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RP-HPLC method development and validation for estimation of doxorubicin and in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of doxorubicin by RP-HPLC technique. Chromatographic conditions used are stationary phase Discovery C18 150mm x 4.6 mm, 5µ ,Mobile phase 0.1% OPA: Acetonitrile in the ratio of 45:55 and flow rate was maintained at 0.9ml/min, detection wave length was 234 nm, column temperature was set to 30°C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 0.7 for intermediate precision. LOD and LOQ are 0.085µg/ml and 0.258µg/ml respectively. By using above method assay of marketed formulation was carried out 99.90% was present. Degradation studies of doxorubicin were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

Keywords: HPLC Doxorubicin, Method development. ICH Guidelines

INTRODUCTION

Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of streptomyces peucetius var. caesius. Doxorubicin binds to nucleic acids; presumably by specific intercalation of the planar anthracycline nucleus with the DNA double helix.Doxorubicin is capable of undergoing 3 metabolic routes: one-electron reduction, two-electron reduction, and deglycosidation. However, approximately half of the dose is eliminated from the body unchanged. Two electron reduction yields doxorubicinol, a secondary alcohol this pathway is considered the primary metabolic pathway.

Literature survey revealed that there were few analytical methods have been reported for doxorubicin in LC MS/MS for doxorubicin has been reported.

However, an extensive literature search didn't reveal any estimation method for doxorubicin in API and Pharmaceutical dosage form. Therefore an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for estimation of

doxorubicin in API and Pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) were purchased from Mark (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of doxorubicin as reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instruments and Chromatographic Conditions

Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC Acuity system equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Doxorubicin solution. The mobile phase used was 0.1% orthophosphoric acid: Acetonitrile (45:55A) at a flow rate of 1ml/min, samples were analyzed at 234 nm detector wave length and at an injection volume of 10 µL using discovery C_{18} 150 x 4.6 mm, 5µ with run time of 5 min.

METHODS

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Buffer

0.1%OPA Buffer

1ml of Perchloric acid was diluted to 1000ml with HPLC grade water.

Standard Preparation

Accurately Weighed and transferred 7.5mg of Doxorubicin working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above stock solution was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation

10 tablet was weighed ,powdered and then was transferred into a 100mL volumetric flask, 10mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity

It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of doxorubicin is taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 18.75ppm, 3.75ppm, 56.25ppm, 75ppm, 93.75ppm, 112.5ppm

Accuracy

Preparation of Standard stock solutions

Accurately Weighed and transferred 7.5mg of doxorubicin working Standard into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above stock solution was taken into a 10ml volumetric flask and made up to 10ml

Preparation of 50% Spiked Solution

0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution

1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each

standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution

1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml of doxorubicin solution was transferred to 10ml volumetric flask and made up with the same diluent.

LOQ sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml of doxorubicin solution was transferred to 10ml volumetric flask and made up with the same diluent.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of doxorubicin (75ppm) solution was injected six times and the parameters like peak tailing, resolution and USP plate count were determined to check whether the results complies with Recommended limits.

Assay of doxorubicin

An Accurately measured weight equivalent to (Adriamycin) 75mg of doxorubicin was used to perform assay by utilizing the method developed and under the optimized chromatographic conditions. Sample solutions were injected in to the HPLC system and scanned at 234 nm from which the % of drug was estimated.

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions

To develop and establish a suitable RP-HPLC method for estimation of doxorubicin in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 45% Ortho phosphoric acid:55% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 234 nm detector wave length and at an injection volume of 10 µL using Discovery C18 4.6 x 250mm, 5µm.with run time of 5 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for doxorubicin, the optimized chromatogram was obtained as shown in (Figure-2).

Validation

Linearity was established (18.75-112.5µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 15456x + 2384, correlation coefficient (R^2) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Figure-3). Retention times of doxorubicin were 2.567min. Where no interfering peaks in blank and placebo at retention time of this drug was not found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 99.00% was shown in (Table-2).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of doxorubicin was obtained as 0.6% and the % RSD for intermediate Precision was obtained as 0.7%, Low % RSD values indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on

Relative standard deviation of response and slope of the calibration curve doxorubicin. The detection limit values were obtained as 0.085 and Quantitation limit were fund to be 0.258 as given in (Table-4).Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (50:50) mobile phase plus (40:60) temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner Table -5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Doxorubicin pure drugs (API) was obtained from spectrum Pharma research solutions and pfizer Ltd (Adriamycin), bearing the label claim 75mg. Assay was performed with the above formulation. Average % Assay obtained was 98.82% the result was shown in (Table-7) and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-4, 5) respectively.

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 8).

