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ORIGINAL ARTICLE

Qualitative and Quantitative Estimation of Related Substances in Marketed Formulation of Epirubicin Hydrochloride using Green RP-HPLC Method

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ABSTRACT

An advanced and reliable approach method was developed for concurrent evaluation of chemotherapy drug Epirubicin (EPI) which is used to treat breast cancer and its impurities (i.e., related substances) namely Doxorubicin (DXR), Doxorubicinone (DXR-one) in marketed formulation. The current study is carried out with the help of a reverse phase high performance liquid chromatography (RP-HPLC) method to measure precisely Epirubicin, Doxorubicin and Doxorubicinone in pharmaceutical dosage form. The separation was done using XDB C18 (Zorbax Eclipse), 150mm. 4.6mm, 5µm column. Epirubicin and its impurities were resolved using a mixture of mobile phase OPA buffer and Acetonitrile in the ratio of 56:44 v/v at optimum temperature (25^0) and pH was maintained at 2.5. The detection was done at wavelength 254nm. The method was found to be linear at the concentration range of 0.04 to 143.8 µg/mL, 0.04 to 3 µg/mL and 0.04 to 5.1 µg/mL for Epirubicin, Doxorubicin and Doxorubicinone respectively. International Council for Harmonization (ICH) prescribed guidelines were applied on Epirubicin, Doxorubicin and Doxorubicinone and further observed that there is no interference between the respective drug and its related substances. The values obtained for Detection Limit & Quantification Limit were 0.0144 µg/mL and 0.479 µg/mL, 0.0149 µg/mL and 0.0496 µg/mL, 0.0142 µg/mL and 0.0474 µg/mL for Epirubicin, Doxorubicin and Doxorubicinone correspondingly. Retention times of Epirubicin, Doxorubicin and Doxorubicinone were measured as 11.06, 7.9 and 2.5 minutes respectively. The results for all the validation parameters were obtained within the acceptance criteria of ICH Q2 guidelines. Hence, the developed Reverse Phase HPLC method is said to be precise, accurate, linear and specific. Along with the validation process the stability of the drug was measured using force degradation studies which proved that the developed method was stable in applied adverse conditions. Green assessment of the developed method was also carried out using two tools viz., AGREE & GAPI to determine the green profile. The developed, validated, stability studies and green assessment can be applied to perform quality control of the Epirubicin and its related substances i.e., Doxorubicin and Doxorubicinone..

KEYWORDS: Doxorubicin, Doxorubicinone, Epirubicin, Green assessment, Related substances & RP-HPLC.

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INTRODUCTION

Epirubicin Hydrochloride [1] (EPI) is an anthracycline semi derivative of daunorubicin and doxorubicin 4' epimer and also a cytotoxic agent used to treat breast cancer [2]. EPI Chemical name is (7*S*,9*S*)-7-[(2*R*,4*S*,5*R*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-mrthoxy-8,10-dihydro-7*H*-tetracene-5,12-dione. EPI along with combination of other drugs can be used to treat breast cancer for the patients who undergo surgeries for tumour removal as EPI exhibits highest rate of survival among the patients with poor prognosis breast cancer [3]. EPI also has Anti-Neoplastic

properties analogous to doxorubicin hydrochloride [4]. EPI's mechanism of action is based on the formation of the complex with DNA by intercalation of its planar rings between nucleotide base pairs which inhibits nucleic acid and protein synthesis. This intercalation causes DNA cleavage by topoisomerase II, resulting in cytocidal activity. The most widely used anthracycline medication for breast cancer treatment is Epirubicin hydrochloride [5].

Related substances (RS) Is the small amount of degradation impurity which is associated with the main drug compound. RS identification, quantification together with the pharmaceutical dosage form is the prime need for the safety and stability of the drug [6]. Doxorubicin and Doxorubicinone are the organic impurities associated with Epirubicin and these impurities are generally categorised as process related impurities. As, Doxorubicin and Epirubicin (4' epimer of Doxorubicin) both the molecular structures are similar, their mode of activity is also same except the cardio toxic activity of Doxorubicin [7]. Doxorubicin (DXR) when compared with EPI drug has highest therapeutic index [8-9], thus EPI is favoured over the DXR. The chemical name of DXR is (7*S*,9*S*)-7-[(2*R*,4*S*,5*S*,6*S*)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihtdro-7*H*-tetracene-5,12-dione.

