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RP-HPLC method development and validation of doxycycline in bulk and tablet formulation

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ABSTRACT

A Simple, accurate and rapid RP-HPLC method has been developed for the estimation of doxycycline (DOXY) in bulk and pharmaceutical dosage forms using an Altima C 18, 150 x 4.6 mm i.d, 5 µm particle size in isocratic mode; with mobile phase comprising of buffer (0.1% of potassium dihydrogen phosphate) and acetonitrile in the ratio 60:40 (v/v). The flow rate was 1 ml/min and detection was carried out by photodiode array detector at 268 nm. The retention time for DOXY was found to be 2-897 min. The proposed method has permitted the quantification of DOXY over linearity in the range of 25 – 150 µg/ml and its percentage recovery was found to be 99.134 – 101.997 %. The % RSD of intraday and inter day precision were found 0.7999% and 1.3%.

Keywords: Doxycycline, RP-HPLC, Validation and method development.

INTRODUCTION

DOXY is an antibiotic. Chemically, DOXY is (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 monohydrate with empirical formula of $C_{22}H_{24}N_2O_8.H_2O$, molecular weight 462.5 and CAS number 17086-28-1.[1]. It is official in IP [2], USP [3], Merck index [4,5]. The chemical structure of DOXY was shown in figure.1. DOXY is readily and almost completely absorbed from the gastrointestinal tract and absorption is not significantly affected by the presence of food in stomach or duodenum. Mean peak plasma concentrations of 2.6µg/ml have been reported hours after a 200-mg dose by mouth, falling 1.45µg/ml at 24 hours. From 80-95% of doxycycline in the circulation reported to be bound to plasma proteins. Its biological half life varies from about 12 to 24 hours. However, the

kinetics of doxycycline has been reportedly altered in patients receiving drugs which induce hepatic metabolism. DOXY is 40% of dose slowly excreted in the urine, although more excreted by this route if the is made alkali. However, the majority of a dose of DOXY is excreted in the faeces following chelation in the intestine[5]. Literature review reveals that few analytical methods were evoked for the estimation of DOXY by spectrophotometry[6,7-9], visible spectrophotometry [10-14], fluorimetry [15], establishment of impurities profile by HPLC [16] and estimation of drug content in bulk and pharmaceutical dosage forms by HPLC was reported [17-20]. The liquid chromatographic method for the determination of DOXY is the choice of some pharmacopoeias [2-4]. We here in report a simple, rapid and reliable RP-HPLC for the estimation of DOXY in bulk and pharmaceutical dosage forms.

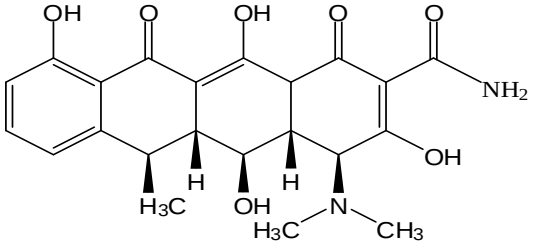


Fig:1. Structure of doxycycline

EXPERIMENTAL

REAGENTS AND MATERIALS

Pure standard of DOXY (99.6%) was obtained as gift sample from Ranboxy Pharma Ltd, New Delhi. HPLC grade acetonitrile (Rankem, avantor chemicals, gujarat), HPLC grade water, potassium dihydrogen phosphate, Triethylamine, and ortho phosphoric acid were obtained from Merck specialities pvt ltd, Mumbai, TETRADOX tablets (Randoxy (Stancare) pharma ltd), high precision weighing balance (wensar instruments, hyderabad), micro pipette (in labs,10-100 µl) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven whenever required. Working environment was maintained in between 20^{o}C ±2.

