

Degradation study of Doxycycline in bulk and formulation by UV-Visible spectrophotometry

Gopika V C*, Hisana M S, Jayakrishnan V, Malavika C P, Mubasher P M, Vismal Vincent, Salman K K and Sajna John

Department of pharmaceutical analysis, ELIMS College of pharmacy, Thrissur, India.

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Abstract

Introduction: Forced degradation is a process that involves degradation of drug products and drug substances at condition more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule.

Aim and objective: To investigate the forced degradation study for the determination of degradation of Doxycycline by UV-Visible spectrophotometric method.

Methods: Doxycycline sample and standard were exposed to different stress conditions (hydrolytic and oxidative degradation). Both standard drug and marketed formulations were used for the degradation study. The amount of percentage degradation of each standard and samples were calculated by taking absorbances at their λ_{\max} with the help of UV-Visible spectrophotometer. Forced degradation of drug substance was done by exposing to acidic, basic and to medium of hydrogen peroxide. The degradation results of each condition were compared with that of standard. This method can be used successfully for studying the stress degradation factors. Because this method is less time consuming and simple and cost effective also.

Results: Forced degradation of selected drugs performed using HCl, NaOH and H₂O₂. Degraded sample were quantified by UV visible spectroscopy. In all the methods used in degradation study, sample undergoes greater degradation compared with that of standard. Among the degradation conditions used in the study it is found that NaOH produce more degradation.

Keywords: Doxycycline; Forced degradation study; UV-Visible spectrophotometry; Percentage degradation; Comparison

1. Introduction

Analytical chemistry is the study of separation, quantification and identification of chemical components of natural and artificial materials constituted with one or more compounds or elements. Analytical chemistry is separated into two main categories; qualitative analysis that is to say the identification with regard to the chemical components exists in the sample, whereas quantitative analysis estimates the amount of elements or compounds in the substance i.e., sample.

UV-visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in the solution [1-5].

* Corresponding author: Gopika V C

Antibiotic, chemical substance produced by a living organism, generally a microorganism, that is detrimental to other microorganisms. Antibiotics commonly are produced by soil microorganisms and probably represent a means by which organisms in a complex environment, such as soil, control the growth of competing microorganisms.

Doxycycline is a broad-spectrum antibiotic of the tetracycline class used in the treatment of infections caused by bacteria and certain parasites. It is used to treat bacterial pneumonia, acne, chlamydia infections, lyme disease, cholera, typhus, and syphilis. It is also used to prevent malaria in combination with quinine. Doxycycline may be taken by mouth or by injection into a vein.

Common side effects include diarrhoea, nausea, vomiting, abdominal pain, and an increased risk of sunburn. Use during pregnancy is not recommended. Like other agents of the tetracycline class, it either slows or kills bacteria by inhibiting protein production. It kills malaria by targeting a plastid organelle, the apicoplast [6-8].

