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Development of stability indicating HPLC method for the assay of epirubicin in the presence of degradents

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ABSTRACT

An HPLC method with UV detection to understand degradation path way of epirubicin and it's quantification in injection as pharmaceuticals form has been developed and validated as per guidelines from ICH. It is to be employed for routine and stability analysis of epirubicin. A linear isocratic elution was employed starting with 44% A, 18% B and 36% C up to 30 min. Mobile phase A was 1.5g/mL of sodium lauryl silicates add 1.0mL of ortho phosphoric acid and adjust pH-3.6 with sodium hydroxide. B and C were methanol and acetonitrile respectively. UV detection was performed at 254 nm. The chromatographic column was Inertsil ODS-3 (150mm X 4.6mm) 5.0µ kept at 25°C. All impurities were separated and it was possible to quantify the epirubicin in formulation with precision and accuracy. The method was validated for determination of epirubicin over linearity range from 67.20µg/mL to 100.80µg/mL with limit of detection was 0.12µg/mL.

INTRODUCTION

Epirubicin Hydrochloride is an anthracycline derivative, Chemotheraptic agent; it is the 4'-epimer of doxorubicin and a semi-synthetic derivative of daunorubicin. Epirubicin has the chemical name (8S-cis)-10-[(3-Amino-2, 3, 6-trideoxy-alpha-L-arabino-hexopyranosyl)oxy]-7, 8, 9,10-tetrahydro-6, 8, 11-trihydroxy-8-(hydroxyacetyl)-1-methoxynaphthacene-5,12-dione hydrochloride. Epirubicin Hydrochloride has a similar spectrum of activity and toxicity as doxorubicin. Epirubicin Hydrochloride is an anthracycline antibiotic with antineoplastic actions similar to those of doxorubicin.

Epirubicin hydrochloride is a red colored powder with molecular formula of $C_{27}H_{29}NO_{11}$. HCl and a molecular weight of 579.99 while epirubicin has molecular formula $C_{27}H_{29}NO_{11}$, and a molecular weight of 543.49.

Epirubicin Hydrochloride is the most important antitumor agent, which is derivative of anthracycline antibiotics. The fungus Strep peucetius var. caesius produces them. Idarubicin, doxorubicin and daunorubicin are the synthetic derivatives. Although they differ slightly in chemical structure. Daunorubicin and Idarubicin have been used primarily in the acute leukemia's where as epirubicin displays broader activity against human neoplasm, including a variety of solid tumors. These all derivatives shown promise in clinical studies including Idarubicin, epirubicin and the synthetic compound mitoxantrone; which is an amino anthracenedione[1-3]

The anthracycline antibiotics have tetracycline ring structure with an unusual sugar, daunosamine, attached by glycosidic linkage[4,5]. Cytotoxic agents of this class all have quinone and hydroquinone moieties on adjacent rings that permit them to function as electron-accepting and donating agents. Although there are marked differences in the clinical use of daunorubicin and

doxorubicin, their chemical structures differ only by a single hydroxyl group on C14. Idarubicin is 4-demethoxydaunorubicin, a synthetic derivative of daunorubicin without methoxy group on C14 of the glycone ring. It is used, alone or with other antineoplastics, in acute leukaemias, lymphomas, multiple myeloma, and in solid tumours including Wilms' tumour, cancer of the bladder, breast, and stomach. The chemical structures are as follows:

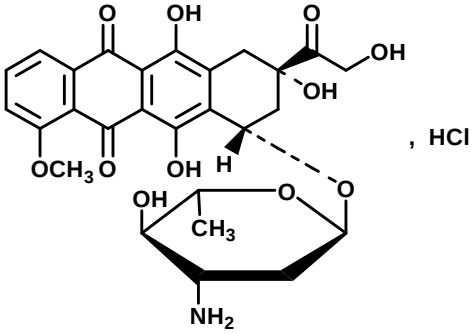

Figure 1 : Epirubicin hydrochloride

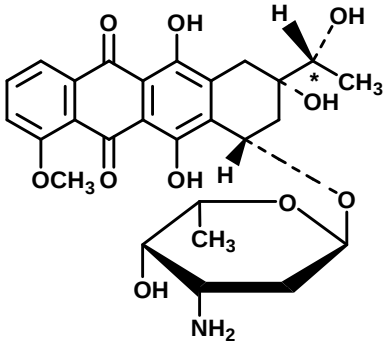

Figure 2 : Doxorubicin

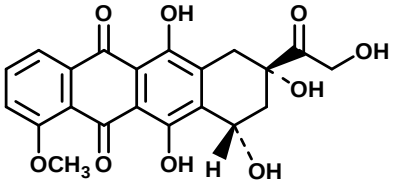

Figure 3 : Doxorubicinone

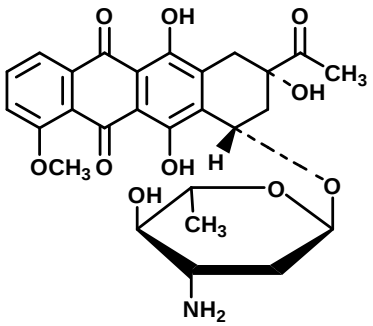

Figure 4 : Daunorubicin

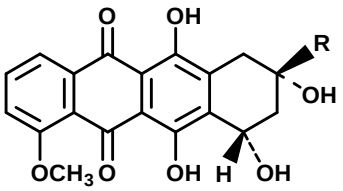

Figure 5 : Daunorubicinone

Epirubicin Hydrochloride is available for intravenous use. The recommended dose is 60 to 75mg/m^2, administered[6] as a single rapid intravenous infusion; it is repeated after 21days.

Literature survey reveals number of analytical method have been reported for the derivatives of epirubicin hydrochloride (Figure 1) but no analytical method has been reported in pharmacopoeia for the quantification of epirubicin in formulations. Only one simple sensitive isocratic high performance liquid chromatographic method has been reported to measure the concentration of epirubicin hydrochloride in raw material with UV detection at 254nm. The method uses a silical gel (250mm X 4.6mm) 6µ as analytical column. The mobile phase consist of 17 volumes of methanol, 29 volume of acetonitrile and 54 volumes of a solution containing 3.7g/mL of sodium laurilsulphate R and 2.8 percent V/V of diluted phosphoric acid. The flow rate is 2.5mL/min with column oven temperature 35°C.[7]

Another high performance liquid chromatography method has been reported in Therauptic Drug monitoring for the quantification of epirubicin and their metabolites in plasma.[8]

One coupled-column liquid chromatographic method has been reported for the quantification of epirubicin and their metabolite in biological material.[9]

Doxorubicin Hydrochloride (Antineoplastic drug) is available for intravenous use. The recommended dose is 60 to 70 mg/m^2, administered as a single rapid intravenous infusion. It is to be repeated after 21 days. Care has to be taken to avoid extravenous infusion since severe local vesicant action and tissue necrosil may result. As for dounorubicin, patients must be advised that the drug may impart a red color to the urine.

Doxorubicin is effective in acute leukemia and malignant lymphomas. A rapid simple and sensitive isocratic HPLC method was developed to measure the concentration of Doxorubicin with UV detection at 254 nm[10].

Daunorubicin Hydrochloride (Antineoplastic drug) is available for intravenous use. The recommended dose

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is 30 to 60mg/m^2 daily for 3 days. The agent is administered with appropriate care to prevent extra-vasation, since severe local versicant action may result and therefore patients should be advised that the drug might impart a red colour to the urine.

Daunorubicin is very useful in the treatment of acute lymphocytic and acute granulocytic leukemia's. A reverse phase HPLC method has been validated for the quantification of Daunorubicin[11].

The Objective of the present work was the development and validation of a stability indicating HPLC method with UV detection for determining epirubicin in different formulation as pharmaceutical form to be employed in routine and stability analysis.

EXPERIMENTAL

Apparatus

An Agilent technologies HPLC 1100 series (Las Rozas, Madrid, Spain) provided with an automatic injector, a diode-array detector and a column oven was used for chromatographic analysis. The chromatographic separation was achieved on Inertsil ODS-3 (150mm X 4.6mm) 5.0µ column with column oven temperature of 25°C.

A linear isocratic elution was employed starting with 44% A, 18% B and 36% C up to 30 min. Mobile phase A was 1.5g/mL of sodium lauryl silicates add 1.0mL of ortho phosphoric acid and adjust pH-3.6 with sodium hydroxide. B and C were a methanol and acetonitrile respectively. The flow rate was 1.3 ml/min and the injection volume was 10µL. UV detection was performed at 227 nm and peaks were identified with retention times as compared with standards and during method development by spiking.

Chemicals

Standard of epirubicin, impurities of epirubicin, injection of epirubicin and placebo were obtained by Cancer Hospital, Gwalior, Sodium lauryl silicate from BDH Limited, Sodium Hydroxide, Ortho phosphoric acid, Methanol and Acetonitrile from Qualigens. HPLC grade water from Merk.

Optimization of HPLC method

Selectivity under RP-HPLC conditions can be controlled by mobile phase composition, pH, temperature and stationary phase chemistry. The parameters were varied to achieve the separation.

Standard solution and sample preparation

Samples were dissolved in solvent A: methanol: acetonitrile 44:18:38 (v/v), being solvent A: 1.5g/mL of sodium lauryl silicates add 1.0mL of ortho phosphoric acid and adjust pH-3.6 with sodium hydroxide. For quantitation Pipette 2mL of epirubicin injection in 50mL volumetric flask, dissolved and rinse with mobile phase and make up the volume with mobile phase to get final concentration of 80µg/mL.