HPLC APPARATUS AND CHROMATOGRAPHIC CONDITIONS

The analysis was performed on Waters 2695 HPLC system with Waters2996 Photodiode Array detector. Data acquisition was performed by using Empower 2 software. Altima, C18 column (150 x 4.6mm, 5µ) was used as stationary phase. Injections were performed by the manual injector with 10µl. Different mobile phases were tested in order of their polarity to find out the best conditions for the separation of doxycycline. The selected mobile phase Acetonitrile and Potassium Dihydrogen Phosphate buffer of 0.1% (pH 3.1) in the ratio of 40:60%v/v gave acceptable retention time (RT). The flow rate was maintained at 1.0 mL min^{-1}, with a run time of 6 min. the mobile phase was filtered by using 0.45µ filter and it was degassed by sonication prior to use. All determinations were made at ambient temperature. The eluent was detected at 268nm.

PROCEDURE RECOMMENDED

PREPARATION OF MOBILE

Potassium Dihydrogen Phosphate Buffer (0.1% of KH_2PO_4) and acetonitrile taken in the ratio 60:40 (v/v) were employed as a mobile phase.

PREPARATION OF STOCK SOLUTION

A stock solution was prepared by accurately weighed and transferred 10mg of DOXY working standards into a 10ml clean dry volumetric flask, add 7ml of diluent (Water and acetonitrile 50:50), sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1ml was pipette out into a10ml volumetric flask and then make up to the final volume with diluent.

CONSTRUCTION OF LINEARITY

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.25 – 1.5 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 25 – 150µg/ml.[21,22] the prepared solutions were filtered through 0.45µm membrane filter and each of the dilutions was injected three times into the column. The

calibration curve for DOXY was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 25-150µg/ml with good correlation in between concentration and mean peak area.

ASSAY

20 tablets were weighed and the contents were removed to obtain the average weight powder. A sample of the powder claimed to contain 100mg of active ingredient, was mixed with 70ml of diluent. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the volume made up with diluent and the resulting solution was passed through 0.45µm membrane filtered. From the filtered stock solution of 1mg/ml an aliquot of this solution (0.2ml) was transferred to a volumetric flask and made up to a sufficient volume with diluent to get desired concentration of 20µg/ml. The prepared dilution was injected three times into the column to obtain chromatogram. From that peak area, the drug content in the tablet was quantified. [23,24]

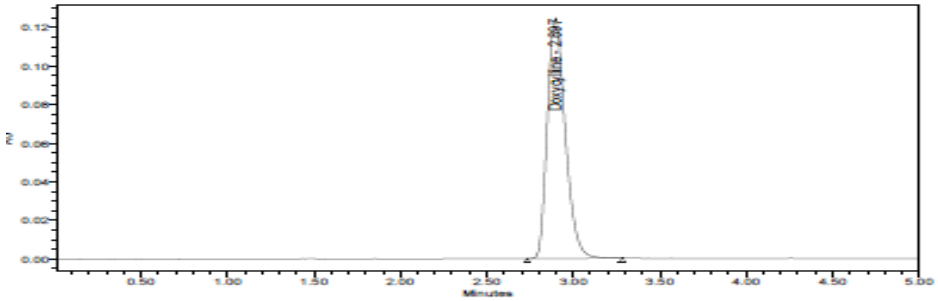


Fig 2: A typical chromate gram of DOXY

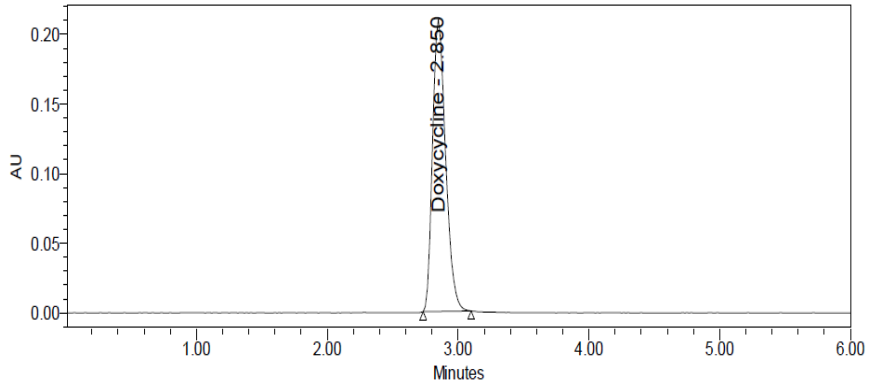


Fig:3.Chromatogram showing the assay of Doxycycline marketed dosage form (TETRADOX tablets).

