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FIRST ORDER UV SPECTROPHOTOMETRY METHOD DEVELOPMENT AND VALIDATION OF DOXYCYCLINE HYCLATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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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 hemiethananolate hemihydrates. It can be obtained from oxytetracycline or methacycline. It is highly stable in normal human serum. There are several methods reported such as HPLC, Spectrophotometry, HPTLC, etc for the estimation of doxycycline hyclate but best of our knowledge no first order spectrometry method is published for the estimation of Doxycycline hyclate. Hence, we developed simple, accurate, rapid, specific the first order UV spectrometry method for estimation of doxycycline hyclate in bulk and pharmaceutical dosage form. The absorption maxima were found to be 266nm in first order. Water was used as a solvent for the experiment. Doxycycline hyclate shows linear response between 14.4 to 33.6µg/ml. And correlation coefficient were found to be 0.99 with linear equation y = 0.000568x+0.00015. % RSD of system precision and method precision were found to be 0.7975 and 1.0172 respectively. Centrifuge and no. 0.45 micron filter were found to be suitable to be suitable for the method. Accuracy were performed at 80%, 100% and 120% level and were found to be 100.5%,100.8% and 99.5% respectively. Therefore, system precision, method precision, accuracy was found to be within the specified limit of ICH guideline.

KEYWORDS

First order validation, UV spectrophotometer, Doxycycline hyclate and First order spectrum.

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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 hemiethananolate hemihydrates[1]. Doxycycline hyclate is freely soluble in water and in methanol, sparingly soluble in ethanol (96 per cent). It also dissolves in solution

of alkali hydroxide and carbonates[3]. Doxycycline hyclate is a salt form of Doxycycline. It's 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 sexual transmitted diseases[2]. 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.

Molecular Formula - C22H24N4O8, HCL, 1/2C2H6O,1/2H2O

Molecular Weight - 513.0

The review of literature revealed that the various analytical spectrometry, HPLC and RP-HPLC methods are published for doxycycline hyclate, but no first order uv validation method was published for doxycycline hyclate[4-9].Therefore, the objective behind this work is to introduce a new analytical pathway or method i.e. first order UV spectrophometry for estimation of doxycycline hyclate.

MATERIAL AND METHODS

Marketed preparation

Doxycycline hyclate containing capsule made by SCOSHIA pharmaceutical was used.

PREPARATION OF STANDARD SOLUTION

The API of Doxycycline hyclate 20 mg was weighted and transferred into a 100 ml of volumetric flask, and then some ml of water is added and sonicates 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 and analysed the standard solution at 266 nm after derivatizing the spectrum.

PREPARATION OF SAMPLE SOLUTUION

10 capsules were weight and drug powder was removed and weight of empty capsule was taken and average weight of powder was calculated. then equivalent weight to 20mg were weight and transferred into 100ml volumetric flask , add some amount of water and sonicate for certain period of time then shake it for 15 minutes. Appropriate aliquots were pipette out into 25 ml volumetric flask and make up the volume with water up to mark. Final solution were filtered from whatmann 41 and analysed at 266 nm wavelength after derivatizing the spectrum.

Absorption maxima

The standard solution were scanned in the range of 200-400nm by using water as a blank and sample solution were also scanned in the range of 200-400nm and the derivatives the zero order spectrum into the first order spectrum.

Both standard and sample solution gave a maximum absorption at 266nm wavelength after derivatizing the spectrum and this wavelength was selected for analysis.

VALIDATION OF ANALYTICAL METHODS

PRECISION

System precision

Same concentrations of six standard solutions were prepared and six time reading were taken by using uv/vis double beam spectrophotometer.

Method precision

Same concentrations of six sample solutions were prepared and six time spectrum were recorded by using uv/vis double beam spectrophotometer and derivatized the zero order spectrum to first order spectrum.

Linearity

Fresh aliquots were prepared from stock solution ranging from 14.4 to 33.6µg/ml and the absorbance value of each concentration was recorded at 266nm by derivatizing spectrum

The drug shows linear response in the range of 14.4 to 33.6 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 determine 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.

System suitability

All the parameters of validation were found to be within the limit of ICH guideline.

RESULTS AND DISCUSSION

System Precision

System precision was performed by analysing the six standard solution of Doxycycline hyclate. The % RSD was found to be within the range i.e. below 2. Therefore, it describes that method is precise.

Method Precision

Method precision was performed by analysing the six solution of sample preparation. And % RSD was found to be 1.0172. It describes that the method is precise.

Linearity

Linearity was performed in the range of 60% to 140% and correlation coefficient was found to be 0.99. So, it reveals that the doxycycline hyclate shows linear response in the range of 14.4 µg to 33.6 µg per ml concentration.

