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Research Article

Development and Validation of Stability Indicating Assay Method of Doxycycline Hyclate by using UV-Spectrophotometer

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Abstract

In the present study a simple, rapid, sensitive, and cost-effective stability indicating UV-spectrophotometric method was developed of doxycycline hyclate in bulk form and validated as per ICH guidelines. The stability-indicating study of doxycycline hyclate was based on the forced degradation reactions of the drug under different ICH-prescribed stress conditions like acid, alkali, neutral, oxidation, thermal, and photolysis. The absorption maximum of the drug was shown at 270nm and methanol was selected as solvent on the basis of the solubility of the drug. A linear relation with a regression coefficient value (R^2) of 0.999 indicates that the method follows Beer's-Lambert's law within the concentrations range of 5 - 50µg/ml. The detection limit and quantitation limit were found to be 0.53 µg/ml and 1.55 µg/ml respectively. The % of recoveries was found to be within the range of 98.87 -99.88 with a low % of RSD indicating the accuracy of the method for estimation of the drug. Under stressed conditions, significant degradation was found in alkali, neutral and oxidation conditions and found to be sensitive under thermal and photolytic conditions but stable to acidic hydrolytic conditions. The developed method was validated according to ICH guidelines and found accurate, precise, and specific, suggesting that the developed stability-indicating spectroscopic method could be successfully adopted to estimate doxycycline hyclate.

Keywords: Doxycycline Hyclate; Stability Indicating method; UV spectrophotometer; Validation; Stress degradation.

1. INTRODUCTION

In recent times, there is an increased tendency towards the development of a stability-indicating assay method (SIAM), using the approach of stress testing as mentioned in the ICH guidelines[1]. A stability method is a validated analytical technique used to measure the concentration of active constituents accurately in the presence of its degradation products, excipients, and other impurities. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance[2]. Stress testing encompasses the influence of temperature, humidity, light, oxidizing agent as well as susceptibility over a wide range of pH values[3, 4]. Generally, HPLC and turbidimetric methods are employed to monitor the changes in concentration on exposer of drug to different stressed conditions[5-7]. A UV spectroscopic SIAM may have advantages over chromatographic techniques in terms of simplicity, economic and time consumption. In forced degradation study protocol, the intensity of stress parameters depends on physico-chemical properties of drug substance, nature of drug/product and specific storage requirement. The objective of forced degradation studies is to generate degradation products in order to evaluate suitability and efficiency of analytical method as a stability indicating method.

Doxycycline (DOX) is a synthetic broad-spectrum, tetracycline group of antibiotics having a chemical name (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 was illustrated in Fig. 01, used for the treatment of bacterial and parasitic infections like skin, dental, urinary tract infection, respiratory infection. It binds reversibly to the 30S ribosomal sub-unit as well as the 50S sub-unit and blocks the binding of aminoacyl-tRNA to the mRNA-ribosome complex and inhibits protein synthesis[8]. DOX is a yellow crystalline powder having a molecular weight of 444.4 g/mol and is freely soluble in methanol and sparingly soluble in ethanol and distilled water. Official books like Indian Pharmacopoeia, British Pharmacopoeia, and United States Pharmacopoeia described chromatographic and titrimetric methods for the estimation of DOX[9-11]. From the literature survey, it was revealed that articles for the estimation of DOX in bulk, dosage forms, and biological fluid by UV-Vis spectroscopy[12,13], HPLC[14-16], LC-MS[17], and HPTLC[18] are reported but so far no attempt has been reported regarding the development of a validated UV- spectroscopic method of stability indicating assay method for DOX, hence in the present work it was felt necessary to develop a simple, economic and validated stability-indicating method for analysis of DOX and its degradants formed under ICH suggested stress conditions (hydrolysis, oxidation, photolysis, and thermal stress)[19].

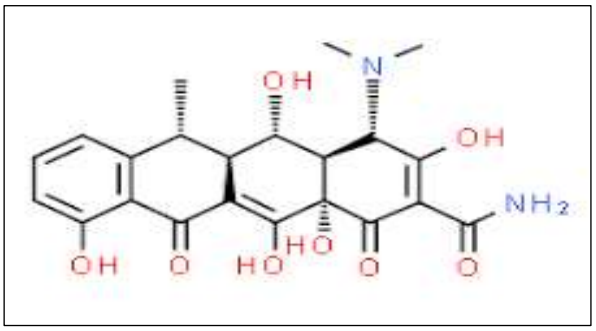

Figure 1: Chemical Structure of Doxycycline

MATERIALS AND METHODS

Materials

The working standard of pharmaceutical-grade DOX was obtained as a gift sample from Mepero Pharmaceutical Pvt. Ltd, Ahmedabad, India, and was used without further purification. Methanol was purchased from Merck Ltd, Mumbai, India and all other chemicals and reagents used during this study were of analytical grade.