RESULTS

The present RP-HPLC method for the quantification of DOXY in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method with significant shorter retention time of 2.897.min. The linearity for the detection of DOXY was 25-150µg/ml with (R^2 = 0.999; y = 15790x + 1138.7) the coefficients of variation based on mean peak area for three replicate injections were found to be 0.999. Results were shown in table-1 and statistical data of calibration curves were shown in table-2. The intraday and inter day variations of the method were determined using five replicate injections analyzed on the same day and next day over a period of 24 hours.[21] The result revealed the precision with %RSD of 0.7999 and 1.3, respectively for intraday and inter day. Results were shown in table-3. To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analyzed sample and contents were reanalyzed by

the proposed method.[21,27] Accuracy was evaluated by injecting the solution about three times, at three different concentrations equivalent to 50%, 100% and 150% of the active ingredients, by adding a known amount of DOXY standard to a sample of known concentration and calculating the recovery of DOXY with RSD (%) and recovery for each concentration.[23,24] The mean % recoveries were in between 99.13 – 101.997% and were given in table- 4. The assay for the marketed tablets of TETRADOX tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.31 of the labeled claim and no interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. [23,24] The results were shown in table – 5. To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factors, theoretical plates, limit of detection and limit of quantification and values were shown in table-6. Ruggedness of the method (intermediate precision) was estimated by preparing six Dilutions of the DOXY as per the proposed method and each dilution injected into column. The results were shown in table -7. Robustness of the proposed method was estimated by changing mobile phase composition from buffer: acetonitrile (60:40) v/v to buffer: acetonitrile 65:35 (v/v), changing the flow rate from 1ml to 1.2 ml/min, changing the temperature ($\pm$ 5^{o}c) and system suitability parameters were found to be within acceptable limits.[29] Results were shown in table-8 and indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. The ruggedness and robustness for the method was performed as per ICH guidelines. Limit of detection (LOD) and quantification (LOQ), the limits of detection and quantification were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = 3.3σ/s LOQ=10 σ/s.[30,31] The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method.[28] The typical chromatograms of DOXY standard and tablet dosage form were shown in figure 2,3.

Table 1 Concentration Vs Mean Peak Area

Concentration (µg/ml)	Mean peak area
25	382469
50	793523
75	1182415
100	1591981
125	1974592
150	2356741
Regression Equation	y = 15790x - 1139
Correlation coefficient(r^2)	0.999

Table 2 Statistical Data of Calibration Curves of DOXY

Parameters	DOXY
Linearity	25 – 150 µg/ml
Regression Equation	15790x – 1139
Average of Slope	15790
Average of intercept	1138.7
Standard deviation of intercept	98.44
Correlation coefficient (r^2)	0.999

Table 3; Precision of method

Parameters (n=6)	Intraday	Interday
Mean Peak Area	1498511	1492636
Standard deviation	11987.29	19422
%RSD	0.7999	1.3

Table 4: Recovery Study of Method

Standard of Drug (µg)	% of drug Added	Amount Present (µg)	Mean amount found (n=3)	Mean % recovery
100	50	50	50.68± 0.31	101.17
100	100	100	100.34 ± 0.07	100.34
100	150	150	149.41 ± 0.71	99.496