Doxorubicinone (DXR-one) is a metabolite of DXR [10]. The chemical name of Doxorubicinone is (8S.10S)-7, 8, 9, 10-tetrahydro-6, 8, 10, 11-tetrahydroxy-8(2-hydroxyacetyl)-1-methoxy-5,12-naphthacenedione. DXR-one has its metabolite determination from DXR [11] and is also called as Adriamycin aglycone. The present study represents the impurity profiling of the degradation impurities of EPI formulation. These related impurities namely DXR, DXR-one were quantified by using RP-HPLC separation technique. The structures of EPI, DXR & DXR-one is shown in the Figure 1.

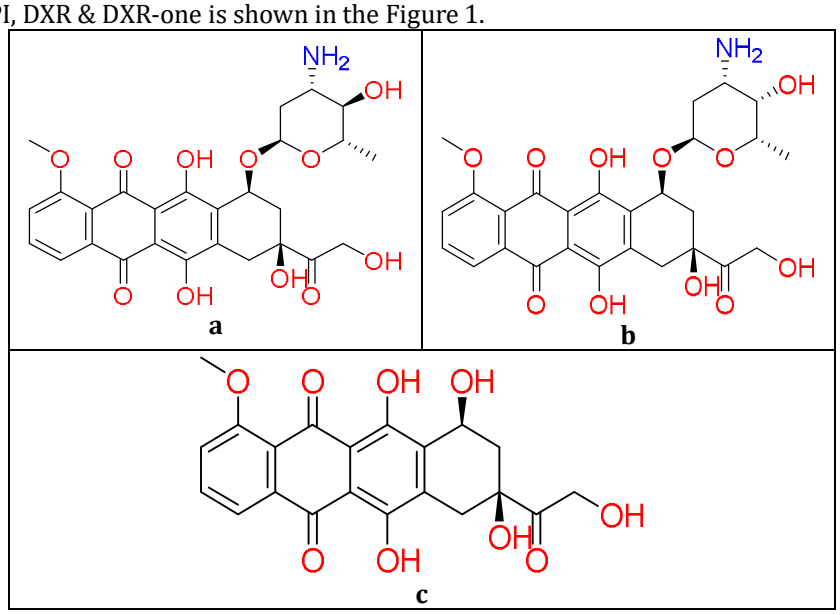


Figure 1: Structure of a) EPI, b) DXR & c) DXR-one

According to the review of literature it was found that DXR and DXR-one are the related substances of Epirubicin. Many advancements are seen in the determinations of impurities [12]. And several methods are available for the determination of the Epirubicin. In these methods single impurity or combination with other impurities were determined using different analytical techniques. HPLC-DAD technique was developed for the purpose of identification and quantification of Epirubicin in combination with topotecan, irinotecan, etoposide where high column temperature is maintained i.e., 40°C and injection volume used was 3µl[13]. LC-Orbitrap-MS gives a brief output regarding the presence of cyclophosphamide and Epirubicin in waste water [14]. LC-MS [15], UPLC-MS/MS [16], LC-FL [17] methods were used for quantifying EPI in human plasma and urine (18). Presently, for the quality control of EPI and RS very few scientific papers were reported using the analytical techniques like RP-LC [19]. Epirubicin along with other impurities was also reported using HPLC [20] method. In the current study an RP-HPLC method was developed and validated for the quantification of the two impurities i.e. DXR, DXR-one. This method aims for the impurity profiling and for the purpose of process improvement. Force degradation & stability studies of the selected drug pave way for the usage of the optimised and developed method in industries and laboratories. Green analytical chemistry (GAC) has been an important aspect in the field of analytical chemistry and pharmacy. The main aim of the GAC is to benefit the industrial and pharmaceutical sectors in cost reduction, operator friendliness and economic

production by removing the usage of harmful & dangerous chemicals and the influence of the chemical processes by employing two green assessment tools like Analytical GREEnness Metric Approach and Software (AGREE) and the Green Analytical Procedure Index (GAPI).

MATERIAL AND METHODS

Reference Standards of drug substances, reagents and chemicals used

EPI, DXR & DXR-one reference standards were gifted by Hetero Labs Ltd, Hyderabad, Telangana. Marketed formulation named Alrubicin 100 was obtained from local laboratories. Sodium dodecyl sulphate, Ortho-phosphoric acid, acetonitrile (ACN) chemicals used to prepare standard, sample and buffer solutions were obtained from the SD fine chemicals, Mumbai, Maharashtra.

