycycline Hyclate in pharmaceutical dosage form.

Molecular Formula - $C_{22}H_{24}N_4O_8$, HCl,$^{1/2}C_2H_6O$,$^{1/2}H_2O$

Molecular Weight – 513.0

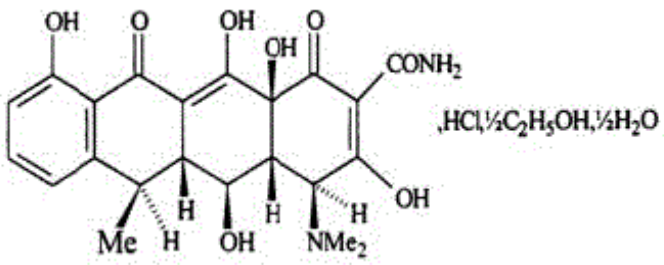


**Figure 1: Structure of Doxycycline Hyclate**

The absorption maxima were found to be 274.4nm. Water was used as a solvent for the experiment. Doxycycline Hyclate shows linear response between 14 to 24.6µg/ml. And correlation coefficient were found to be 0.99 with linear equation y=0.0313x + 0.011.

There are three types of stability studies accelerated, long term stability studies and forced or stressed degradation studies. Forced degradation study was performed by exposing to elevated temperature, acidic, basic condition, UV light and oxidation by Hydrogen peroxide[6]

**Objective:**

The objective of this work is to develop a simple and accurate method for the validation of Doxycycline Hyclate by UV spectroscopy of marketed preparation.

## MATERIALS AND METHODS

### Instrument

**Table 1 List of instruments**

| Instrument | Model no. | Manufacturer |
|---|---|---|
| UV | 1800 | Shimadzu |
| Balance | CA 123 | Contech |
| Centrifuge | BL-135 D | BIO-LAB |
| Sonicator | KI-1.5 | Kroma Tech, India |
| Milli Q water | NV00922/08/70001 | LAB Q ULTRA |
| Oven | EHT-169 | EXPO HI-TECH |



**Marketed preparation-** Doxycycline Hyclate containing capsule made by SCOSHIA pharmaceutical was used.

### Chemicals and reagent

**Table 2: List of chemicals and reagents**

| **Chemical** | **Supplier** |
|---|---|
| Sodium hydroxide | CHEMDYES CHEMICAL |
| Hydrochloric acid | CHEMDYES CHEMICAL |
| Hydrogen peroxide | SD FINE CHEMICALS |

## PREPARATION OF STANDARD SOLUTION

The pure drug of Doxycycline Hyclate 20 mg was weighed and transferred into a 100 ml of volumetric flask, and then some ml of water is added and sonicated the solution for 1-2 minute. Then make up the volume with water up to mark. Aliquots of standard stock solution

were pipette out into 25ml volumetric flask and again make up the volume with water up to mark.

**Preparation of Sample Solution**

10 capsules were weight, drug powder was removed, weight of empty capsule was taken, and average weight of powder was calculated. then equivalent weight to 20mg was weight and transferred into 100ml volumetric flask, add some amount of water and sonicate for certain period of time and shake. Some aliquots were pipette out into 25 ml volumetric flask and make up the volume with water up to mark. Final solution was filtered from whatmann 41 and analysed at 274.4 nm wavelength.

**Absorption Maxima**

The standard solution was scanned in the range of 200-400 nm by using water as a blank and sample solution were also scanned in the range of 200-400 nm.

Both standard as well as sample solution given maximum absorption at 274.4 nm wavelength. And this wavelength was selected for analysis.

**VALIDATION OF ANALYTICAL METHOD**



**PRECISION**

**System precision**

Same concentrations of six standard solutions were prepared and six-time reading was taken by using UV/VIS double beam spectrophotometer.

**Method precision**

Same concentrations of six sample solutions were prepared and six time reading was taken by using UV/VIS double beam spectrophotometer.

**LINEARITY**

Fresh aliquots were prepared from stock solution ranging from 14.4 to 28.6µg/ml and the absorbance value of each concentration was recorded at 274.4nm.

The drug shows linear response in the range of 14.4 to 28.6 µg/ml.

**ACCURACY**

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration level 80%, 100%, and 120% with respect to purity of the standard drug.

**LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION**

The limit of detection and limit of quantification of Doxycycline Hyclate by proposed method were determined using calibration graph.

**FILTER SUITABILITY**

Sample preparation:

Performed the % Assay in Doxycycline Hyclate capsules as per method and filtered through four different types of membrane filter discarding first few ml of the filtrate. Additionally, the solutions from the same vessel were also centrifuged. The filtrate and centrifuge were measured as per method.



**STABILITY OF SAMPLE SOLUTION**

The sample was stored at room temperature and tested after a time interval of 0hr, 2hrs, 8hrs and 24hrs.