A stock solution of epirubicin was prepared with about 50mg of epirubicin Hydrochloride was weighed in 25mL volumetric flask, dissolved in mobile phase and make up the volume with mobile phase. This solution was diluted quantitatively with the same solvent to get final concentration of 80µg/mL of epirubicin Hydrochloride.

System suitability check

System suitability[12] was checked with standards chromatograms. Standard solution was injected in six replicate. The tailing factor of epirubicin peak was not more than 2.0, column efficiency was not less than 4348 theoretical plates. Relative standard deviation of area of epirubicin peak for six replicate injections of standard solution was not more than 2.0%.

Validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceuticals methods, guidelines from the united state pharmacopoeia (USP)[13], International Conference on Harmonization (ICH)[14] and the Food and drug administration (FDA)[15,16] provide a framework for performing such validation.

Selectivity[17,18] was tested by injecting the placebo of the pharmaceutical specialty and checking that there was no interference peak and by spiking the drug substance and the drug product with appropriate levels of standards and demonstrating the separation of these impurities individually and other components in the sample matrix. Moreover, identification of each impurity was confirmed with retention time as compared with those of pure standards and by spiking.

For epirubicin method, linearity[19] is determined by preparing standard solution at five concentration levels over a range from 67.20µg/mL-100.80µg/mL. Linearity was tested by adding proportional weight of the placebo to each flask. The correlation coefficient, slope and intercept were determined. The data is provided in TABLE 1.

TABLE 1 : Linearity data

Sr. No.	Parameter	Concentration	Result's
1.	Linearity	67.20µg/mL-100.80µg/mL.	R^2=1.0000
2.	Slope		10403
3.	Intercept		888.21

For pharmaceutical studies, the most widely used approach to test accuracy is the recovery study, which is performed by spiking analyte in blank matrices. It was tested at three different concentration level which were 67.20µg/mL, 84.20µg/mL and 100.80µg/mL. The percent recovery was in the range of 99.19% to 100.54%.

Intra-assay precision data were obtained by repeatedly analyzing the samples which was independently prepared according to the method procedure. Data to evaluate intermediate precision were obtained by repeating the intra-assay experiment on a different day with a newly prepared mobile phase and samples. Relative standard deviation of results was 0.47%. The data is provided in TABLE 2.

TABLE 2 : Intermediate precision data

Sr.No	Equipment	Analyst	Reagent	Assay	Mean	RSD
1	Instrument-1	Analyst-1	Water, HPLC grade (Milli Q) Acetonitrile, S.D.Fine Chemicals (B.No-H02/1476/0602/53) Methanol:Qualigens, (B.No-NL45536112H2) Ortho Phosph Acid, Qualigens, (B.No-NL00566208S) Sodium Hydroxide, Qualigens, (B.No-NL45716203S)	2.10mg/mL		
2	Instrument-2	Analyst-2	Water, HPLC grade (Milli Q) Acetonitrile, Qualigens, (B.No-58956209T) Methanol:S.D.Fine Chemicals (B.No-J02A/1597/1002/53) Ortho Phosph Acid, Qualigens, (B.No-NL00566208S) Sodium Hydroxide, Qualigens, (B.No-NL45716203S)	2.12mg/mL	2.11mg/mL	0.47%
3	Instrument-3	Analyst-3	Water, HPLC grade (Milli Q) Acetonitrile, Merck, (B.No-RH2NF52026) Methanol:S.D.Fine Chemicals (B.No-B02A/1724/2812/53) Ortho Phosph Acid, Qualigens, (B.No-NL00566208S) Sodium Hydroxide, Qualigens, (B.No-NL45716203S)	2.11mg/mL		

LOD & LOQ[20] of epirubicin was determined and precision was established at the predicted concentration. % Relative standard deviations was found with in Limit for LOQ at 0.40 µg/mL with %RSD=0.59%.

The data is provided in TABLE 3.

TABLE 3 : Precision at LOQ level.

Concentration µg/mL	Injection Volume	Area Count	Mean	RSD
0.40µg/mL	10µL	10.15 10.02 10.03 10.00 10.02	10.04	0.59%

RESULT AND DISCUSSION

A typical chromatogram of epirubicin is shown in Figure 6 and peak purity of the samples was also determined by area normalization method. Throughout this study the peak purity was confirmed by photo diode array detector (PDA), which incorporates 512 diodes over UV-VIS spectral range to evaluate UV spectra

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across the peak of interest hence the method is specific. Presented method is sensitive enough and is found to be linear in the range of 67.20 µg/mL to 100.80 µg/mL with R^2 1.0000. Accuracy of the method was established by recovery, the recovery values are with in the acceptable limit at different concentration level (50%, 100% and 150% of specification). Over all recovery ranges from 99.19% to 100.54% and this indicates that the method is accurate. Repeatability, reproducibility and intra day precision were performed as system precision, method precision intermediate precision and ruggedness values obtained were very well within the acceptance criteria. Limit of quantitation of the method was 0.4 µg/mL and limit of detection was 0.12 µg/mL.