RP-HPLC Equipment used

Shimadzu LC 20 AD equipped with Elico pH meter was used for chromatographic separation. The HPLC instrument consists of an auto-sampler, binary pump, a degasser, temperature-controlled column compartment and a wavelength detector. Separation was achieved by using mobile phase combination of buffer and acetonitrile with pH maintained at 2.5 in the ratio 56:44 v/v with a flow rate of 1.0 mL/min. Mobile phase was also used as diluent. Zorbax Eclipse XDB C18, (150mm × 6.6mm, 5µm) column was used for isocratic elution of the compounds. Column temperature was maintained at 25°C and injection volume was 10µL with a total run time of 45 minutes.

Preparation of buffer solution and mobile phase

2.3 g of sodium dodecyl sulphate (SDS) was dissolved in 900 mL of water and the pH was maintained at 2.5 using 30% ortho-phosphoric acid and the volume was made up to the mark in 1 L volumetric flask. Buffer solution and acetonitrile in the ratio 56:44 v/v were mixed and pH was adjusted to 2.5. The mobile phase solution was degassed prior to use.

Diluent

Mobile phase was used as a diluent.

Placebo solution

100 mg of placebo powder was weighed and transferred into a 100 mL volumetric flask, which was further diluted up to the mark with the diluent.

Preparation of EPI standard solution

100 mg of the working standard of Epirubicin hydrochloride was weighed and transferred into a 100 mL volumetric flask, which was dissolved and diluted up to mark with the diluent.

Preparation of EPI stock solution

5mL of the above mixture is transferred into 50mL volumetric flask, and diluted with the diluent.

Preparation of DXR and DXR-one RS stock solutions

Transfer 5 mg of working standard DXR into a 250mL volumetric flask and dissolve with diluent until the desired level. In the same way, transfer 2.5 mg of the working standard of DXR-one into 250 mL volumetric flask and dissolve with the diluent up to the mark.

Preparation of DXR and DXR-one RS sample solutions

Transfer accurately 5 mL of DXR RS standard stock solution into 50mL volumetric flask and. In the same way, transfer 5 mL of the DXR-one RS standard stock solution into 50 mL volumetric flask and dilute both the solutions with the diluent up to the mark to prepare DXR RS and DXR-one sample solutions respectively.

Method development

The developed method deliberates over EPI impurities DXR and DXR-one. DXR and DXR-one were injected along with EPI to confirm the retention times. All the peaks were resolved well. The mobile phase and pH were chosen cogitating the nature and elution of the related substances was performed by conducting number of trials. The buffer solution was combined with acetonitrile in different combinations, which plays a crucial role in selecting the appropriate ratio of the mobile phase for the method. Overall, the pH 2.5 was found to be suitable and the method was compassed with XDB C18, 150mm × 4.6mm, 5µm column. The temperature was maintained at 25°C which is ambient and suitable for the method. The run time for the developed method was 45 minutes, where all the impurities were eluted and well resolved peaks were obtained. In addition to this, forced degradation studies were also carried out to assess the degradation peaks with respect to acid, base, peroxide, thermal and photolytic stress. In order to ensure the safety and efficacy of the drug these studies aid in evaluating the drug stability and impurity profiles. Further, various parameters like temperature, pH, and flow rate were changed and the robustness was evaluated, the values were found to be within the range. The developed & optimised method was also applied for green assessment using two tools viz., AGREE & GAPI.

Method Validation

Method validation was performed by preparing all the sample solutions by diluting the stock solution with the diluent. Parameters viz., linearity, range, selectivity, system suitability, precision, accuracy, Detection Limit & Quantification Limit, robustness and stability of the solutions were validated as per the ICH guidelines for the developed method [21-25].

RESULTS AND DISCUSSION

A high resolved column with suitable pH was selected for the stability and resolution of peaks. SDS buffer and acetonitrile are suitable for the separation with column XDB C18, 150mm ×4.6mm,5µm in isocratic elution. pH 2.5 was maintained for the system which was suitable for the effective elution of the related substances. Separation was carried out using the mobile phase combination of buffer and acetonitrile in the ratio of 56:44 v/v by maintaining pH at 2.5. Isocratic elution was maintained in the separation. The retention time of Epirubicin was 11.06 minutes with respect to impurities which was nominal and run time was 45 minutes. This method was effective in estimating the assay of Epirubicin and its impurities. Method validation parameters results were found to be within the acceptance criteria. The developed method was validated according to the ICH guidelines. Later, forced degradation studies were carried out and the degradation peaks found were well separated from specified impurities and unspecified impurities, thus this method was concluded as stability indicating method. The method validation parameters results proved that the method is precise, selective, accurate, specific and robust.

