WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 2037-2050. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF UV SPECTROMETERIC METHOD FOR ESTIMATION OF DOXYCYCLINE HYCLATE

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Article Received on
09 May 2020,

Revised on 31 May 2020,
Accepted on 21 June 2020,

DOI: 10.20959/wjpr20207-17946

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ABSTRACT

The UV-Spectrometric method was developed and validate for the estimation of the Doxycycline hyclate. For this purpose the SHIMADZU-1800 spectrometer was used. The identification of drug was done by the FTIR Spectrophotometer IRAffinity-1S. The working wavelength 260nm was selected using water as solvent. Using this wavelength the assay and validation for doxycycline hyclate was done. The linearity study was show R^2=0.999 in range 0 ppm to 100 ppm. % recovery of doxycycline at 80%, 100% and 120% Concentration level was found to be 91%, 82%, and 76.8% respectively. The relative standard deviation was found to be within 0.007-0.010 for interday precision. % RSD was found to be less than 2.0%, which is within limit. The relative standard deviation was found to be within 0.0021-0.00280 for intraday precision. % RSD was found to be more than 2.0%, which is not within limit. LOD & LOQ was found to be 2.84 & 8.61 µg/ml for doxycycline drug. In robustness it was observe that minor change in wavelength, the absorbance was not affected. In ruggedness it was observe %RSD was found to be less than 2% which is within the limit.

KEYWORDS: UV-Spectrometric, Doxycycline Hyclate, SHIMADZU, Linearity, precision, Spectrophotometer.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involve a series of process for identification, determination quantification and purification of a substance, separation of the components of a solution or mixture or determination of structure of chemical compounds.

The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, micro-organisms, minerals and various synthetic products.

Spectroscopy is study of interaction between electromagnetic radiation and matter. This interaction takes place in many different ways and over a wide range of radiant energies, most of this interactions are not visible to human eye, but can be measured with suitable instruments. If a beam of white light is passed through a beaker of water, it remains white. If potassium permanganate is added to the water, the white light appears purple after it passes through the solution. This is one example of interaction of electromagnetic radiation or light with matter.

UV-Spectroscopy

The wavelength range of UV radiation starts at the blue end of the visible light about 4000 A^0 and ends at 2000 A^0.

The ultraviolet region is subdivided into two spectral regions.

i. The region between 2000 A^0-4000 A^0 is known as near ultraviolet region.
ii. The region below 2000 A^0 is called the far or vacuum ultraviolet region.

The principle of UV-spectroscopy is based on Lambert's Law and Beer's Law.

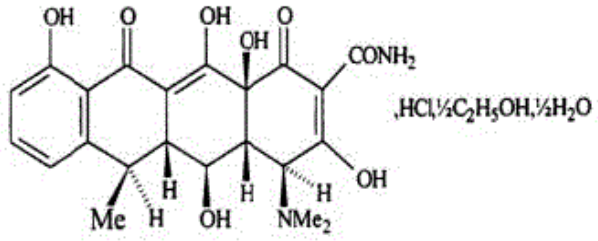
Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

A Source of Radiation
A Wavelength Selector
A Sample Holder
A Detector

The IP has published specific guidelines for method validation for compound evaluation. IP defines eight steps for validation: Specificity, Linearity and Range, Accuracy, Precision, Limit of detection, Limit of quantitation, Ruggedness, Robustness.

Doxycycline hyclate i.e. (4S,4Ar,5S,5Ar,6r,12As)-4-dimethylamino-1,4,4a,5,5a,611,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphacene-2-carboxamide hydrochloride hemiethanolate hemihydrate is antibacterial drug belonging to tetracycline category. Which is orally administered and freely soluble in water, methanol, sparingly

soluble in ethanol. For validation the marketed preparation of doxycycline hyclate DOXY-T having strength 100mg was used.



