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Development and Validation of RP-HPLC Method for the Determination of Doxorubicin Hydrochloride in Pure and Pharmaceutical Dosage Forms

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ABSTRACT

A simple, linear, rapid, precise and stability-indicating RP-HPLC method was developed for the estimation of Doxorubicin hydrochloride (DXRH) in pure and pharmaceutical dosage forms. The separation of DXRH was achieved on a reversed phase Syncronis C_{18} (10µm, 250 x 4.6 mm, ID with 3.5 microns particles column) using UV detection at 254 nm. The mobile phase was consisted of (40:60, v/v) of acetonitrile - buffer (pH 3.0). The linear range of detection for DXRH was found to be 40 to 140 µg/mL (r^2 = 0.9989). Intra and inter day assay relative standard deviations were less than 1 %. The method has been applied successfully to the determination of DXRH in various pharmaceutical preparations. There was no interference from drugs commonly administered with DXRH. The method has been shown to be linear, reproducible, specific and rugged.

Keywords: Doxorubicin hydrochloride, RP-HPLC, Pharmaceutical dosage forms

I. INTRODUCTION

Doxorubicin hydrochloride/Adriamycin HCl ((7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione; hydrochloride) is antineoplastic agent, also called as anticancerous drug. Doxorubicin (HCl) is the prototype agent of anthracycline antibiotic, isolated from Streptomyces peucetius var caesius. It contains an amino sugar and an anthracycline ring. Doxorubicin (HCl) is among the most useful cytotoxic anticancerous drugs. It has a broad spectrum of potent activity against many different types of cancers especially haematologic malagnancies. Doxorubicin (HCl) is used in combination with different anticancerous drugs to obtain best therapeutic effects and to reduce the side effects or toxicities. Doxorubicin (HCl) is of Semi Synthetic origin and belongs to Anthracycyline. It belongs to intercalation (DNA acting) pharmacological group on the basis of mechanism of action and also classified in Antineoplastic Agent, Antibiotic pharmacological group. DXRH (Doxorubicin HCl liposome injection) is indicated for the treatment of patients with ovarian cancer whose disease has progressed or recurred after platinum-based chemotherapy. DXRH is indicated for the treatment of AIDS-related Kaposi's sarcoma in patients after failure of prior systemic chemotherapy or intolerance to such therapy.

Many HPLC methods [1-11] have been reported for the determination of DXRH in biological fluids or in pharmaceutical formulations. The reported methods are not satisfactory for routine quality assurance for one or the other reason. Some of these methods use MS detection [2,3] that needs special requirements and is not affordable for most laboratories. Other methods that utilize UV or low sensitivity [4,5] or long analytical run

times [1]. On the other hand, sample preparation methods in these studies include protein precipitation which would increase the analytical column pressure [1, 4, 6] , solid phase extraction [7, 8] that is expensive and labour-intensive or liquid- liquid extraction with high amounts of organic solvent [9-11]. Hence, it was felt necessary to develop a simple and sensitive HPLC method, which does not suffer from the above limitations for the determination of DXRH in pharmaceutical formulation.

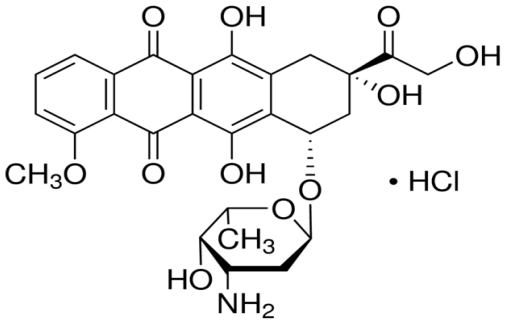

Fig. 1 Chemical structure of Doxorubicin hydrochloride

II. EXPERIMENTAL

2.1 Materials and apparatus

Pharmaceutical grade (> 99%) Doxorubicin hydrochloride was obtained from Sigma Aldrich, Acetonitrile (s.d Fine-Chem, Ltd., India) and water (Rankem Ltd., India) used were of HPLC grade. All HPLC measurements were made on a Shimadzu Corporation system (Analytical Instruments division, Kyoto, Japan) consisting of a LC-2010 CHC binary gradient solvent pump, SPD-10A detector and a data station with win chrome software version 3.1. The elusion was performed on reversed phase Syncronis C_{18} (10µm, 250 x 4.6 mm i.d) column. A 850:150 v/v mixture of buffer: acetonitrile was used as a mobile phase at a rate of 1.5 ml/min. Hamilton 702 µR injector with a 25 µl loop was used for the injection of the samples. Detection was done at 254 nm. The mobile phase was filtered through 0.45µ Millipore membrane filter and degassed. The separation was carried out at room temperature.