In addition the robustness of the method makes it easy for an operator to generate reproducible data. The method is robust under different conditions like change in mobile phase, column, flow, temperature and wavelength. The data is provided in TABLE 4.

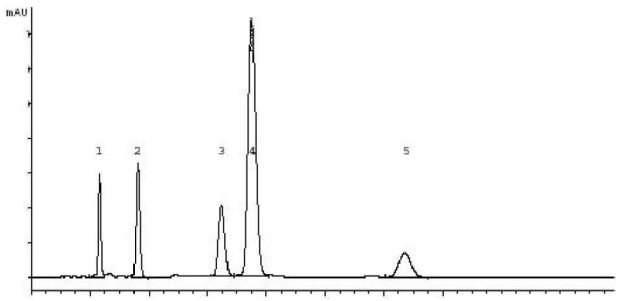

Figure 6 : Chromatogram of Doxorubicinone (1), Daunorubicinone (2), Doxorubicin (3), Epirubicin (4) and Daunorubicin (5).

TABLE 4 : Robustness data

Sr. No	Column	Mobile Phase	Flow rate	Temperature	Wavelength	Assay of Epirubicin
1.0	C18, Inertsil ODS3, 5µ (150X4.6) mm	Buffer:MeOH:ACN (40:20:35)	1.2ml/min	35°C	256nm	2.05mg/mL
2.0	Water's Novapack ODS, 4µ (150X3.9) mm	Buffer:MeOH:ACN (43:18:37)	1.0ml/min	30°C	252nm	2.11mg/mL
3.0	C18, Inertsil ODS3, 3µ (150X4.6) mm	Buffer:MeOH:ACN (45:17:37)	1.0ml/min	34°C	258nm	2.12mg/mL

In order to validate the stability indicating power of the analytical procedure, epirubicin standard and sample were treated with acidic, basic, oxidative media, heat and light.

For Heat stressing epirubicin Injection sample was kept at temperature 105°C for 5.0 Hrs in oven to observe degradation. Degradation was very rapid, one major polar degradant doxorubicin was observed having RRT 0.99.

For base stress epirubicin injection was diluted in 0.01 N NaOH, as degradation was very severe in 0.01 N NaOH, duration of studies was very small no UV active degradation was observed but epirubicin was degraded therefore sodium hyperoxide was further diluted to 0.001 N and study was carried out and observed degradation pattern was same.

For light stressing epirubicin Injection sample was placed under white fluorescent light at the rate of 2400-kilo lux per Hours at 15°C and analyzed at regular interval of time. No degradation observed during the study.

For peroxide stress epirubicin injection was diluted in 30% H_2O_2. Degradation was observed with in hours of time. A polar lipophilic impurity A having RRT 0.84 and nonpolar impurity B having RRT 1.45 was generated in peroxide stressing. Epirubicin was degraded within 23 hours and converted into impurity A and Impurity B.

For Acid stressing epirubicin Injection was diluted with 0.1N HCl. Degradation was very slow at room temperature so study was carried out up to 71 hours in order to establish the degradation. One major polar degradant doxorubicin was observed having RRT 0.99.

Data of forced degradation study presented in TABLE 5.

TABLE 5 : Forced degradation study data

Sr. No.	Condition	Duration	Peak of Degradent	Peak Integrity
1.	Heat 105°C	5 Hours	Yes	Yes
2.	Light (2400 Kilolux/hour) Fluoresent Lamp	15 Days	No	Yes
3.	0.1 N HCl	71 Hours	Yes	Yes
4.	0.01 N NaOH	Initial	Yes	Yes
5.	0.001 N NaOH	30 Hours	Yes	Yes
6.	30% H_2O_2	23 Hours	Yes	Yes

The stressed samples were analyzed on HPLC. The purity of the samples was also determined by area normalization method. Throughout the study peak purity was confirmed by photo diode array

detector (PDA), which incorporates 512 diodes over UV-VIS spectral range to evaluate UV spectra across the peak of interest.

CONCLUSION

The presented work describing a reverse phase HPLC method for the determination of epirubicin is specific, accurate, rugged, precise, linear and can be applied for the quantification of epirubicin in different drug products. Validated method permits the separation and identification of four impurities of epirubicin. The method was successfully applied for the determination of epirubicin in injectables and can be applied to other drug products and drug substances with minor modification and optimization.

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