System Suitability

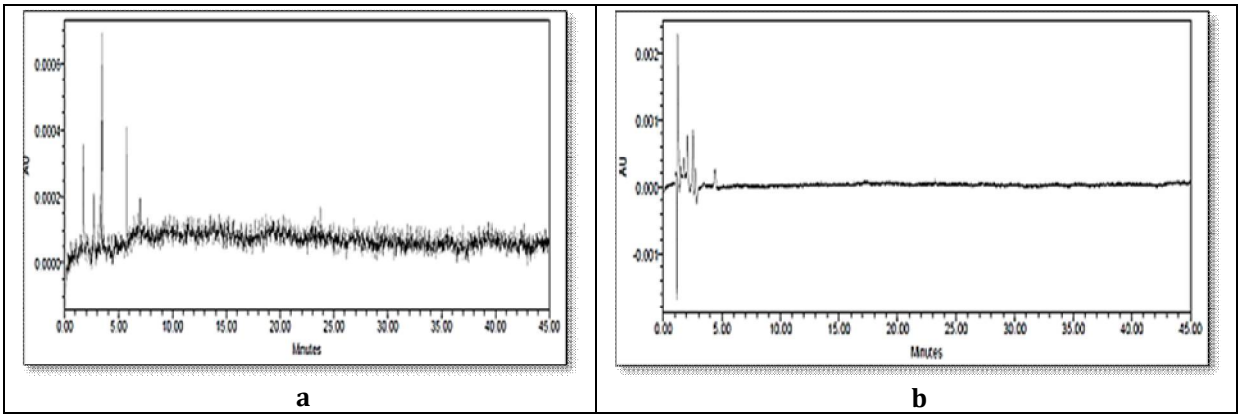
The blank solution and standard solutions of EPI, DXR & DXR-one was injected six times consecutively. The retention time and relative retention time were measured and the suitability of the system was established. The system suitability data is given in the Table 1.

Table 1: System Suitability Parameters

Sl. No.	Name of the Drug	Retention time (mins) (n=6)	Relative Retention Time (mins) (n=6)
1.	Epirubicin	11.06	--
2.	Doxorubicinone	2.5	0.20
3.	Doxorubicin	7.9	0.80
4.	Unspecified Impurity	0.85	0.85
5.	Any Individual Impurity	NA	NA

Specificity

The specificity of the method was demonstrated by injecting blank, standard and spiked sample solutions containing impurities. Placebo solution was also injected to check the specificity of the method. Epirubicin peak and the peaks corresponding to the impurities were well resolved. There was no interference detected with respect to blank, placebo solution at the retention time of EPI and its impurities DXR & DXR-one. Chromatograms of placebo, blank, standard and sample solutions are shown in the Fig 2.