Filter suitability

Filter suitability was performed by using filter whatmann 41, no.45 micron and centrifudge. The correlations of no.45 micron filter and centrifuged were found to be 0.98 and 1 respectively. Therefore, centrifuge and no. 45 micron filter were found to be suitable.

Stability of sample solution

Stability of sample solution was analysed at 2 hours, 4 hours, 6 hours and 24 hours with respect to initial sample solution. Therefore, it shows that the sample solution is stable up to 24 hours.

Accuracy

Accuracy was performed at 80%, 100% and 120% and it was found to be 100.5%, 100.8% and 99.5% respectively that is within the range from 98.0% to 102%. Therefore, method is accurate.

Instrument

S.No	Instrument	Model no	Manufacturer
1	Balance	CA 123	contech
2	UV	1800	shimadzu
3	Sonicator	KI-1.5	Kroma Tech, India
4	Centrifuger	BL-135 D	BIO-LAB
5	Milli Q water	NV00922/08/70001	LAB Q ULTRA
6	Oven	EHT-169	EXPO HI-TECH

Chemicals and reagent

S.No	Chemical	Supplier
1	Sodium hydroxide	CHEMDYES CHEMICAL
2	Hydrochloric acid	CHEMDYES CHEMICAL
3	Hydrogen peroxide	SD FINECHEMICALS

Table No.1: System precision

		Interday	Intraday
S.No	**Sample**	**Absorbace**	**Absorbance**
1	STD-1	0.0132	0.0132
2	STD-2	0.033	0.0133
3	STD-3	0.0132	0.0131
4	STD-4	0.0131	0.0131
5	STD-5	0.0130	0.0130
6	STD-6	0.0132	0.0132
7	Avg	0.013166	0.0131
8	SD	0.001033	0.0001049
9	% RSD	0.7844	0.7975

SD=standard deviation, RSD= relative standard deviation, Avg= average

Table No.2: Result of method precision

S.No	Sample	Absorbance	Absorbance
1	SMPL-1	0.0135	0.0135
2	SMPL-2	0.0136	0.0136
3	SMPL-3	0.0138	0.0135
4	SMPL-4	0.0135	0.0134
5	SMPL-5	0.0135	0.135
6	SMPL-6	0.0136	0.0138
7	**Avg**	0.01383	0.01355
8	SD	0.0001169	0.0001378
9	% RSD	0.8606468	1.0172

SD= standard deviation, RSD = relative standard deviation, Avg= average

Table No.3: Result of linearity study

S.No	Concentration(µg/ml)	Absorbance
1	14.4	0.0081
2	19.2	0.0111
3	24	0.014
4	28.8	0.017
5	33.6	0.0188
6	slope	0.00056875
7	Intercept	0.00015
8	correlation	0.99

Table No.4: Result of filter suitability

S.No	Sample ID	% ASSAY	Correlation
1	Whatmann no. 41	99.59	-
2	Centrifuge	100.07	1
3	No.0.45micron	98.67	0.98

Table No.5: Result of stability of sample solution

S.No	Time interval	% Assay	Correlation
1	initial	99.82	-
2	2 Hours	97.02	0.98
3	6 Hours	98	0.99
4	24 Hours	98.10	0.99

Table No.6: Result of accuracy

S.No	Level	Test amount(µg/ml)	Spiked std amount(µg/ml)	Total amount recovered (µg/ml)	% Recovery
1	80	10	10	19.10	100.5
2	100	12	12	24.2	100.8
3	120	15	15	29.80	99.5