**FORCED DEGRADATION STUDY**

Degradation study of Doxycycline Hyclate capsule was performed by acid, base, peroxide oxidation, and by heat

**Acid degradation**

Performed % assay as per the method as described in preparation of sample solution with prior degradation by 5ml of 1N HCl and kept the sample for 2 hrs, after 2 hours neutralise with 1N NaOH and make up the volume and dilute as per the method.

**Base degradation**

Performed % assay as per the method as described in preparation of sample solution with prior degradation by 5ml of 1N NaOH and kept the sample for 2 hrs, after 2 hours neutralise with 1N HCl and make up the volume and dilute as per the method.

**Oxidative degradation**

Performed experiment as per the method of preparation of sample solution by priory adding 5 ml 30% $H_2O_2$ and kept it for 2 hours and neutralised with solution of sodium metabisulphite and make the volume and dilute as per the method described in preparation of sample solution.

**Thermal degradation**

Performed experiment as per the method as described in preparation of sample solution and kept the solution in oven at 60°C for 2hr, 4hr and 6hr

**UV degradation**

Performed experiment as per the method described in preparation of sample solution and kept the solution in UV chamber for 2 hours.



**RESULTS AND DISCUSSION**

**Table 3: System Precision**

| **STD** | **Absorbance** |
|---|---|
| STD-1 | 0.7245 |
| STD-2 | 0.7251 |
| STD-3 | 0.7254 |
| STD-4 | 0.7251 |
| STD-5 | 0.7248 |
| STD-6 | 0.7247 |
| **AVG** | 0.724933333 |
| **SD** | 0.000326599 |
| **% RSD** | 0.04505223 |

**Table 4: Method Precision**

| Sample | Absorbance |
|---|---|
| smpl-1 | 0.7433 |
| smpl-2 | 0.7435 |
| smpl-3 | 0.744 |
| smpl-4 | 0.7446 |
| smpl-5 | 0.7449 |
| smpl-6 | 0.7449 |
| **AVG** | 0.7442 |
| **SD** | 0.0007043 |
| **% RSD** | 0.0946349 |

**Linearity**

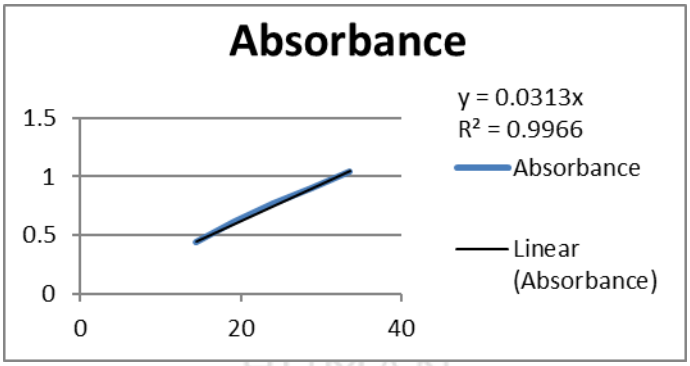


**Figure 2: linearity graph of doxycycline hyclate**

**Table 5: Table of Linearity**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 14.4 µg/ml | 0.441 |
| 19.2µg/ml | 0.612 |
| 24µg/ml | 0.77 |
| 28.8µg/ml | 0.895 |
| 33.6µg/ml | 1.04 |
| **slope** | 0.0313 |
| **Intercept** | 0.0111 |
| **correlation** | 0.997 |

**Table 6: Table of Accuracy**

| Level | Test amount (µg/ml) | Spiked std amount (µg/ml) | Total amount recovered (µg/ml) | % Recovery |
|---|---|---|---|---|
| 80 | 10 | 10 | 19.92 | 102.58 |
| 100 | 12 | 12 | 24.62 | 99.6 |
| 120 | 15 | 15 | 29.85 | 99.5 |

**Table 7: Table of Filter Suitability**

| Sample ID | % ASSAY | Correlation |
|---|---|---|
| Whatmann no. 41 | 99.73 | - |
| Centrifuge | 99.43 | 0.9969 |
| No.0.45micron | 98.83 | 0.9909 |

**Table 8: Table of Stability of Sample Solution**

| Time interval | % Assay | Correlation |
|---|---|---|
| initial | 99.30 | - |
| 2 Hours | 97.50 | 0.98 |
| 6 Hours | 99.20 | 0.99 |
| 24 Hours | 98.10 | 0.99 |

**FORCED DEGRADATION STUDY**

**Acid degradation**

**Table 9: Table of Acid Degradation**

| Time | % Assay | Time | % Assay |
|---|---|---|---|
| Initial | 99.86 | After 2 hours | 92.63 |