a **b**

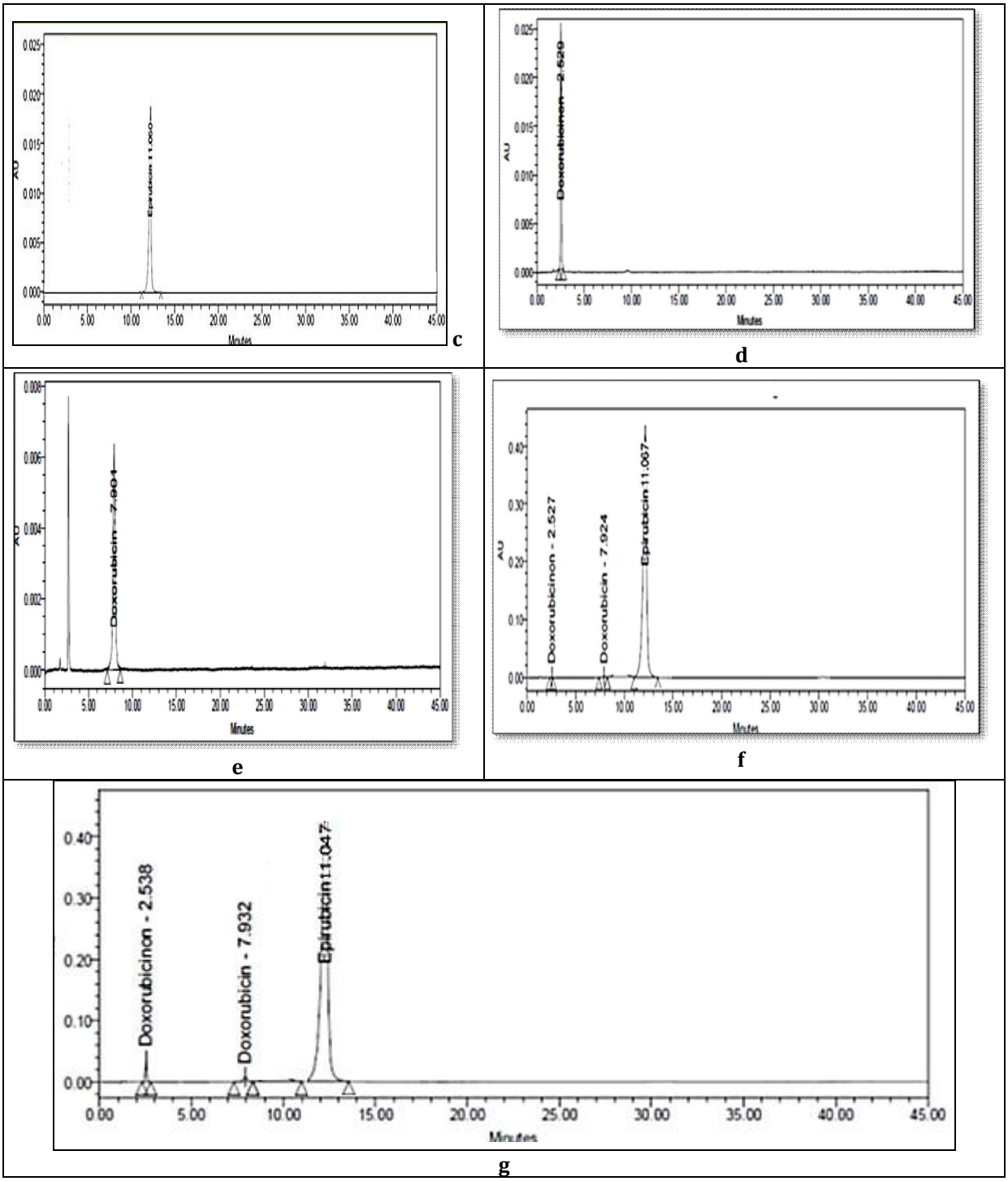


Figure 2: System suitability Chromatograms: a) Placebo b) Blank c) Epirubicin d) Doxorubicin e) Doxorubicinone f) Sample g) Spiked Sample

Linearity & Concentration range

The linearity solutions for the EPI drug and its specified impurities i.e., DXR & DRX-one was determined from the concentration ranging between the LOQ to 150% for EPI and LOQ to 300% for DXR & DXR-one respectively. The correlation coefficient, slope, and intercept for Epirubicin and its impurities was calculated and the data is given in the table 2 and the linearity graphs are shown in the fig 3.

Table 2: Linearity results of EPI, DXN & DXR-one

Sr. No.	Linearity Levels	EPI		DXR		DXR-one	
		Conc.	Peak area	Conc.	Peak area	Conc.	Peak area
1.	LOQ	0.0479	5840	0.0496	4860	0.0472	5790
2.	50	47.950	4852816	0.500	37758	0.851	87686
3.	80	76.720	7718425	0.801	65315	1.361	147089
4.	100	95.900	9590267	1.001	80346	1.701	179164
5.	120	115.080	11536197	1.501	118926	2.552	262250
6.	150	143.850	14422026	2.002	155194	3.403	342667
7.	200	--	--	3.003	235577	5.104	511489
6.	300	--	--	0.0496	4860	0.0472	5790
Correlation Coefficient		1.000		0.999		0.999	
Slope		100098.6219		77981.38		99472.03	
Y-Intercept		20581.1803		1035.43		6020.66	