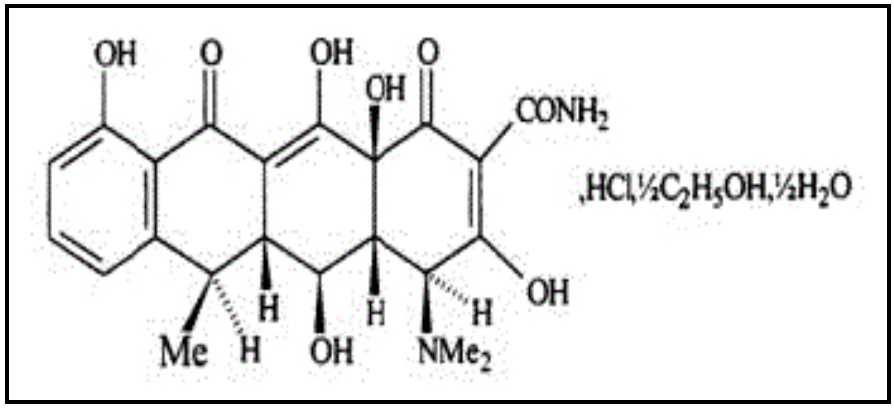

Figure No.1: Structure of doxycycline hyclate

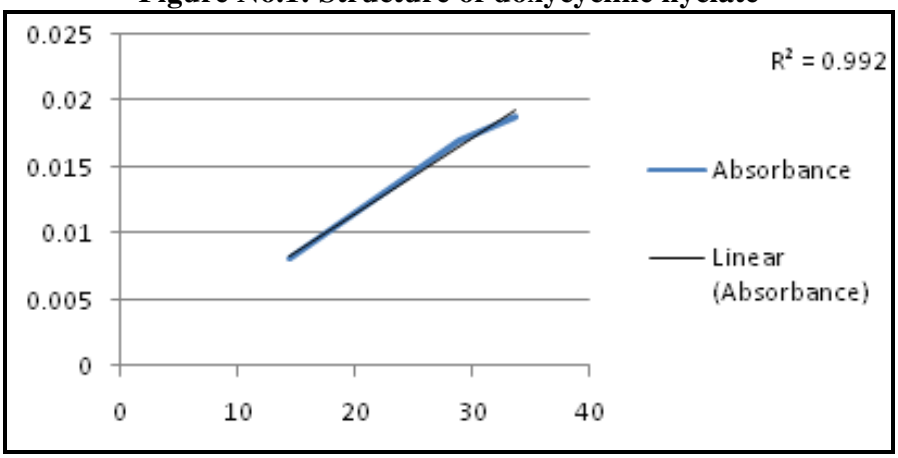

Figure No.2: Calibration curve of linearity

SPECTRUM

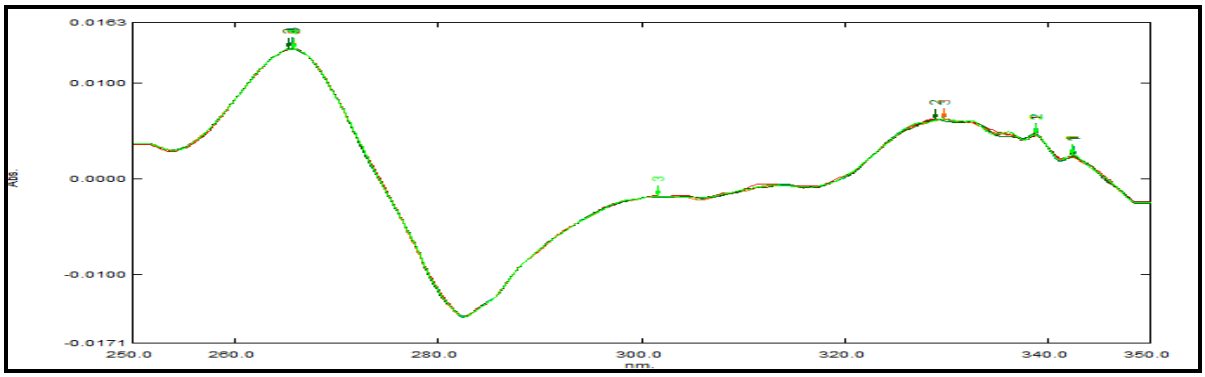

Figure No.3: Overall spectrum of system precision

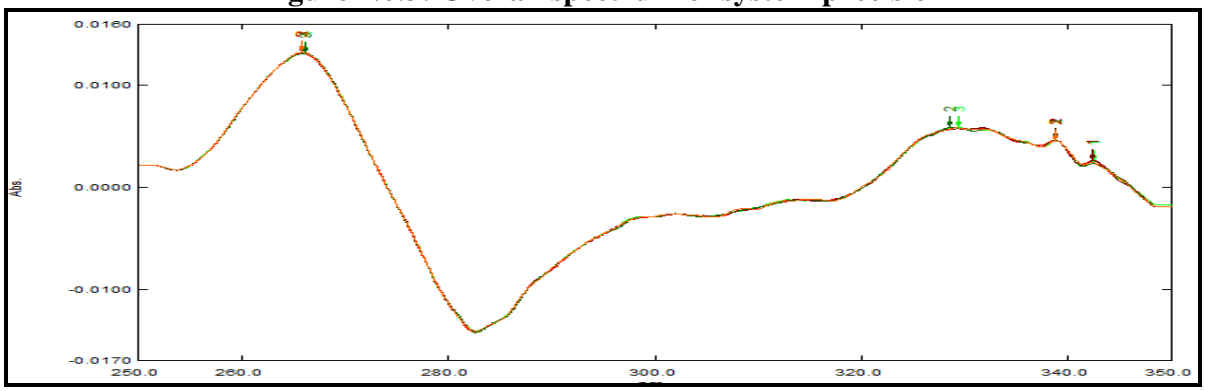

Figure No.4: Overall spectrum of method precision

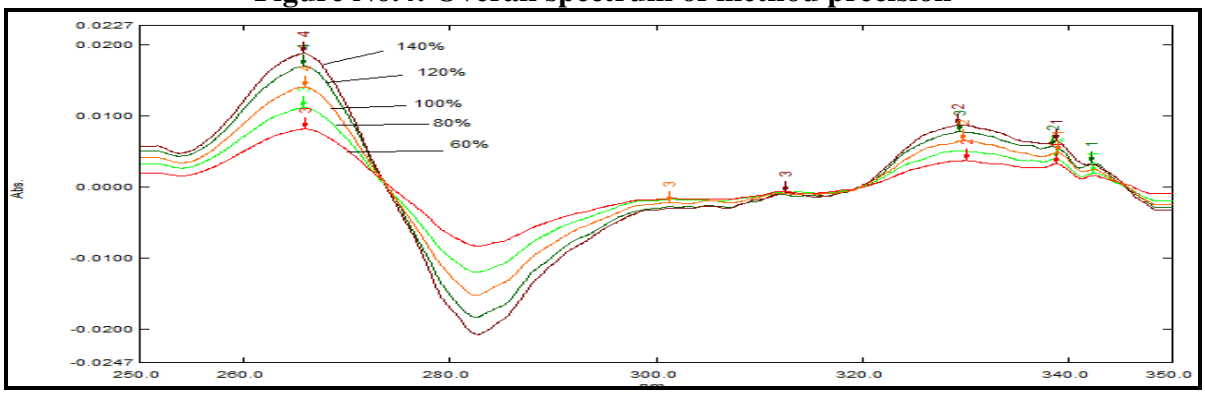

Figure No.5: Spectrum of linearity

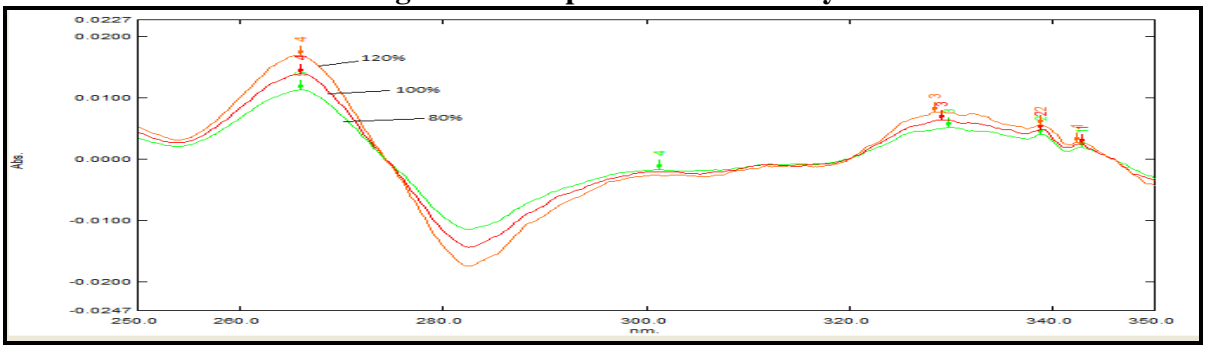

Figure No.6: Spectrum of accuracy

CONCLUSION

The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, filter suitability, stability of solution. This method can be used for the routine analysis and quality control assay of doxycycline hyclate in bulk and pharmaceutical formulation. The % RSD of system precision and method precision were found to be 0.7975 and 1.0172 respectively and sample solution were found to be stable for 24hours. Therefore, it concludes that the method is time saving, economical for the estimation of Doxycycline hyclate in bulk and pharmaceutical dosage form.

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CONCLIFICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Indian Pharmacopoeia 2010(2), doxycycline hyclate, 1257-1258.