Instrumentation

For the purpose of developing a stability-indicating assay method of DOX, a double beam UV- spectrophotometer (Systonic) having spectral bandwidth 3nm and of wavelength accuracy ±1nm with 1 cm quartz cell was used for spectral and absorbance measurement. The equipment was controlled by a PC workstation. The work was carried out in an air-conditioned room maintained at a temperature 25 ± 2^{0}C. A precision mantel heater (Biotech, Mumbai) with temperature regulator equipped with a reflux condenser was used for degradation study in acid, alkali and neutral conditions. A dry-air oven was used to study the effect of dry heat. The photolytic study was carried out by exposing the drug to direct sunlight for 4hrs.

Preparation of Standard Stock Solution and determination of λmax

The standard stock solution of 1000 µg/ml for DOX was prepared by dissolving 100mg of pure drug in methanol in a 100ml volumetric flask by adjusting the final volume with methanol. Further, a working standard solution of 100 µg/ml of DOX was prepared by diluting 10ml of the above-prepared solution to 100ml with methanol. Then further dilutions of 5-50 µg/ml were made by diluting the required aliquot of 100 µg/ml solution with methanol. A solution with a concentration of 30 µg/ml was then scanned in the range of 200-400nm against methanol as blank. The drug solution was showing maximum absorbance (λ_{max}) at 270nm which is considered as the analytical wavelength for further study (Fig. 02).

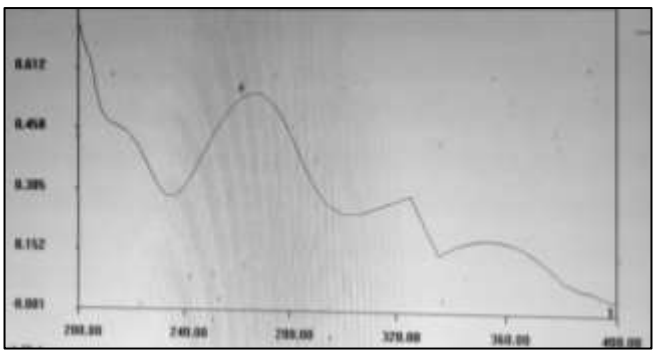

Figure 2: λmax of DOX at 270nm

Forced Degradation Studies

Forced degradation studies were performed to provide an indication of the stability indicating property of the drug. Drug at a concentration of 1mg ml^{-1} was used in all degradation studies. Conditions employed for stability studies were as follows[20-22].

Acid Hydrolytic study

For acid hydrolysis study, 10 ml of 1mg ml^{-1} standard stock solution of the DOX was added to 10 ml of 1.0N HCl and refluxed for 12hrs at 60^{0}c. From this 2ml of aliquot was taken at regular interval of time in separate 10ml volumetric flask which was then neutralized and adjusted the volume with methanol to form a solution containing 30 µg/ml and absorbance was recorded at 270nm against methanol as blank.

Alkaline Hydrolytic Study

For alkaline hydrolysis study, 10 ml of 1mg ml^{-1} standard stock solution of the DOX was added to 10 ml of 1.0N Naoh and refluxed for 12hrs at 60^{0}c. From this 2ml of aliquot was taken at regular interval of time in separate 10ml volumetric flask which was then neutralized and adjusted the volume with methanol to form a solution containing 30 µg/ml and absorbance was recorded at 270nm against methanol as blank.

Neutral Hydrolysis

In separate volumetric flask 10 ml of 1mg ml^{-1} standard stock solution of the DOX was added to 10 ml of distilled water and refluxed for 12hrs at 60^{0}c. From this 2ml of aliquot was taken at regular interval of time in separate 10ml volumetric flask which was then adjusted the volume with methanol to form a solution containing 30 µg/ml and absorbance was recorded at 270nm against methanol as blank.

Oxidation induced degradation

For the study oxidative-induced degradation, 10 ml of 1mg ml^{-1} standard stock solution of the DOX is taken in a separate volumetric flask and mixed with 10 ml of 3% H_2O_2. The solution was kept at room temperature for a period for 24hrs. From this 2ml of aliquot was taken after 24hrs in a separate 10ml volumetric flask which was then adjusted the volume with methanol to form a solution containing 30 µg/ml and absorbance was recorded at 270nm against methanol as blank.