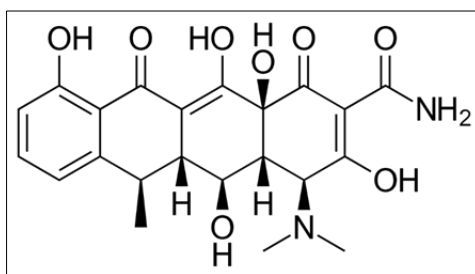


Figure 1 Molecular structure of Doxycycline

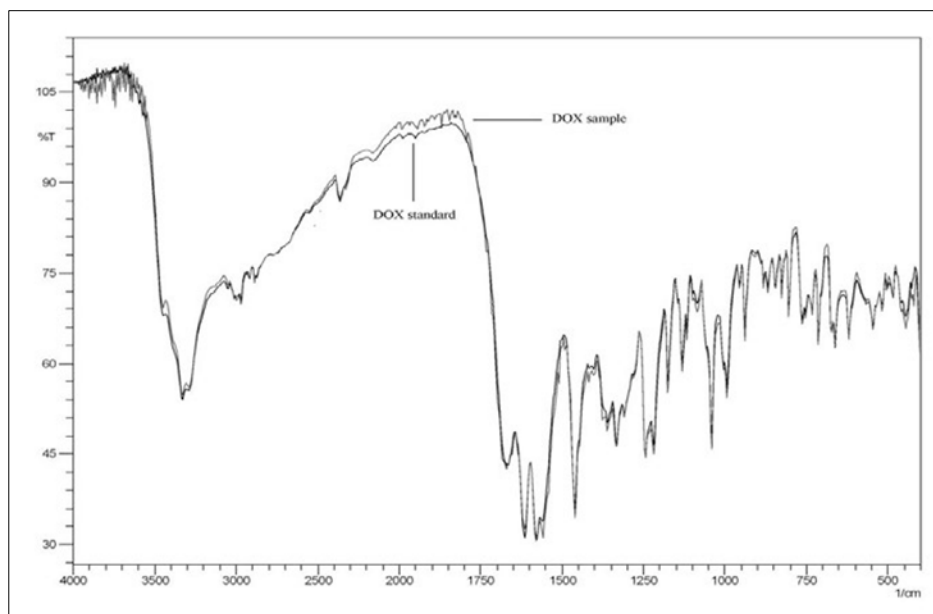


Figure 2 IR spectrum of Doxycycline

2. Material and methods

2.1. Standard drugs

Doxycycline RS obtained from Cipla Ltd.

2.2. Test product

Doxycycline capsule (Doxicip 100 mg) was purchased from local medical shop.

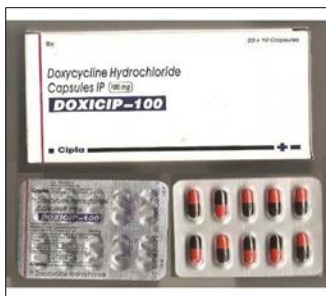


Figure 3 Marketed formulation of Doxycycline

2.3. Chemicals and solvents used for degradation

- 0.1M Sodium hydroxide
- 0.1M Hydrochloric acid
- Hydrogen peroxide (3% W/V)
- Distilled water

2.4. Instruments used

- UV-Visible Spectrophotometer – 1900 (Shimadzu)
- Electronic balance – (Wensar ISO 9001: 2000 Certified)

2.5. Methodology adopted

- Preparation of standard solutions of Doxycycline.
- Study of spectral characteristics.
- Preparation of calibration curve of drug in the obtained λ_{max} .
- Preparation of solutions for degradation study of each drug standard and sample in 0.1M NaOH, 0.1M HCl and 3% W/V H_2O_2
- Measurement of absorbance of each drug solution at 0, 30, 60 and 90 minutes in their λ_{max} .
- Calculation of percentage degradation for each solution in the selected degradation method.
- Comparison of percentage degradation of standard and sample drug with standard drug under normal condition.

2.6. Preparation of reagents

Preparation of 0.1M sodium hydroxide.

4mg of sodium hydroxide pellets were weighed and dissolved in small amount of distilled water then made up the volume to 1000 ml.

Preparation of 0.1M Hydrochloric acid

8.33ml of concentrated hydrochloric acid was measured and diluted with distilled water to 1000ml.

2.7. Degradation study of Doxycycline

Standard preparation

100 mg of Doxycycline was transferred to volumetric flask and dissolved in 100 ml of distilled water to achieve a concentration of 1 mg/ml. The solution was kept at room temperature. An aliquot solution was then diluted with distilled water to get final concentration of 20 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$, 60 $\mu\text{g/ml}$, 80 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$. The solution was scanned in the UV region and the maximum absorbance was recorded at 274 nm.

Sample preparation

Weighed and transferred 0.518 g of doxycycline capsule which contains 100 mg of Doxycycline to a 100 ml standard flask and made up the volume with distilled water. Solution was filtered and prepared five concentrations such as 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml & 100 µg/ml. Absorbance was measured at 274 nm.