2. British pharmacopoeia 2009(1), doxycycline hyclate, 729.
3. Indian Pharmacopoeia 2010(2), doxycycline hyclate, 2, 1258.
4. Kogawa A C and Salgado H R N. Quantification of Doxycycline Hyclate in Tablets by Ultraviolet Spectrophotometric Method, *World Research Journal of Pharmaceutical Research,* 1(2), 2013, 021-024.
5. Hasan *et al.* Quantification of doxycycline hyclate in different pharmaceutical samples, *European Journal of Biological Research,* 6(4), 2016, 275-286.
6. Pavagada J R. *et al,* Development and validation of RP-HPLC method for the determination of doxycycline hyclate in spiked human urine and pharmaceuticals, *Journal of Pre-Clinical and Clinical Research,* 4(2), 2010, 101-107.
7. Nahla S. Elshater *et al,* Validation of HPLC Method for Determination of Doxycycline Hyclate in Turkey Serum, *Egypt, J. Chem. Environ. Health,* 2(2), 2016, 282 -291.
8. Jain P *et al,* Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation, *Int. J. Drug Dev. and Res.,* 3(4), 2011, 194-199.
9. Chaudhari R. Developments and validation of first order derivative spectrophotometric method for estimation of lacosamide in bulk and tablet dosage form, *Asian journal of pharmaceutical and clinical research,* 6(3), 2013, 162-164.

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