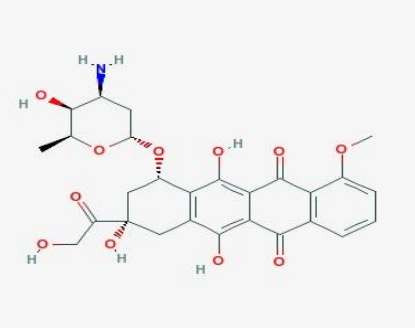

Figure-1: Chemical Structure of doxorubicin

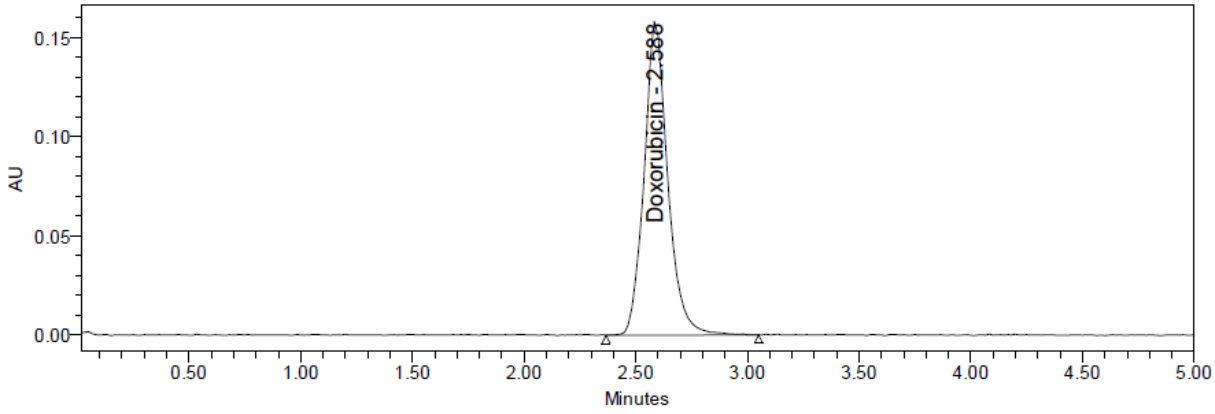

Figure-2: Optimized Chromatogram of Doxorubicin

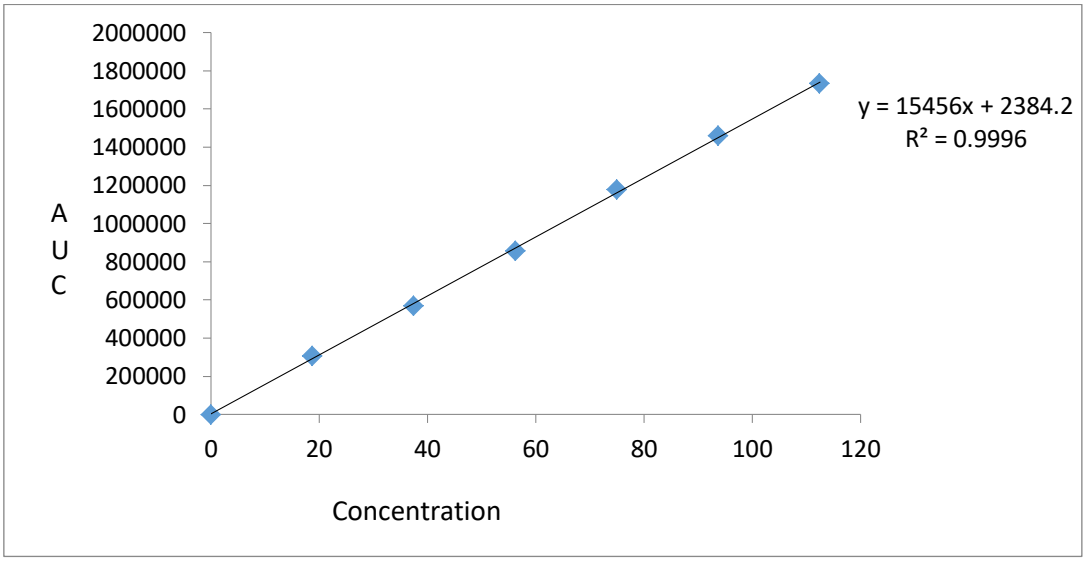

Figure-3: Linearity Curve of Doxorubicin

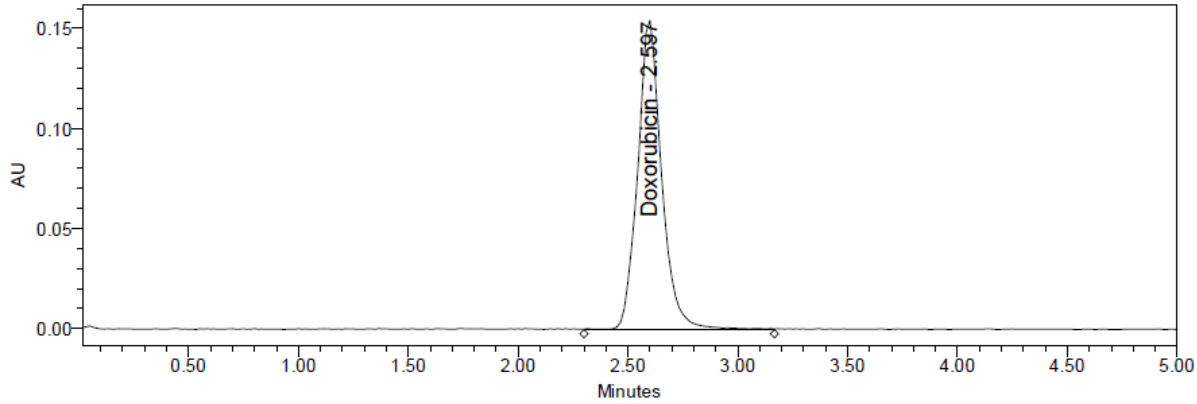

Figure-4: Standard Chromatogram of Doxorubicin

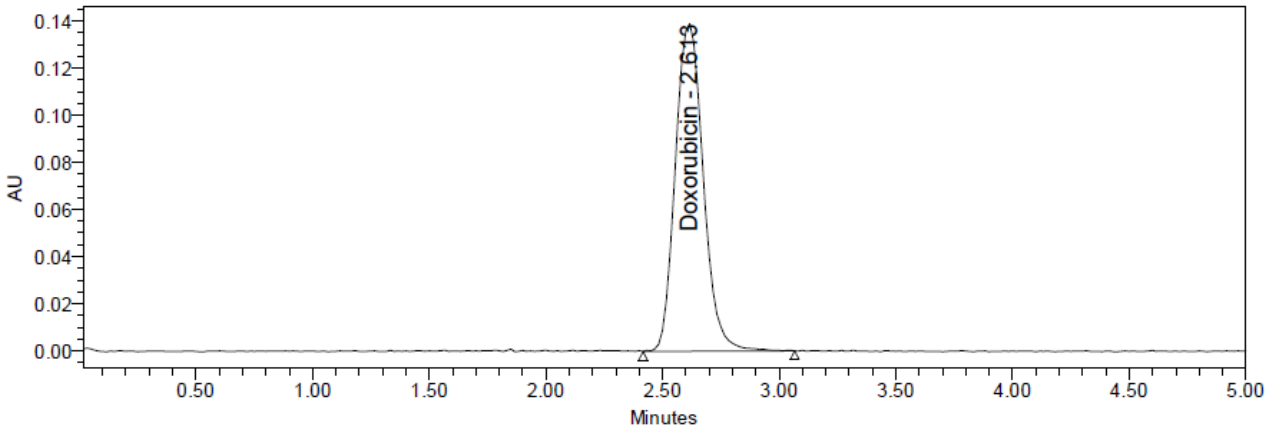

Figure-5: A Sample Chromatogram of Doxorubicin in Pharmaceutical Dosage FormTable-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	45% OPA (0.1%) : 55% Acetonitrile

Flow rate	1ml/min
Column	Discovery 250x4.6mm, 5µ
Detector wave leng	234nm
Column temperatu	30°C
Injection volume	10µL
Run time	5 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Doxorubicin 2.588 min
Theoretical Plates	Doxorubicin 2820

Table-2: Accuracy results of Doxorubicin

Sample	Amount added (µg/ml)	Recovery (%)	% RSD
	37.5	99.16	0.44
	75	98.99	0.46
	122.5	98.84	0.040

Table-3: Precision Result of Doxorubicin

S.no	Repeatability	Intermediate precision
1.	1145785	1080737
2.	1147001	1070565
3.	1153316	1068333
4.	1165564	1060334
5.	1157097	1078539
6.	1156369	1078539
Mean	1154189	1072841
S.D	7289.1	7863.9
%RSD	0.6	0.7

Table-4: LOD and LOQ values of Doxorubicin and Elbasvir

Molecule	LO	LO
Doxorubic	0.085	0.258

Table-5 Robustness Data of Doxorubicin