MATERIAL AND METHOD

Instrument used

Sr. No.	Instrument and Glassware	Manufacturer and Model
1	UV-Visible Spectrophotometer	SHIMADZU-1800
2	FTIR Spectrophotometer IRAffinity-1S	SHIMADZU
3	Ultrasonicator	LABLINE
4	Weighing Electronic Balance	SHIMADZU Corporation (0.001 g readability)
5	Pipettes, Beakers and Volumetric Flask	Borosil
6	Whatman Filtered Paper No.	31

METHOD

UV Method

1) Solubility study: Solubility study is carried out by using water as a solvent.

Procedure

1. Take 2ml of water, add sufficient quantity of drug.
2. Prepare the saturated solution.
3. Filter and make suitable dilution with same.

2) Selection of working wavelength

Procedure

1. The standard solution was scanned in the range of 200-400 nm by using water as a blank
2. Sample solution where also scanned in the range of 200-400 nm.
3. Both standard as well as sample solution given maximum absorption at 260 nm wavelength.
4. Hence, this wavelength was selected for analysis.

Assay Method

1) Selection of solvent: Distilled water is used as a solvent

2) Preparation of standard solution:

1. The pure drug of Doxycycline Hyclate 50mg was weighed and transferred into a 50ml of volumetric flask, make up the volume up to the mark using distilled water and sonicated it for 1-3 minutes.
2. Then pipette out 1ml of this solution and transferred into 100ml volumetric flask and make up the volume up to mark using distilled water as solvent.

3) Preparation of Sample Solution

1. 8 tablet were weight, transferred into a mortar and crushed to fine powder to uniformly mix it.
2. 396mg of average weight of powder was calculated using 100mg equivalent weight.
3. 198mg of this powder was weighed and transferred into a 50ml of volumetric flask, make up the volume up to the mark using distilled water and sonicated it for 1-3 minutes.
4. Then pipette out 1ml of this solution and transferred into 100ml volumetric flask and make up the volume up to mark using distilled water as solvent.
5. Measure the absorbance of standard and sample solution using wavelength 260nm.

1) Linearity

Preparation for standard solution

10mg of doxycycline Hyclate transfer in100ml of volumetric flask make up volume up to 100ml with distilled water (100ppm)

Procedure

1. Pipette out above solution and make different concentration in range of 20-100ppm as per procedure.
2. The Standard solution which is prepared using distilled water which is scan in range of 200-400nm for measurement of wavelength.

2) Precision

Preparation for standard solution

1. Give 100mg Doxycycline Hyclate transfer in100ml volumetric flask make up the volume up to 100ml water (1000ppm).

2. Pipette out 10ml of given solution. Transfer in 100ml volumetric flask make up the volume upto100ml distilled water (100ppm).
3. Pipette out above solution and make a different concentration as per procedure.
4. Measure absorbance of above solution at wavelength 260nm.

Preparation for sample solution

1. Take 0.396gm of crushed Doxycycline Hyclate transfer in 100ml volumetric flask makeup volume up to 100ml with water.
2. Pipette out 1ml of given sample solution transfer in 10ml of volumetric flask make up volume up to 10ml with distilled water.
3. Measure absorbance of this solution and calculate concentration of Doxycycline Hyclate in given solution.

3) Accuracy

Procedure

1. Take 9 volumetric flask the 25ml and label them.
2. Prepare three volumetric flask for the 80%, three volumetric flask for the100%and three flask for the 120%.
3. In 80%solution containing volumetric flask. add 1.5ml sample solution (solution B) and then add 1.2ml of standard solution in that three volumetric flask (solution A)
4. In 100%volumetric flask add 1.5ml of sample solution(solution B) and add 1.5ml of standard solution (solution A) in that volumetric flask which contains label as 100%
5. In 120%volumetric flask add 1.5ml of sample solution (solution B) and add 1.8ml of standard solution (solution A) in that three volumetric flask.
6. Make up the volume up to 25ml all of 9volumetric flask with water.
7. Measure the absorbance of all sample solution at wavelength 260nm.