Thermal Degradation

For thermal degradation, accurately weighed 100 mg of DOX was kept at 80°C for 24hrs. Then required amount was dissolved in methanol and a solution of 30 µg/ml was prepared then the absorbance was recorded at 270nm on UV spectrophotometer against methanol as blank.

Photo degradation

The pure drug sample accurately weighed 50 mg of DOX and was exposed to direct sunlight for 6hrs. Further a dilution of 30 µg/ml was prepared by dissolving the required amount in methanol and absorbance was recorded at 270nm against methanol as blank.

Validation of Method [23,24]

Linearity

The prepared dilutions of 5-50 µg/ml were scanned at 270nm and absorbance was measured for them. The calibration curve was constructed by plotting absorbance v/s concentration of standard solutions and the regression equation was determined. The experiment was carried out in five replicates.

Accuracy study

The accuracy of the developed method was confirmed by performing recovery study. The recovery study was carried out by standard addition method in which pre-analyzed samples were spiked with the extra standard drug of DOX in 80,100,120% and the mixture was analyzed at 270nm by UV-Spectrophotometer. The study is carried out in triplicate.

Precision

The precision of the method was established by repeatability and intermediate precision. Three solutions of concentration 10, 20 and 30 µg/ml of DOX were prepared to perform the precision study.

Repeatability

Repeatability (intra-day) was assessed by analyzing DOX in three different concentrations (10, 20 and 30 µg/ml) of three times a day at an interval of 1hr. The % relative standard deviation (RSD) was calculated for absorbance thus obtained, to analyze the intra-day variation.

Intermediate precision

Intermediate precision (inter-day) was established by analyzing three different concentrations (10, 20 and 30 µg/ml) of DOX for three different days. The results was reported in terms of % RSD.

Detection and Quantitation limit

The detection limit (DL) is the lowest amount of analyte in a sample, which can be detected and quantitation limit (QL) is the lowest amount of analyte in a sample, which can be quantitatively determined by the developed method. The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve by using eq. 1 and eq.2, where, σ is the standard deviation of the intercept of the calibration plot and S is the slope of the calibration curve.

$LOD = 3.3\ \sigma / S$---------- Eqn. 01

$LOQ = 10\ \sigma / S$---------- Eqn. 02

RESULT AND DISCUSSION

UV Spectroscopic method development

In the present study, a stability-indicating assay method was developed and validated as per ICH guidelines. Methanol was selected as the solvent for the development and validation of the stability-indicating assay method of DOX in bulk drug, and λ_{max} 270nm was taken as the analytical wavelength for estimation of the drug. The present method is found to be simple and economical as compared to the previously reported HPLC method as it required less sample preparation and solvent consumption.

Validation of the developed method

Linearity and range

Linearity was evaluated by analysis of working standard solutions of DOX for concentration 5-50 µg/ml. The absorbance of these solutions was recorded at 270 nm and a plot was made between the absorbance and concentration of standard solutions as shown in Figure 3. The results of the regression analysis are summarized in (Table 1). The result of the study showed that the drug obeyed Beer-Lambert's law in the above concentration range with a correlation coefficient of 0.999. The mean absorbance was found in between 0.042-0.931.

Table 1: Regression analysis of DOX for developed UV Spectrophotometric method

S.NO	Parameters	Results
1	Absorption maxima λ_{max}	270 nm
2	Beer's-Lambert's range	5-50 µg/ml
3	Regression equation	y = 0.0194x - 0.0542
4	Correlation coefficient	0.999
5	Slope	0.0194
6	Intercept	-0.0542

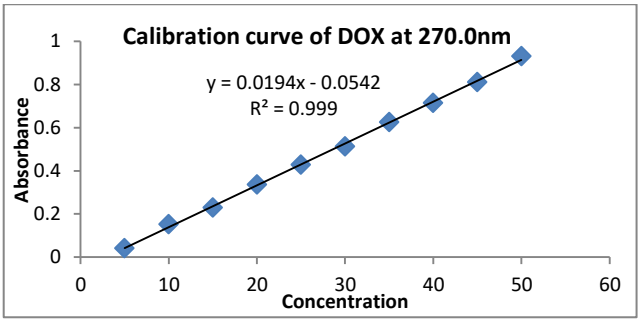

Figure 3: Linearity of DOX at λ_{max} 270 nm

Accuracy Study

The accuracy was carried out to check the sensitivity of the method for the estimation of DOX. The recovery study was investigated by analyzing three concentrations of standard drug solution previously analyzed using the standard addition technique. The standard addition technique was carried out by adding 80,100 and 120 % of the DOX concentration in the sample. The % recoveries of the three concentrations were found to be 98.87-99.88 %. The respective values of % recovery and % RSD are displayed in Table 2.