**Base degradation**

**Table 10: Table of Base Degradation**

| Time | % Assay | Time | % Assay | Time | % Assay | Time | %Assay |
|---|---|---|---|---|---|---|---|
| Initial | 100 | After 2hrs | 99.73 | After 4hrs | 98.21 | After 6hrs | 77.69457 |

**Oxidative degradation**

**Table 11: Table of Oxidative Degradation**

| Time | % Assay | Time | % Assay |
|---|---|---|---|
| Initial | 98.55 | After 2 hours | 72.402 |

**Table 12: Table of Thermal Degradation**

**Thermal degradation**

| Time | % Assay | Time | % Assay | Time | % Assay | Time | % Assay |
|---|---|---|---|---|---|---|---|
| Initial | 100.03 | After 2 hr | 99.73 | After 4 hr | 95.49032 | After 6 hr | 84.64805 |

**Table 13: Table of UV Degradation**

**UV degradation**

| Time | % Assay | Time | % Assay |
|---|---|---|---|
| Initial | 98.55 | After 2 hours | 83.57 |

**SPECTRUM**

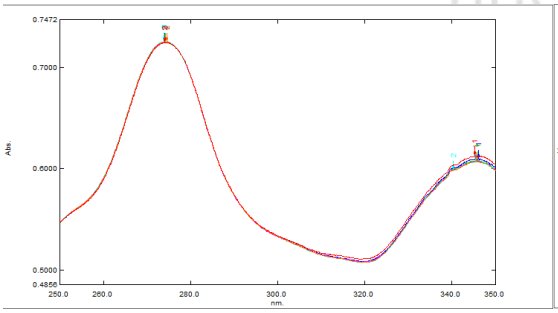

**Figure 3: spectrum of method precision**

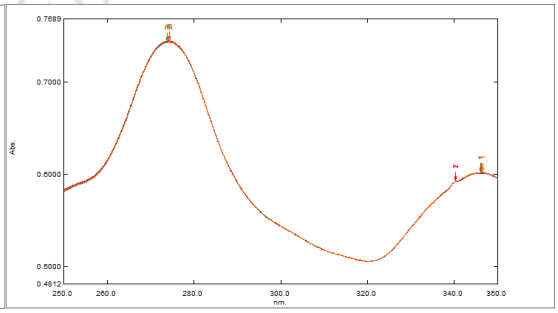

**Figure 4: spectrum of method precision**

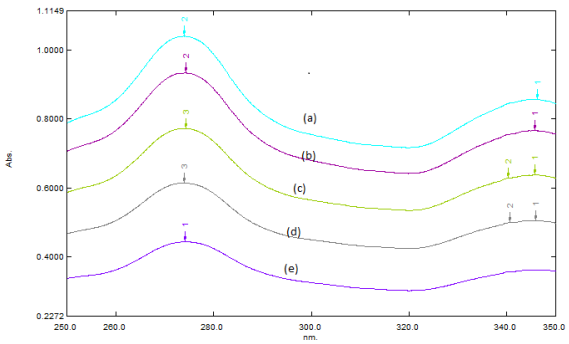


**Figure 5: Linearity spectrum**

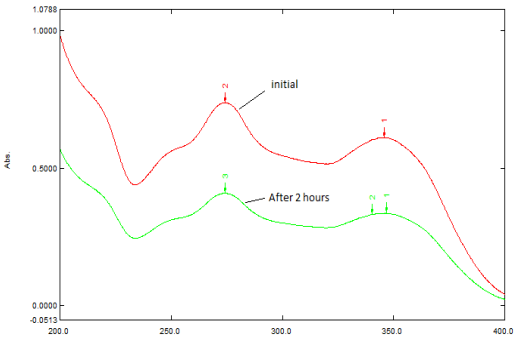


**Figure 6: Acid degradation**

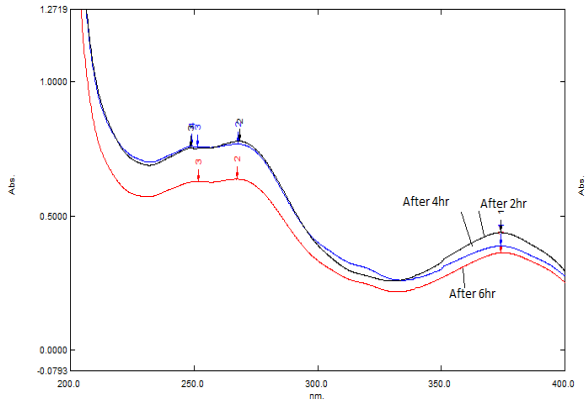


**Figure 7: Base degradation**

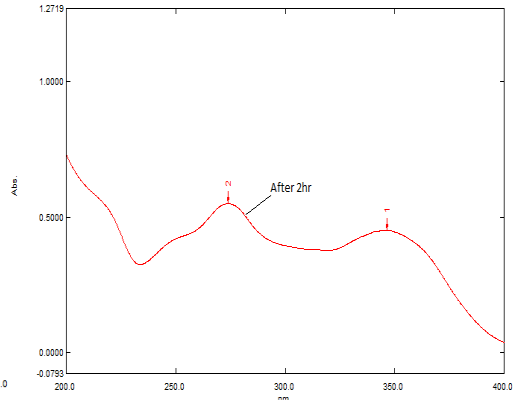


**Figure 8: Oxidative degradation**

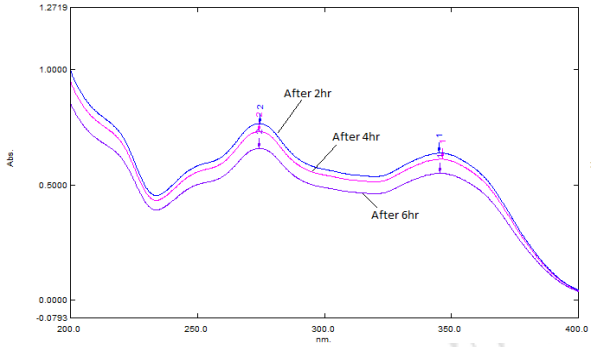


**Figure 9: Thermal degradation**

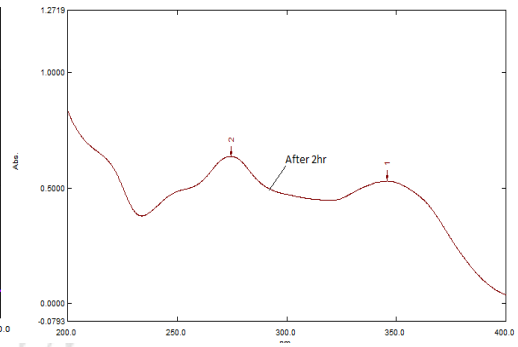


**Figure 10: UV degradation**



**First Order Derivative Spectrum**

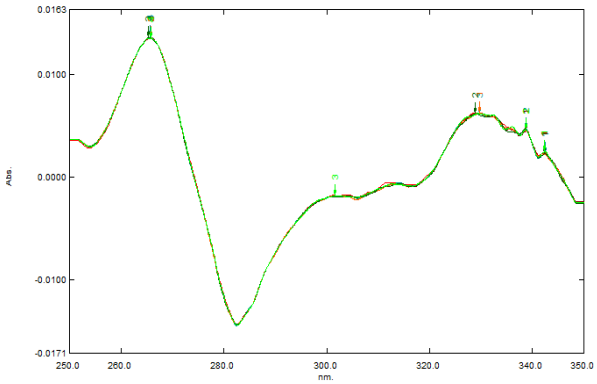


