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Spectrophotometeric Methods for Determination of Doxycycline in Tablet Formulation

AMIT KUMAR[1*], SANJU NANDA[2] AND RAJIV CHOMWAL[3]

[*1,3]Swami Keshvanand Institute of Pharmacy Bikaner, Rajasthan. 334001,India

[2]M D University, Rohtak, Haryana,India

[*]Corres.author: amitchomwal@yahoo.co.in
Telephone No. – 09928265745

Abstract: Two simple, economical, precise and reproducible visible spectrophotmetric methods have been developed for the estimation of doxycycline in tablet formulation. The developed methods are based on the formation of chloroform extractable complex of doxycycline with bromo phenol blue and safranine in double distilled water. The complex with bromo phenol blue (method I) shows absorbance maxima at 413 nm and linearity in the concentration range of 5-40 µg/ml. The extracted complex with safranine (Method II) shows absorbance maxima at 525.6 nm and the linearity in the concentration range of 2.5-20 µg/ml. Results of analysis for both the methods were validated statistically and by recovery studies.
Key words: Doxycycline; visible spectrophotometry; quantitative estimation; bromo phenol blue; safranine.

Introduction and Experimental

Doxycycline, chemically (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro tetracene-2-carboxamide, is an well known broad spectrum antibiotic, was derived synthetically from oxytetracycline. It is a bacteriostatic inhibiting the bacterial protein synthesis due to the disruption of transfer RNA and messenger RNA at the ribosomal sites[1]. It is also used for malaria treatment[2]. It is official in IP[3], BP[4], USP[5] and Merck Index[6]. This describes thin layer chromatographic method for its quantitation[2]. Literature survey reveals two HPLC methods [7, 8], has been developed for estimation of doxycycline in human plasma. The objective of the present investigation was to develop simple, accurate and economical spectrophotometric methods for quantitation of doxycycline in tablet formulation. Shimadzu UV 1700, UV/Vis double beam spectrophotometer with spectral band width of 1 nm, wavelength accuracy of ± 0.3 nm and 1.0 cm matched quartz cells was used for analytical method development. All the chemicals and reagent used were of analytical grade. Bromo phenol blue (Loba Chemie. Ltd., Mumbai) reagent and safranine (S. D. Fine Chem. Ltd., Mumbai) reagent were prepared in double distilled water. Both the reagents were extracted several times with chloroform so as to remove chloroform soluble impurities. Tablet formulations of doxycycline [Doxit (Dr.Reddy Laboratories Industries, Ahemdabad), Doxicip (Cipla Limited Mumbai) were procured from local pharmacy. Standard solution of doxycycline was prepared by dissolving 10 mg in 100 ml of double distilled water to give stock solution of concentration 100 µg/ml of drug.

Procedure for preparation of calibration curve

For method I, in a series of 10 ml volumetric flask, aliquots of standard drug solution (100 µg/ml) in double distilled water were transferred and diluted with same so as to give several dilutions in concentration range of 5-40 µg/ml of doxycycline. To 5 ml of each dilution taken in a separating funnel, 5 ml of bromo phenol blue (0.25 % w/v) reagent and 5 ml of chloroform was added. Reaction mixture was shaken gently for 5 min and allowed to stand so as to

separate aqueous and chloroform layer. The chloroform layer was separated out and absorbance maxima measured at 413.0 nm (Fig. 1) against a reagent blank. Calibration curve was plotted between concentration of doxycycline and measured absorbance. Spectral characteristics of doxycycline are given in table 1.

For method II, in a series of 10 ml volumetric flask, aliquots of standard drug solution (100 µg/ml) in double distilled water were transferred and diluted with same so as to give several dilutions in concentration range of 2.5-20 µg/ml of doxycycline. To 5 ml of each dilution taken in a separating funnel, 5 ml of safranine reagent (0.25 % w/v) and 5 ml of chloroform was added. Reaction mixture was shaken gently for 5 min and allowed to stand so as to separate aqueous and chloroform layer. The chloroform layer was separated out and absorbance maxima measured at 525.6 nm (Fig. 2) against a reagent blank. Calibration curve was plotted between concentration of doxycycline and measured absorbance. Spectral characteristics of doxycycline are given in table 1.