Table 2: Result of Recovery Study.

Initial Concentration (µg/ml)	Level of spiking %	Concentration of spiked sample (µg/ml)	% Recovery	% RSD
20	80	16	99.88	0.104
20	100	20	99.21	0.098
20	120	24	98.87	0.112

Precision Study

The results of the repeatability and intermediate precision study are given in Table 3. The developed method was found to be precise as the % RSD values for repeatability and intermediate precision studies were < 2.0.

Table 3: Result of Precision Study

Repeatability Study			Intermediate Precision study		
Sample concentration (µg/ml)	Mean Absorbance	% RSD	Sample concentration (µg/ml)	Mean Absorbance	% RSD
10	0.151 ± 0.012	0.132	10	0.150 ± 0.023	0.098
20	0.321 ± 0.021	0.109	20	0.315 ± 0.054	0.194
30	0.527 ± 0.019	0.289	30	0.507± 0.037	0.272

Detection and Quantitation limits

The detection limit (DL) and quantitation limit (QL) were determined as per the ICH guidelines and were found to be 0.53 and 1.55 µg/ml for DOX respectively (Table. 4).

Table 4: Result of LOD and LOQ

Ingredients	LOD (µg/ml)	LOQ (µg/ml)
DOX	0.53	1.55

Forced Degradation Study

The forced degradation study for DOX was performed at different stressed conditions prescribed as per ICH guidelines. The result of the study indicated that DOX was undergoes significant degradation in alkali, neutral, oxidation conditions and sensitive to thermal and photolytic conditions but stable at acidic hydrolytic conditions. The results of forced degradation study summarized in (Table 5).

Hydrolytic studies

Acidic condition.

In stress degradation study under acidic conditions, DOX dissolved in 1.0 N HCl and refluxed for 12hrs at 60^0c. It was observed that the drug gets slowly degraded about 5-8 % in strongly acidic conditions over a period of time (Fig.4).

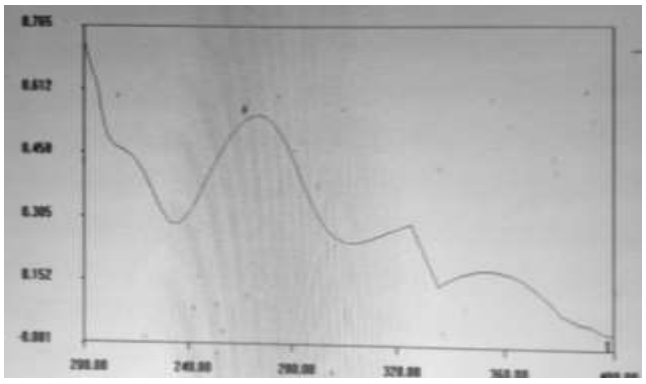

Figure 4: Acid induced degradation of DOX

Degradation in alkali

Alkaline hydrolysis study of DOX was performed by mixing the drug in 1.0N NaOH solution and refluxed for 12hrs at 60^0c. The drug was found to be highly degraded in alkaline medium and the percentage of degradation was found to be 85-95% in this stress condition (Fig. 5).

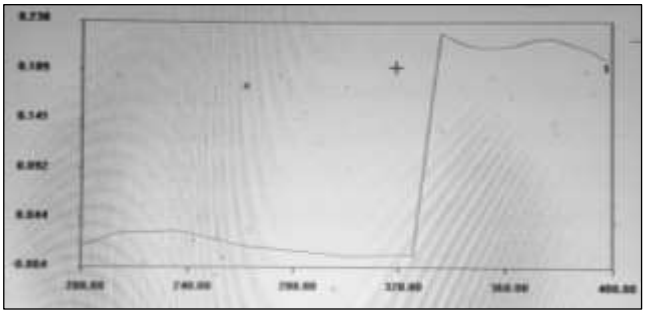

Figure 5: Alkaline-induced degradation of DOX

Neutral (water) condition

In neutral conditions when the prepared drug solution was refluxed for 12hrs at 60^0c. The percentage of degradation was found to be 80-90% (Fig. 6).