4) Precision

Procedure

1. Take 6 volumetric flask of 10ml.
2. Pipette out 1ml of standard solution (solution A) in each volumetric flask.
3. Make up volume up to 10ml with water.
4. Measure the absorbance initially, after 1hour,and after hour of sample solution and also measure absorbance intraday initially,1 hour and 2 hour of sample solution at wavelength 260nm

5) Preparation of standard solution for LOD, LOQ, Ruggedness, Robustness

10mg of doxycycline Hyclate transfer in100ml of volumetric flask make up volume up to 100ml with distilled water (100ppm)

6) LOD and LOQ

LOD and LOQ is defined as the absorbance is measured by taking smallest quantity of drug

Procedure

1. Take 6 volumetric flask of 10ml.
2. Pipette out 2ml of standard solution and transferred in 10ml volumetric flask.
3. Make up volume up to 10 ml with water.
4. Measure the absorbance at 260nm.
5. Calculate standard deviation and slope and put it in following formula

Formula:

$$LOD = 3.3 * \frac{\sigma}{s}$$

$$LOQ = 10 * \frac{\sigma}{s}$$

7) Robustness

Procedure

1. Take 6 volumetric flask of 10ml.
2. Pipette out 2ml of standard solution and transferred in 10ml volumetric flask.
3. Make up volume up to 10 ml with water.
4. Measure the absorbance at wavelength 257nm, 258nm, 259nm, 261nm, 262nm, 263nm.

8) Ruggedness

Procedure

1. Take 6 volumetric flask of 10ml.
2. Pipette out 2ml of standard solution and transferred in 10ml volumetric flask.
3. Make up volume up to 10 ml with water.
4. Measure the absorbance at wavelength 260nm.

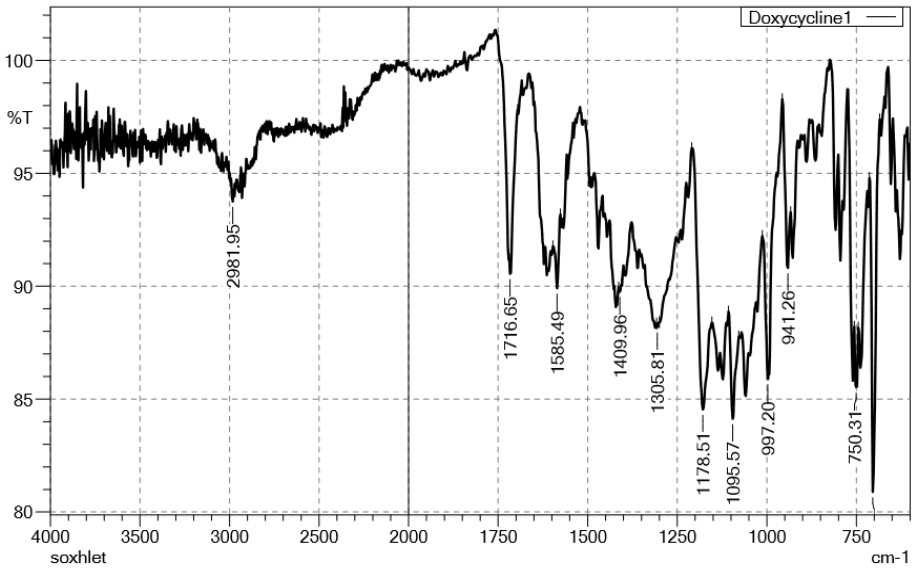
RESULT AND DISCUSSION

1. Solubility study

Table No. 1: Solubility study of doxycycline Hyclate studied using different solvent.

Sr. No.	Solvent	Solubility status
1	Water	Freely soluble
2	Methanol	Freely soluble
3	Ethanol	Sparingly soluble
4	Chloroform	Practically insoluble