Procedure for Analysis of Tablet Formulation

For analysis of tablet formulation 20 tablets (100 mg) of doxycycline were weighed accurately and finely powdered. An accurately weighed powdered sample equivalent to 10 mg of doxycycline was taken in a 100 ml volumetric flask containing 40 ml of double distilled water, sonicated for 10 min. The resultant solution was filtered through Whatman filter paper no. 41 into another 100 ml volumetric flask. The filter paper was washed several times with double distilled water. The washings were added to the filtrate and final volume was made up to the mark with double distilled water.

For method I, 2 ml of filtrate of the sample solution was diluted to 10 ml with double distilled water. These were treated as per the procedure for preparation of calibration curve and amount of the drug present in sample was computed from respective calibration curve.

For method II, 1 ml of filtrate of the sample solution was diluted to 10 ml with double distilled water. These were treated as per the procedure for preparation of calibration curve and amount of the drug present in sample was computed from respective calibration curve.

The procedure of analysis from tablet formulations for both the methods was repeated five times with two different tablet formulations and results are reported in table 2.

Recovery Studies

Recovery studies were carried out for both the developed methods by addition of known amount of standard drug solution of doxycycline to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by proposed methods. The recovery was in the range of 98-99 % for Method I and 98-100 % for Method II. The results of recovery studies are reported in table 2.

Result and Discussion

These proposed methods were found to be simple, accurate, economical and rapid. Recovery studies were found close to 100 % that indicates accuracy and precision of the proposed methods. The statistical analysis was carried out and results of which were found satisfactory. Standard deviation values were found low that indicated reproducibility of the proposed methods. It was observed that excipients did not interfere in the determination of doxycycline. Hence these developed methods could be used for routine estimation of doxycycline from tablet formulations.

TABLE 1: SPECTRAL CHARACTERISTICS OF DOXYCYCLINE

PARAMETERS	METHOD I	METHOD II
λ max	413.0 nm	525.6 nm
Beer's law limit (µg/ml)	5-40 µg/ml	2.5-20 µg/ml
Regression equation* (y = a + bc)	y = 0.3802+ 0.0236c	y = 0.0633+ 0.0814c
Slope (b)	0.0236	0.0814
Intercept (a)	0.3802	0.0633
Correlation coefficient (r^2)	0.9997	0.9996

* y = a + bc, where c is the concentration in µg/ml and y is the absorbance unit of five replicate samples.

TABLE 2: RESULTS OF ANALYSIS AND RECOVERY STUDIES OF COMMERCIAL FORMULATIONS OF DOXYCYCLINE

METHOD	FORMULATION	LABEL CLAIM (mg/tab)	% LABEL CLAIM*	% RECOVERY**	SD
I	DOXT	100	99.53	98.99	± 0.449
	DOXICIP	100	99.52	98.76	± 0.544
II	DOXT	100	99.60	98.96	± 0.668
	DOXICIP	100	99.42	99.71	± 0.466

* Average of five determinations.
** Average of determinations at three different concentration levels.
SD- Standard Deviation