Average mean % recovery = 100.31, Standard deviation = 0.8511, %RSD = 0.8484

Table 5: Estimation of amount of DOXY

Brand name of Tablet	Label claim (mg)	Amount of drug estimated (mg)	Mean amount	%RSD
TETRADOX	100	99.87	99.87± 0.32	0.32

Table 6: System suitability Parameters

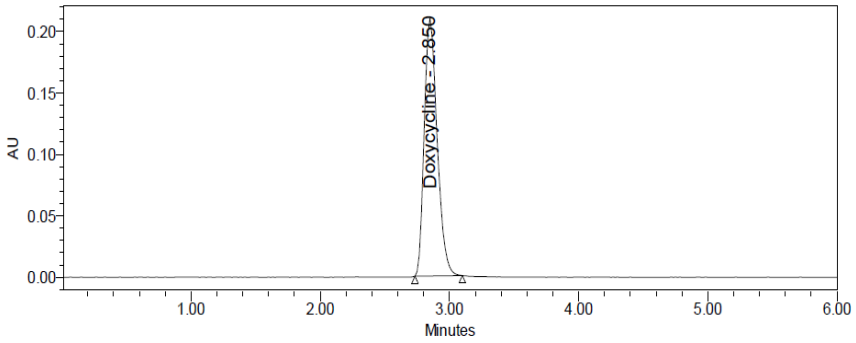
Parameters	DOXY
Retention time (min)	2.897
Theoretical plates	3677
Tailing factor	1.28
Linearity Range (µg/ml)	25 - 150
Limit of Detection (LOD) (µg/ml)	0.02
Limit of Quantification (LOQ) (µg/ml)	0.06
Relative standard deviation (RSD)	0.999

Table 7: Ruggedness of method

S.No	Labeled Claim (mg)	Amount estimated (mg)	Mean ± S.D	%RSD
Analyst - 1	100	99.87	99.87 ± 0.32	0.32
Analyst - 2	100	100.31	100.31 ± 0.85	0.848

Table 8: Robustness of method

Parameter	Variation	Theoretical Plates	Tailing factor	%RSD
Standard	-	3744	1.29	0.79
Flow rate	0.8 ml	3961	1.31	0.8
	1.2 ml	3476	1.26	0.7
Mobile phase	55:45	3758	1.32	1.4
	65:35	3845	1.31	1.1
Temperature	-5°C	3879	1.30	1.2
	+5°C	3865	1.31	0.6



DISCUSSION

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products.[25,26] The goal of this study was to develop and validate a RP-HPLC method for the estimation of DOXY in bulk and pharmaceutical commercial preparations. The main objective of method development was to determine the drug content present in the formulation and its % purity. The chromatographic conditions like mobile phase composition, flow rate was optimized and the method was developed, validated success fully. The selected mobile phase system gave a single sharp peak without interfering peaks. Initial development of the method various mobile phases were tried to get sharp peak, finally buffer: acetonitrile in the ratio of 60:40 (v/v) was selected which gave a single sharp peak with retention of 2.897 min and tailing factor 1.28. Commercial marketed formulation of DOXY was analyzed for its contents and % of content was calculated. The proposed method was found to be simple, rapid, economic and accurate and the method was applicable to routine laboratory analysis. The method was validated statistically for various parameters like standard deviation, % relative standard deviation, slope and intercept. The proposed method was following linearity in the concentration range of 25-150 µg/ml and obeys the beers lamberts law and above 25-150 µg/ml the linear plot showing deviation from beers law. Every concentration was injected into chromatographic system about three times and peak areas were noted. Greater reproducibility was obtained for calibration plots and it was determined by calculating the slope, intercept and %RSD for each standard plot. The method was found to be robust as there was no significant change in the peak area and retention time. The system suitability tests were performed to asses the quality performance of the method. The method was found to be more specific, robust and rugged and most suitable for routine analysis.

CONCLUSION

The proposed method was simple, accurate and sensitive HPLC method for the estimation of DOXY in bulk and pharmaceutical dosage forms.

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