2. Identification of Drug

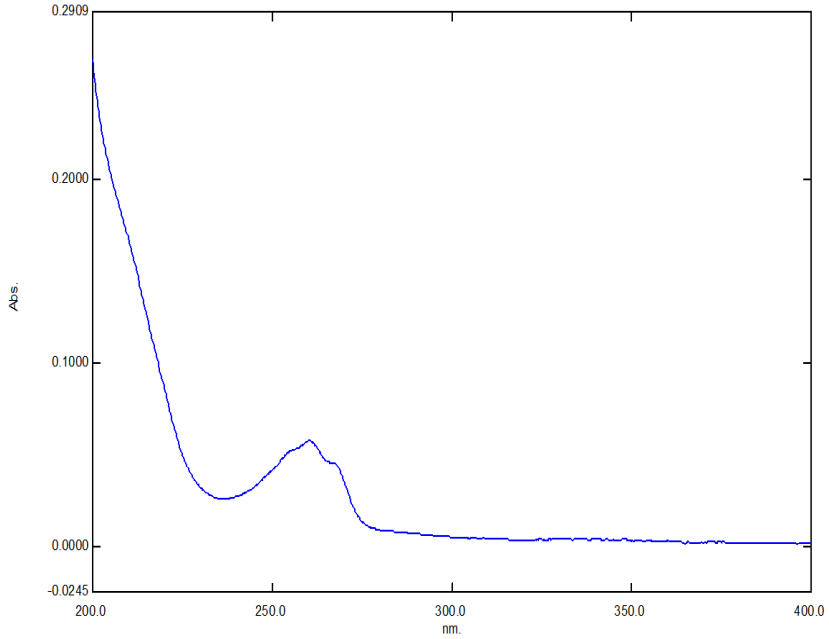


"FIG. 1" Identification of Doxycycline hyclate by IR Spectra.

3. Development and optimization of Analytical Method

1) Determination of Working Wavelength in Distilled water

Wavelength of Doxycycline succinate in distilled water was found to be 260nm. The resulting spectacular are shown in figure which shows the absorption Maxima at 260nm for doxycycline succinate.



"Fig.2" UV Spectra of Doxycycline hyclate in distilled water.

2) Assay method Development

Table No. 2 Results for estimation of doxycycline in tablet dosage form by spectrophotometric method.

Tablet formulation	Label claim	Sample absorbance	Standard absorbance	Wavelength	LOD	%assay
Doxycycline Hyclate	100mg	0.330	0.083	260	2.48	103.3%

3) Validation of analytical method for UV

1. Linearity

Linearity of doxycycline drug

Table No. 3: Linearity range of doxycycline hyclate in water as solvent.

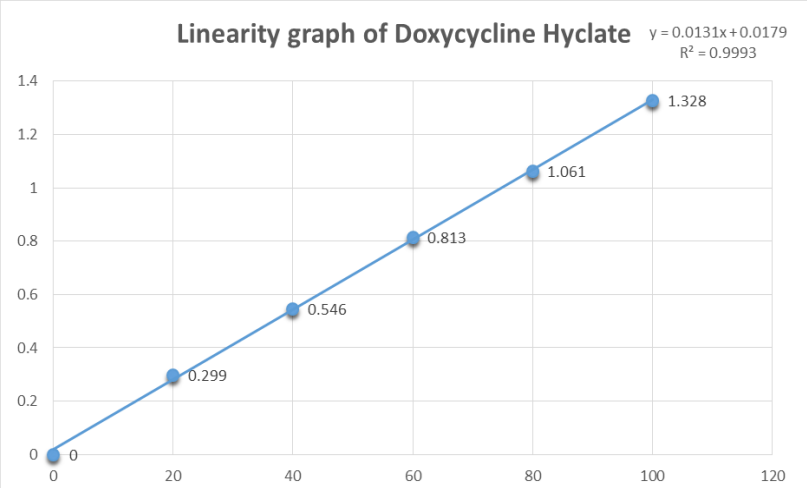
Sr. No.	Concentration(ppm)	Absorbance
1.	0	0.0
2.	20	0.299
3.	40	0.546
4.	60	0.813
5.	80	1.061
6.	100	1.328

R^2=0.999

Linearity Equation:-Y=0.013x+0.0179

Acceptance criteria:-correlation coefficient (r) should not less than 0.997.

Graph



"Fig.3" Linearity graph of Doxycycline hyclate by UV Spectra.

2. Accuracy

Table No. 4: Reading of accuracy.