2.2 Stock solutions

A stock solution of Doxorubicin Hydrochloride was prepared by dissolving appropriate amount of substance in water and Acetonitrile (1:1). Working solutions of 100µg /mL were prepared from the above stock solutions. Studies on the stability of analytes in standard working solution showed that there was no decomposition product in the chromatogram and difference in area-ratios during the analytical procedure and even after storing for 2 days at +4ºC.

2.3 Pharmaceutical preparation

Twenty tablets of the selected drug were finely powdered. An amount equivalent to 20 mg of the drug was weighed accurately and transferred into a 50 ml volumetric flask. 25 ml of mobile phase was added and the powder was completely disintegrated. The solution was filtered and the filtrate was made up to 50 ml with the

mobile phase. Further transferred 3.0 ml of the solution into 25.0 ml volumetric flask, diluted up to the mark with diluent and mixed well.

III. PROCEDURE

3.1 Chromatographic conditions

LC analysis was performed by isocratic elution with flow rate of 0.75 ml/min. The mobile phase of 850:150 v/v mixture of buffer : acetonitrile acetonitrile was used throughout. Buffer solution was prepared by dissolving 1cm^3 of orthophosphoric acid into 1000 cm^3 of distilled water. After addition of buffer, the pH of mobile phase was 3.0. All solvents were filtered through a 0.45µ Millipore membranes filter before use and degassed in an ultrasonic bath. The flow rate was maintained at 0.75 mL/min. Quantification was effected by measuring at 254 nm and the chromatographic run time was 6 min.

Throughout the study, the solubility of the chromatographic system was monitored by calculating the capacity factor (k), the resolution (R), the selectivity (σ) and the peak asymmetry (T).

3.2 Establishment of calibration

Working standard solutions of DXRH (40 to 140 µg/mL (r^2 = 0.9989) was prepared in mobile phase. Triplicate 20 µl injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of standard was plotted against the concentration of the drug to obtain the calibration graph. The results were subjected to regression analysis to calculate calibration equation and correlation coefficients. A typical chromatogram is obtained as show in Fig. 2.

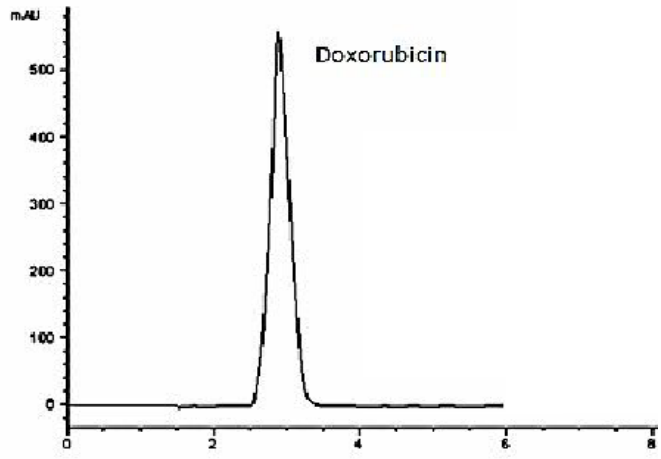

Figure 2. Chromatogram for DXRH (retention time = 3.0 min)

3.3 Analysis of tablet

An aliquot of the drug obtained by following the procedure described for analysis of pharmaceutical preparations was taken and analysed. The chromatogram at 254 nm showed a complete resolution of the peak.

IV. RESULTS AND DISCUSSION

4.1 Method development

The mobile phase was chosen after several trials with methanol, acetonitrile, isopropyl alcohol, triethylamine, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of buffer: acetonitrile (850:150 v/v) was selected to achieve the maximum separation and sensitivity. The effects of flow rates in the range of 0.5, 0.75, 1.0 and 1.5 ml/min were examined. A flow rate of 0.75 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using reverse phase C18 column, the retention time was observed to be of 3.0 min. The total time of analysis was less than 10 min.

The solution containing DXRH exhibited maximum absorption at 254 nm and hence, this wavelength was chosen for the analysis.