S.no.	Condition	%RSD of Doxorubicin
1	Flow rate (-) 0.9ml/min	0.2
2	Flow rate (+) 1.1 ml/min	0.2
3	Mobile phase (-) 50B:50A	0.5
4	Mobile phase (+) 60B:40A	0.6
5	Temperature (-) 25°C	0.5
6	Temperature (+) 35°C	0.3

Table-6: System Suitability Parameters Result of Doxorubicin

S no	Doxorubicin		
Inj	RT(min)	USP Plate Count	Tailing
1	2.585	2513	1.13
2	2.585	2640	1.12
3	2.588	2922	1.15
4	2.590	2291	1.12
5	2.597	2900	1.13
6	2.603	2234	1.12

Table -7: Assay Results of Doxorubicin

S. No.	Doxorubicin %Assay
1	98.10
2	98.20
3	98.74
4	99.79
5	99.07
6	99.00
AVG	98.82
STDEV	0.6241
%RSD	0.63

Table 8. Degradation Data of Doxorubicin

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.98	0.368	0.665
2	Alkali	2.79	0.402	0.712
3	Oxidation	1.98	0.381	0.517
4	Thermal	0.51	0.358	0.526
5	UV	0.54	0.416	0.571
6	Water	0.54	0.316	0.532

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the doxorubicin in injection dosage form. Retention time of doxorubicin was found to be 2.58min. %RSD was found to be 0.6. %Recover was obtained as 99.90%. LOD, LOQ values were obtained from regression equations of doxorubicin was 0.085µg/ml and 0.258µg/ml respectively. Regression equation of doxorubicin is y = 15456x + 2384. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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