Sr.	Conc. Level	Sample	Standard		Absorbance	Mean of Absorbance	% Recovery
1.	80%	1.5ml 1.5ml 1.5ml	1.2ml 1.2ml 1.2ml	Make up Volume 25ml	0.231 0.245 0.236	0.237	91%
2.	100%	1.5ml 1.5ml 1.5ml	1.5ml 1.5ml 1.5ml	With Distilled Water	0.257 0.256 0.253	0.255	82%
3.	120%	1.5ml 1.5ml 1.5ml	1.8ml 1.8ml 1.8ml		0.277 0.275 0.275	0.275	76.8%

Observation: % recovery of doxycycline at 80%, 100% and 120% concentration level was found to be 91%, 82%, and 76.8% respectively.

3. Precision

Inter day reading

Table No. 5 Inter day reading of precision.

Volume of standard	Initial reading	After 1 hour	After 2 hour
1ml	0.110	0.109	0.107
1ml	0.108	0.110	0.109
1ml	0.111	0.112	0.111
1ml	0.109	0.108	0.109
1ml	0.110	0.107	0.108
1ml	0.113	0.111	0.112
Mean	0.110	0.109	0.109
S.D.	0.00173	0.00194	0.001897
RSD	0.01584	0.017	0.0174
%RSD	1.58%	1.77%	1.74%

Acceptance criteria: % RSD should be less than 2.0%

Observation: The relative standard deviation was found to be within 0.007-0.010 for interday precision. % RSD was found to be less than 2.0%, which is within limit.

Intraday reading

Table No. 6 Intraday reading of precision.

Volume of standard	Initial reading	After 1 hour	After 2 hour
1ml	0.109	0.113	0.112
1ml	0.112	0.109	0.109
1ml	0.114	0.109	0.108
1ml	0.107	0.110	0.111
1ml	0.111	0.108	0.108
1ml	0.110	0.105	0.104
Mean	0.110	0.109	0.108
S.D.	0.00248	0.00260	0.00279
RSD	0.0225	0.02385	0.0256
%RSD	2.25%	2.38%	2.56%

Acceptance criteria- %RSD should be less than 2%.

Observation: The relative standard deviation was found to be within 0.0021-0.00280 for intraday precision. % RSD was found to be more than 2.0%, which is not within limit.

4. Limit of Detection and Limit of Quantification.

Table No. 7 Reading of LOD & LOQ.

Sr. No.	Conc. in ml		Absorbance
1.	2ml	Make up	0.245
2.	2ml	the volume	0.235
3.	2ml	up to 10ml	0.220
4.	2ml	with	0.224
5.	2ml	Distilled water	0.219
6.	2ml		0.216

Observation- LOD & LOQ was found to be 2.84 & 8.61 µg/ml for doxycycline drug.

5. Robustness

Table No. 8 Reading of Robustness.

Sr. No.	Wavelength	Concentration in ml	Absorbance
1.	257	2ml	0.116
2.	258	2ml	0.117
3.	259	2ml	0.119
4.	261	2ml	0.120
5.	262	2ml	0.122
6.	263	2ml	0.124

Acceptance criteria- %RSD should be less than 2%.

Observation- It was observe that minor change in wavelength, the absorbance was not affected and %RSD was found to be more than 2%which is not within the limit.

6. Ruggedness

Table No. 9: Reading of ruggedness.

Sr. No.	Concentration in ml		Absorbance
1.	2ml	Make up	0.111
2.	2ml	the volume	0.108
3.	2ml	up to 10ml	0.115
4.	2ml	with	0.110
5.	2ml	Distilled water	0.113
6.	2ml		0.109

Acceptance criteria- %RSD should be less than 2%.

Observation- It was observe %RSD was found to be less than 2% which is within the limit.

CONCLUSION

The result of the experiment indicate that the analytical UV method was developed for the quantitative determination of Doxycycline Hyclate 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 260 nm 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.

ACKNOWLEDGEMENTS

I offer my sincere adoration to God almighty who created me the strength and courage to complete my dissertation and given me opportunity to thank all those people through whom this groove delivered to me. I would like to bring to light all those who helped me in completion of my research work.

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