4.2 Linearity

The calibration curves were linear in the studied range of 40-140 µg/ml. The calibration curve equation is x = bx + c and the response was measured as peak area. The mean equation of the calibration curve (n = 9) obtained was y = 33217x + 62583. Excellent linearity was obtained for DXRH between peak area and concentrations of 40 to 140 µg/mL with $r^2 = 0.9989$.

4.3 Limit of detection and limit of quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (*S/N*) of 3 while limits of quantification (LOQ) were established at a signal-to-noise ratio (*S/N*) of 9. The LOD and LOQ were experimentally verified by nine injections of DXRH at the LOD and LOQ concentrations. The limit of detection was calculated to be 0.35 µg/ml and the limit of quantification was calculated to be 0.75 µg/ml.

4.4 Suitability of the method

The chromatographic parameters such as tailing factor, selectivity and peak symmetry were evaluated for the selected drug. The tailing factor for DXRH in standard solution was found to be 1.50 while the theoretical plate was observed to be 1500. The capacity factor (k^1) was found to be 1.0 while the peak asymmetry (*T*) value was observed to be 1.10.

4.5 Precision

The precision (Table 1) of the method (within-day variations of replicate determinations) was checked by injecting nine times of DXRH at the LOQ level. The precision of the method expressed as the relative standard deviations (R. S. D., %) at the LOQ level, were 2.52.

Table 1. Precision parameters

Compound	λ (nm)	Peak area (n = 9, mean)	R. S. D. (%)
DXRH	254	9617797	1.24

R. S. D. (%) = ((S.D./mean) x 100)

4.6 Accuracy

A standard working solution containing DXRH to give final concentration 20µg/ml was prepared. The prepared solution of standard was injected nine times as a test sample. From the area counts, the concentration of the DXRH was calculated using the detector responses. The accuracy, defined in terms of % deviation of the calculated concentrations from the actual concentrations is listed in Table 2.

Table 2 Accuracy parameters (recovery)

Drug	Spiked concentration (µg/ml)	Measured concentration (µg/ml; mean ± S. D.)	R. S. D. (%)	Deviation (%)
DXRH	15.25	15.17	101.2	0.7
	20.80	20.70	100.7	1.4
	25.50	25.28	102.6	0.8

R. S. D. (%) = ((Spiked concentration – mean measured concentration) x 100)/spiked concentration.

4.7 Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis of the standard working solution, the same chromatographic system and the same column on different days. Small differences in area ratios and good constancy in retention times were observed after 48 h time period. The R.S.D. value of less than 0.21% for areas was observed. The R.S.D. values for nine determinations were found to be 0.21 % (for intra-day analysis), and 0.47 % (for inter-day analysis). The low R.S.D. values indicated the ruggedness of the method. The comparable detector responses obtained on different days indicated that the method is capable of producing results with high precision on different days.

Similarly, injecting the standard working solution into a different HPLC unit tested the ruggedness of the method. The high degree of reproducibility of the detector response and the retention times indicate that the method is fairly rugged.

4.8 Specificity of the method

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Doxorubicin HCl was carried out in the presence of its impurities namely Epi rubicin and Methyl paraben. Stress studies were performed for Doxorubicin HCl bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254nm), heat (70°C), acid (0.1N HCl), base (0.1N NaOH), and Oxidation (3.0 % H_2O_2) to evaluate the ability of the proposed method to separate DXRH from its degradation products. For all degradation studies, period was 24 hours. Assay and related substance studies were carried out for stress samples against qualified DXRH reference standard. Assay was also calculated for DXRH samples by spiking all for impurities at the specification level (i.e., 0.1%).

4.9 Analysis of pharmaceutical preparation

The proposed method was successfully applied to the analysis of DXRH in tablet and the results were shown in Table 2. The low values of relative standard deviation indicated high precision of the method.

V. CONCLUSIONS

The RP HPLC method developed for quantitative and related substance determination of Doxorubicin hydrochloride is linear, accurate, precise, rugged, rapid and specific. The method was fully validated showing

satisfactory data for all method validation parameters tested. The developed method is stability indicating and can be conveniently used by quality control department to determine the related substance and assay in regular Doxorubicin Hydrochloride production samples and also stability samples. In the RP HPLC technique all the impurities were separated within a short time.

VI. ACKNOWLEDGEMENTS

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