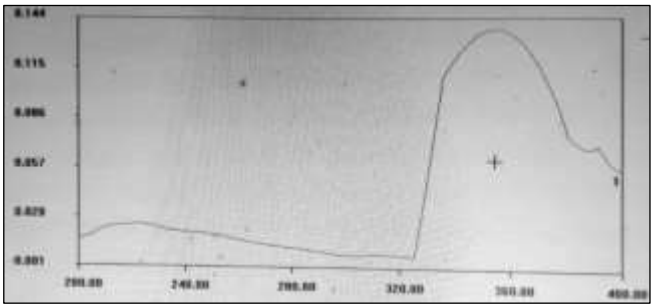

Figure 6: Neutral-induced degradation of DOX

Oxidation studies

In oxidative stressed conditions the drug is exposed to a 3% H_2O_2 solution for 24hrs at room temperature. The result of the study indicates that the drug was degraded by 65-70% in these conditions (Fig.7).

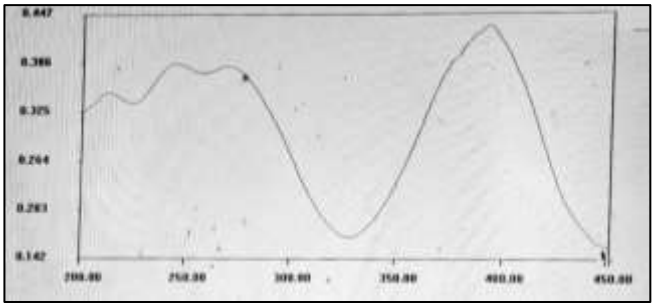

Figure 7: Oxidative degradation of DOX in 3% H_2O_2

Thermal stress study

In stress degradation study under thermal conditions, DOX exposed at 80º C for 24hrs. The degradation was found to be 10-20 % in this stress condition indicates the drug is sensitive to thermal degradation conditions (Fig. 8).

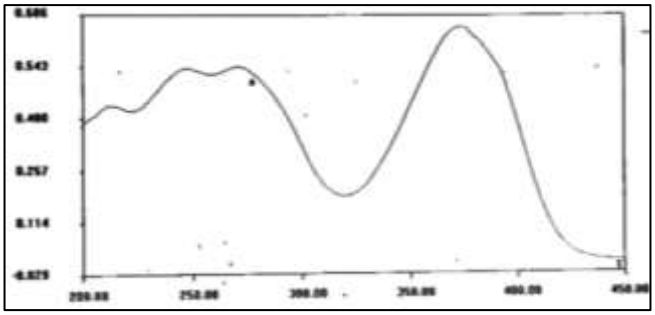

Figure 8: Thermal degradation of DOX

Photolytic studies

Photolytic study was carried out in dry form. Here the drug was directly exposed to the sunlight for 6hrs on a hot sunny day. The drug was found to be labile to sunlight and degraded to 15-20% under this stress condition (Fig.9).

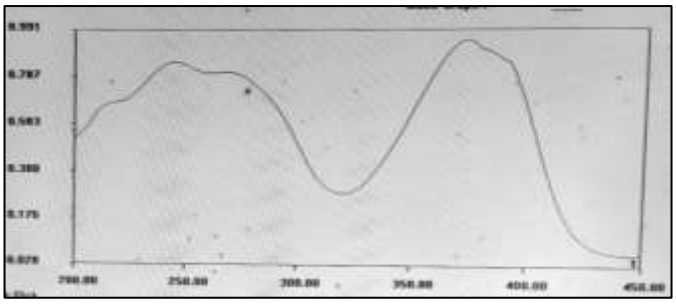

Figure 9: Photolytic degradation of DOX

Table 5: Results of Degradation study of DOX at different stress conditions in developed UV Spectrophotometric method

Different stressed conditions	Percentage of degradation
Acidic condition	5-8
Alkali condition	85-95
Neutral condition	80-90
Oxidation	65-70
Thermal	10-20
Photolysis	15-20

CONCLUSION

The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s). Therefore, it is essential to know the purity profile and behavior of a drug substance under various environmental conditions. In the present study a UV spectrophotometric stability indicating assay method has been developed and validated taking methanol as solvent using absorbance maxima method according to ICH guidelines. The present spectrophotometric method was found to be simple, rapid and economic as compared to reported RP-HPLC method for estimation of drug in its stability samples. The results of validation parameters also showed that the present method was linear, accurate, precise and sensitive. The present method can be used for routine stability indicating assay of doxycycline in fixed dose formulation before going to HPLC or HPTLC methods to save the time.

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Conflict of Interest

The authors have no conflict of interest.

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