2.8. Intraday hydrolytic degradation study using 0.1M NaOH

Standard preparation (Stress)

100 mg of Doxycycline standard was weighed and transferred to volumetric flask, containing 0.1M NaOH & dissolved then make up the solution to 100 ml. 6 ml of the resulting solution was diluted to 100 ml to produce 60 µg/ml. The absorbance of the solution was taken at 274 nm in zero minutes. The same procedure was repeated for 30 min, 60 min and 90 min time interval

Sample preparation (stress)

100 mg equivalent of Doxycycline (0.518 g Capsule) were crushed weighed and transferred to volumetric flask, dissolved in 0.1 M NaOH & made up the solution to 100 ml. 6 ml of the resulting solution was diluted to 100 ml to achieve a concentration of 60 µg/ml. The absorbance of the solution was taken at 274 nm in zero minutes. The same procedure was repeated for 30 min, 60 min and 90 min time interval.

Blank preparation

Blank solution was prepared by pipetting 6 ml NaOH to volumetric flask & made up to 100 ml with distilled water.

2.9. Intraday hydrolytic degradation study using 0.1M HCl

Standard preparation (stress)

100 mg of Doxycycline standard was weighed and transferred to volumetric flask, containing 0.1M HCl & dissolved then make up the solution to 100 ml. 6 ml of the resulting solution was diluted to 100 ml to produce 60 µg/ml. The absorbance of the solution was taken at 274 nm in zero minutes. The same procedure was repeated for 30 min, 60 min and 90 min time interval

Sample preparation (stress)

100 mg equivalent of Doxycycline (0.518 g capsule) were crushed weighed and transferred to volumetric flask, dissolved in 0.1 M NaOH & made up the solution to 100 ml. 6 ml of the resulting solution was diluted to 100 ml to achieve a concentration of 60 µg/ml. The absorbance of the solution was taken at 274 nm in zero minutes. The same procedure was repeated for 30 min, 60 min and 90 min time interval

Blank preparation

Blank solution was prepared by pipetting 6 ml HCl to volumetric flask & made up to 100 ml with distilled water.

2.10. Intraday oxidative degradation study using 3% H₂O₂

Standard preparation (stress)

100 mg of Doxycycline standard was weighed and transferred to volumetric flask, containing 3% H₂O₂ & dissolved then make up the solution to 100 ml. 6 ml of the resulting solution was diluted to 100 ml to produce 60 µg/ml. The absorbance of the solution was taken at 274 nm in zero minutes. The same procedure was repeated for 30 min, 60 min and 90 min time interval

Sample preparation (stress)

100 mg equivalent of Doxycycline capsule (0.518 g) were crushed weighed and transferred to volumetric flask, dissolved in 3% H₂O₂ & made up the solution to 100 ml. 6 ml of the resulting solution was diluted to 100 ml to achieve a concentration of 60 µg/ml. The absorbance of the solution was taken at 274 nm in zero minutes. The same procedure was repeated for 30 min, 60 min and 90 min time interval

Blank preparation

Blank solution was prepared by pipetting 6ml 3% H₂O₂ to volumetric flask & made up to 100 ml with distilled water [9-18].

