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Stability indicating method development and validation for the estimation of Doxorubicin by using RP-HPLC method in a bulk and pharmaceutical dosage form

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ABSTRACT

A simple and selective RP-HPLC method is described for the determination of Doxorubicin dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of HPLC water (pH 5.8): ACN (55:45v/v), with detection of 281 nm. Linearity was observed in the range 75-150 µg/ml for Doxorubicin (R^2 =0.994) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form. The developed RP-HPLC and UV spectrophotometric method were successfully applied for the quantitative determination of cited drugs in pharmaceutical dosage form.

Keywords: Doxorubicin, RP-HPLC method, C_{18} column.

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 [1].

METHODOLOGY

Chemicals

Water	HPLC Grade
Methanol	HPLC Grade
Potassium dihydrogen ortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade

Instrument and chromatographic condition

All solvent used in this work are HPLC & AR grade. Instrument and chromatographic condition RP-HPLC Shimadzu LC20AT infinity series separation model equipped with UV Detector was employed in this method. The Lab solutions software was used for LC peak integration along data acquisition and data processing. The column used for separation of analyte is *Hypersil ODS* (150×4.6mm) 5µ. Mobile phase consisting of phosphate buffer: A mixture of 55 volumes of HPLC Water pH5.8:45 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases [2].

Preparation of phosphate buffer

1.625 gm of Potassium Di Hydrogen orthophosphate and 0.3 gm of Di Potassium Hydrogen orthophosphate was weighed and dissolve in 100 ml of water and volume was made up to 550 ml with water. Adjust the P^H using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of mixed standard solution

Weigh accurately 5mg of Doxorubicin in 25ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100µg/ml of Doxorubicin is prepared by diluting 5ml of Doxorubicin to 10ml with mobile phase. This solution is used for recording chromatogram [3, 4].

Preparation of sample solution

5 tablets (each tablet contains 5mg of Doxorubicin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 1000µg/ml were prepared by dissolving weight equivalent to 5mg of Doxorubicin dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Doxorubicin was made by adding 1ml of stock solution to 10 ml of mobile phase.

Table 1: Summary of chromatographic conditions

Mobile phase	Water: ACN
Ratio	55:45
Column	Zodiac, C18 (250×4.6× 5µ)
Wavelength	281nm
Flowrate	1ml/min
pH	5.8

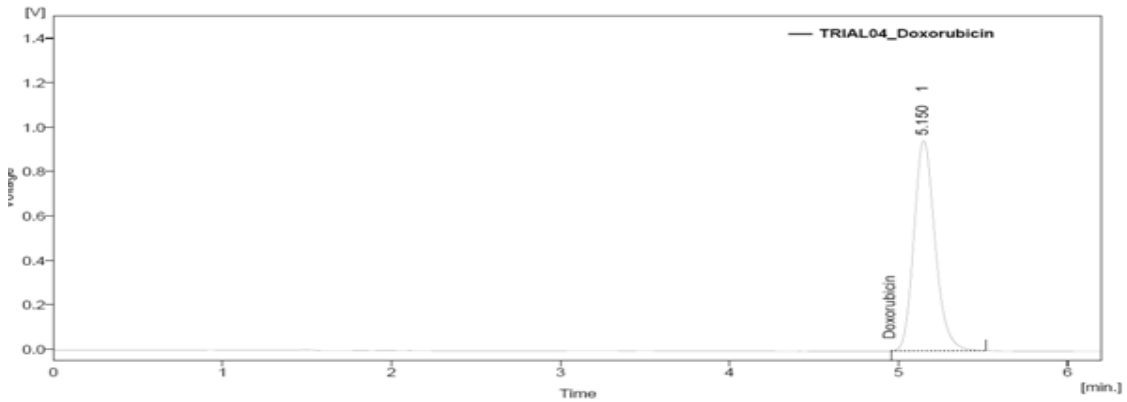

Fig 1: Optimized trial for estimation of Doxorubicin

Method validation

The validation of method was carried out as per ICH guideline. The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ. Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation product and related substances [5].

Accuracy

The accuracy was determined by calculating % recoveries of Thiacetazone, Isoniazide & Pyridoxine. It was carried out by adding known amount of each analyte corresponding to three conc. Levels (80, 100, 120) of the label claims to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by proposed method.

PRECISION

Method precision

Precision of an analytical method is usually expressed as the standard deviation. Method precision was demonstrated by preparing six samples as per test method representing single batch and were chromatographed. The precision of the method was evaluated by computing the %RSD of the results. The individual values and the low % RSD observed on the values are within acceptance criteria and indicates that method is precise.

Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits linear responses and directly proportional over the relevant conc. Range for the target conc. of the analyte. The linear regression data for the calibration plot is the indicative of a good linear relationship between peak and concentration over wide range. The correlation coefficient was indicative of high significance.

Robustness

Robustness of method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no effect on the peak tailing, peak area and theoretical plates finally the method was found to be robust.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. % RSD Assay values between two analysts not greater than 2.0%, hence the method was rugged.

Limit of Detection and Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable responses and LOQ was determined as the lowest amount of the analyte that was reproducibly quantified. These two parameters were calculated using formula based on standard deviation of the response and slope. LOD and LOQ were calculated by equation, LOD=3.3 x σ/s and LOQ= 10 x σ/s, where s = standard deviation, S = slope of calibration curve.