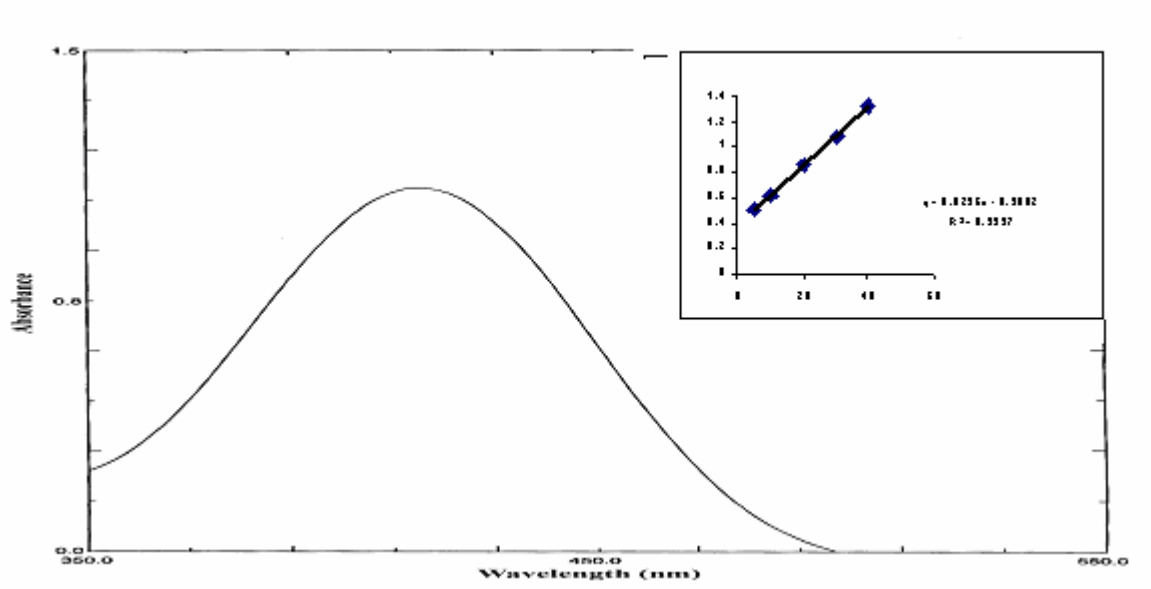

Fig. 1: Spectra of Doxycycline using Method I

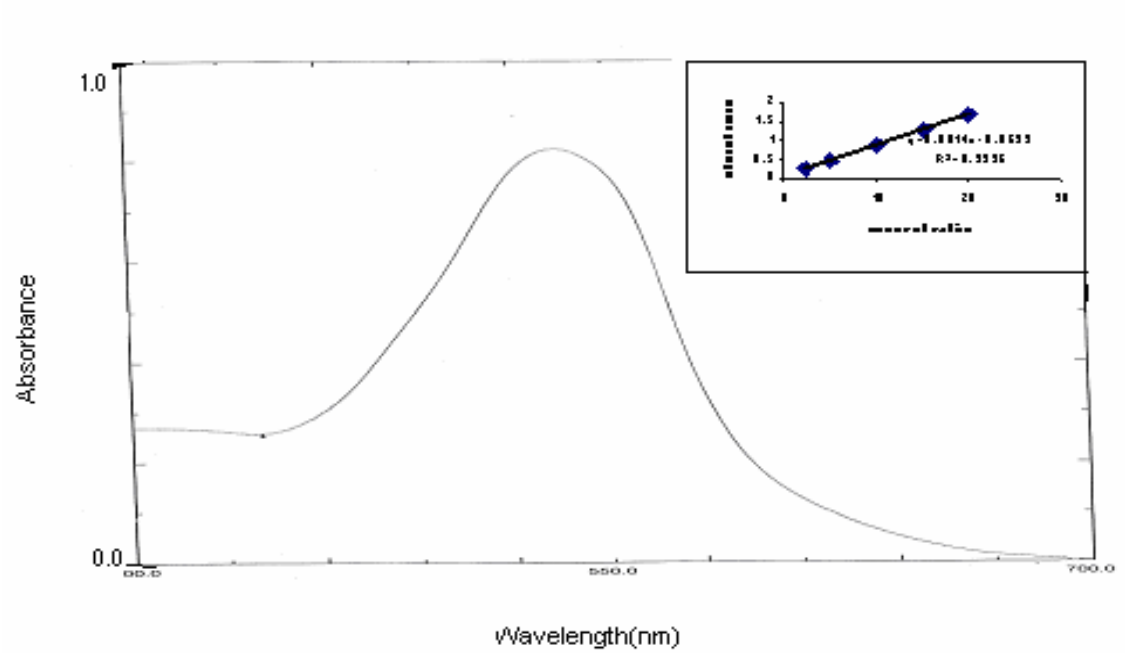

Fig. 2: Spectra of Doxycycline using Method II

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