3. Results and discussion

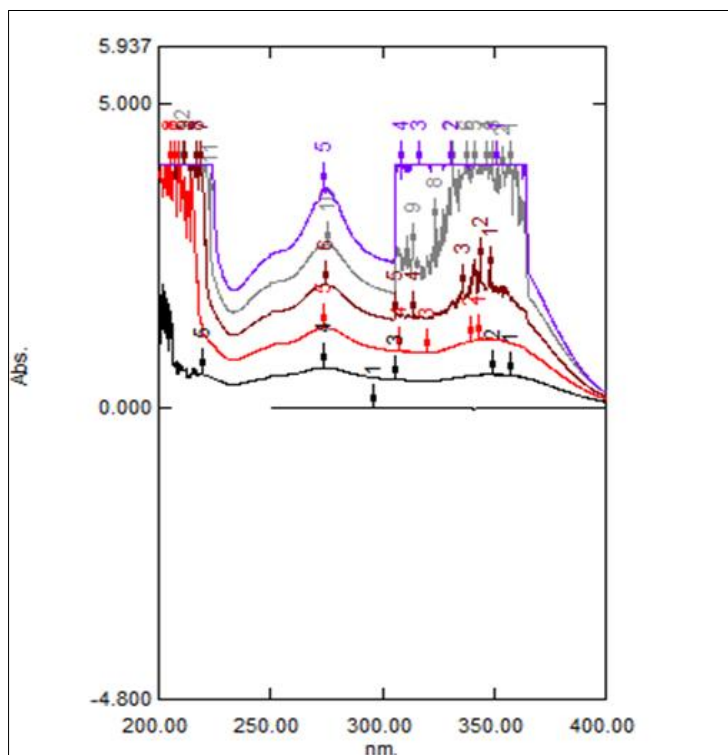


Figure 4 UV-Visible overlay spectrum of Doxycycline standard

Table 1 Absorbance of Doxycycline RS in distilled water at 274 nm

Concentration	Absorbance
20µg	0.605
40µg	1.281
60µg	1.980
80µg	2.592
100µg	3.165

Table 2 Intraday results of hydrolytic degradation of Doxycycline using 0.1 M NaOH

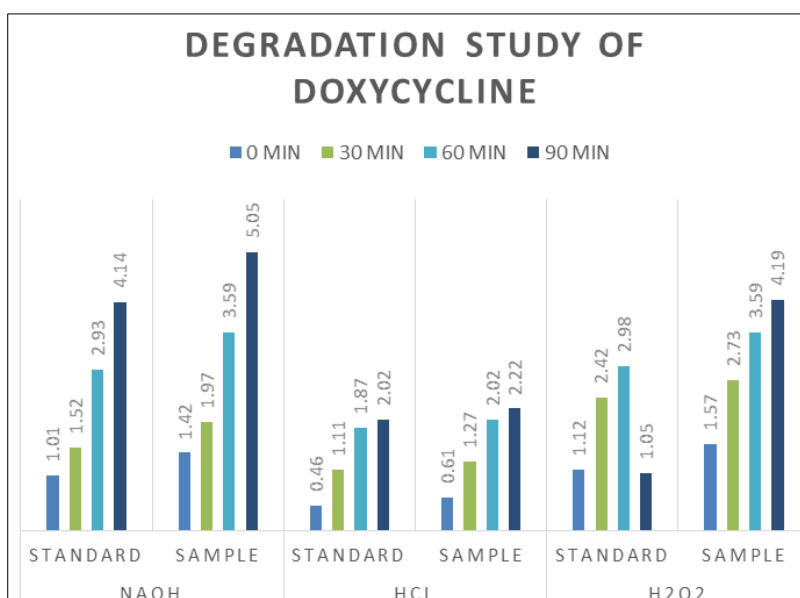
SL NO	Drug	Absorbance			
		0 min	30 min	60 min	90 min
1	Standard	1.960	1.950	1.922	1.898
2	Sample	1.952	1.941	1.909	1.880

Table 3 Intraday results of hydrolytic degradation of Doxycycline using 0.1 M HCl

SL NO	Drug	Absorbance			
		0 min	30 min	60 min	90 min
1	Standard	1.971	1.958	1.943	1.940
2	Sample	1.968	1.955	1.940	1.936

Table 4 Intraday results of oxidative degradation of Doxycycline using 3% H₂O₂

SL NO	Drug	Absorbance			
		0 min	30 min	60 min	90 min
1	Standard	1.958	1.932	1.921	1.900
2	Sample	1.949	1.926	1.909	1.897

**Figure 5** Comparison of percentage degradation of Doxycycline standard and sample with RS under normal condition

4. Conclusion

The present study involves the forced degradation studies such as acid and alkali hydrolytic degradation and oxidative degradation of Doxycycline bulk and formulation. Doxycycline is soluble in water, so λ_{\max} of drug was identified by measuring UV visible spectrum in the range of 800-200 nm in distilled water. Calibration curve of drug plotted in the obtained λ_{\max} (274 nm) and the calibration curve found to be linear in the selected concentration range (20-100 $\mu\text{g}/\text{mL}$). Forced degradation of drug was performed by using HCl (0.1 M), NaOH (0.1 M) and 3% H₂O₂. Degraded sample were quantified by UV visible spectroscopic method and the percentage degradation of each drug was re calculated. In all the methods used in degradation study, sample undergoes greater degradation when compared with that of standard. Among the methods selected for the study it was found that hydrolytic degradation using NaOH cause greater degradation.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest in the manuscript.

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