Assay of Doxorubicin in pharmaceutical dosage form

Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP –HPLC system. The sample solution was scanned at 281 nm. The percentage drug estimated was found to be 99.91% respectively. The chromatogram showed single peak of Doxorubicin, observed with retention time 5.150 min respectively.

Forced degradation studies

Stress studies are performed according to ICH guidelines under following conditions.

Acid degradation

To 5 ml of sample solution add 1ml of 0.1N HCL and sonicate, place it aside for 3hrs, then neutralize the solution with 1 ml of base and then transfer above solution into10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Alkaline degradation:

To 5 ml of sample solution add 1ml of 0.1N NaOH and sonicated, place it aside for 3h, then neutralize the solution with 1 ml of acid and then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Peroxide degradation

To 5 ml of sample solution add 1ml of 3% H_2O_2 and sonicate, place it aside for 3hrs, then transfer the above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Photolytic degradation

Expose about 100 mg of sample in UV light chamber at 365 nm for3hrs. Weigh accurately this powder equivalent to 10 mg of Ornidazole and 15 mg of Diloxanide furoate into a 100ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram.

Thermal degradation

Expose about 100 mg of sample in to dry heat to 80˚ C for 3hrs.Weigh accurately this powder equivalent to 10 mg of Ornidazole and 15 mg of Diloxanide furoate into a 100ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram.

Record the peak area of stressed samples then compare it with peak area of unstressed sample to determine the

$$\% \text{ degradation} = \frac{\text{Response of unstressed sample} - \text{response of stressed sample}}{\text{Response of unstressed sample}} \times 100$$

RESULT AND DISCUSSION

A simple and selective RP-HPLC method is described for the determination of Doxorubicin dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of HPLC water (pH 5.8): ACN (55:45v/v), with detection of 281 nm. Linearity was observed in the range 75-150 µg /ml for Doxorubicin (r^2 =0.994) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form. From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Doxorubicin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

Table 2: Linearity

Concentration(µg/mL)	Area
50	1904.438
75	2901.665
100	3680.717
125	4620.500
150	5220.440

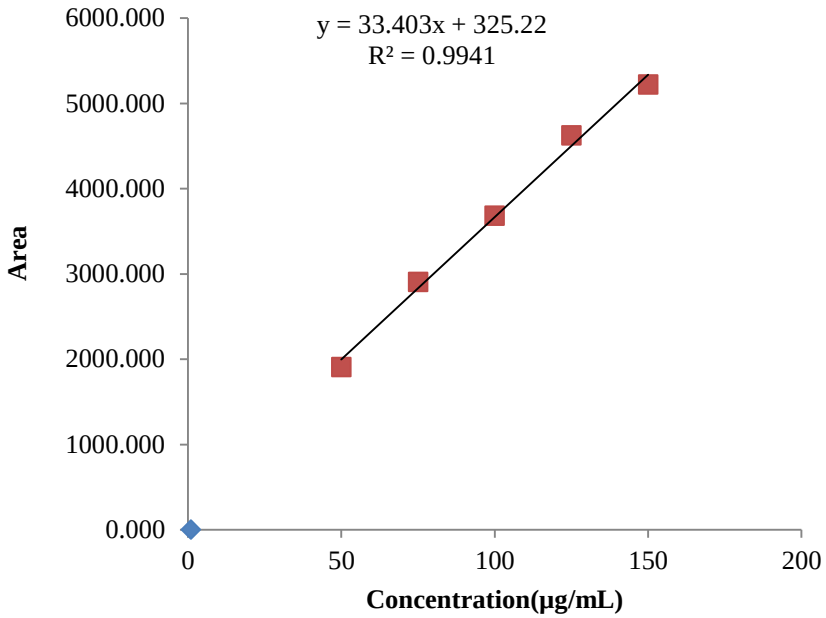

Fig 2: Linearity graph of Doxorubicin

Table 3: Method precision

Method Precision		
Doxorubicin		
S. No.	RT	Area
1	4.987	3259.295
2	4.973	3291.558
3	4.950	3314.065
4	4.983	3293.678
5	4.98	3315.153
6	4.987	3267.163
Average	4.9767	3290.152
SD	0.0141	23.203
%RSD	0.28	0.70

Table 4: Accuracy

	75	3318.54
	100	3519.65
	125	4853.020
		75 mcg
		3404.393
		3069.834
		3296.104
	Average	3256.777
Result		73.60 mcg
%recovery		98.14%
		100 mcg

		3838.43
		3285.17
		3327.673
	Average	3483.758
Result		98.98 mcg
%recovery		98.98%
		125 mcg
		4838.317
		4781.051
		4663.219
	Average	4760.862
Result		122.63 mcg
%recovery		98.10%

Table 5: Assay

Doxorubicin		
Standard Area	1	3323.905
	2	3320.771
	3	3293.678
	4	3274.549
	5	3193.689
	Average	3312.785
Sample area	1	3315.153
	2	3258.634
	3	3399.478
	4	3304.543
	5	3167.163
	Average	3288.994
Standard weight		5
Sample weight		10.58
Average Wt.		10.58
Label claim		5
Standard purity		99.8
Assay in mg		4.95
%Assay		99.08

Table 6: System Precision

System Precision		
Doxorubicin		
S.No.	RT	Area
1	4.987	3259.295
2	4.973	3291.558
3	4.950	3314.065
4	4.983	3293.678
5	4.98	3315.153
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Average	4.9767	3290.152
SD	0.0141	23.203
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CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Doxorubicin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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