**Figure 11: Spectrum of standard solution**

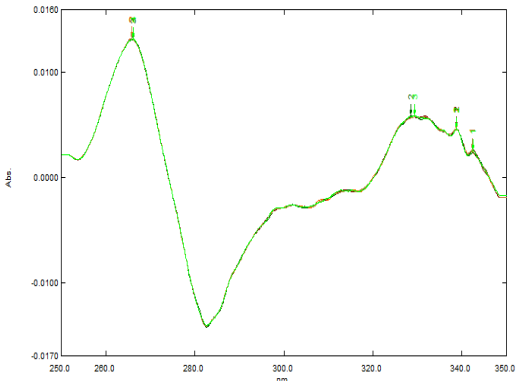


**Figure 12: spectrum of sample solution**

A reliable UV spectrometric method was developed for the estimation of Doxycycline Hyclate. Beers law was obeyed in concentration range of 14 to 24.8 µg/ml at the wavelength of 274.4 nm. The correlation coefficient was found to be 0.99 with line of equation y= 0.0313x + 0.111. The percent recovery found to be in the range of 99.5-102.58% and the

limit of assay by USP specified that the content should not be less than 95% and not more than 105% of labelled amount for filter suitability, Whatmann no.41, 0.45µ filter and centrifuge are suitable. Sample solution is stable for 24 hours. Sample is degraded by acid (0.1N HCl), base (0.1N NaOH), oxidation ($H_2O_2$), thermal, and by UV after 2 hours, 6 hours, 2 hours, 6 hours and 2 hours respectively.

## CONCLUSION

The result of the experiment indicate that the method is precise, accurate, rapid and simple .this spectroscopic method found to be suitable for determination of Doxycycline Hyclate in bulk and pharmaceutical preparation. Degradation products generated from forced degradation studies are potential degradation product that may or may not be formed under relevant storage conditions but they assist in the developing stability indicating method. From our experiment, we can conclude that the sample highly degraded in acid, hydrogen peroxide and in presence of UV light compare to basic condition. This method can save time and money.

## ACKNOWLEDGEMENT

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