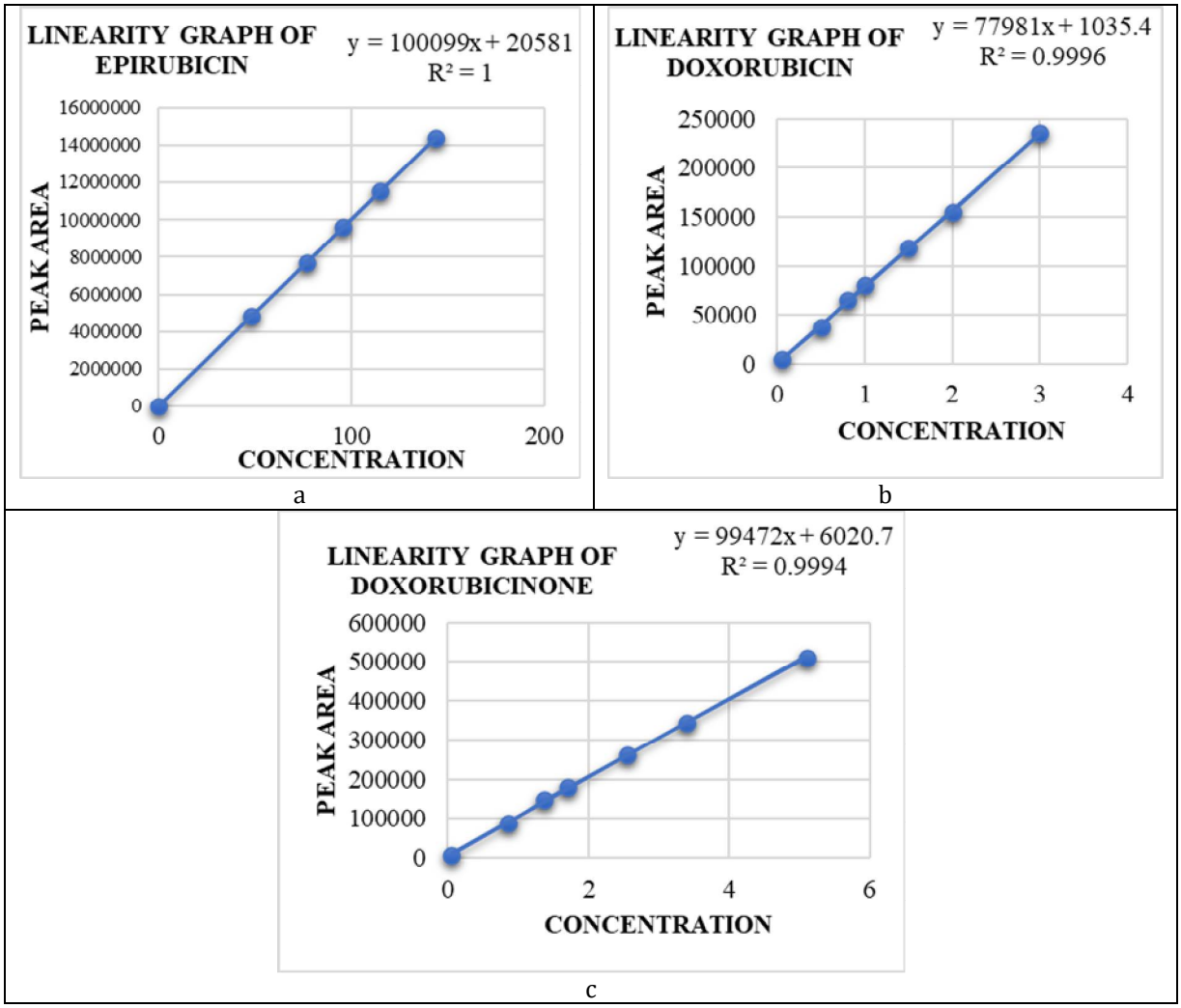


Figure 3: Linearity Graphs of a) EPI, b) DXR and c) DXR-one

Accuracy

The Accuracy was determined by injecting the blank solution, 50% to 150% of assay solutions for EPI drug and for its specified impurities DXR & DXR-one the spiking levels were from 50% to 300%. % Mean recovery & % RSD values were calculated and were found to be within the acceptance criteria. The accuracy data is given in the Table 3.

Table 3. Accuracy data

Sl. No.	Name of the Drug	Recovery Level	Placebo added (n=3)	% Mean Recovery (n=3)	Acceptance criteria	% RSD	Acceptance criteria % RSD
1.	Epirubicin	50 %	100 %	102.1	98-102 %	0.2	<2
		100%	100 %	100.7	98-102 %	0.1	<2
		150%	100 %	101.8	98-102 %	0.1	<2
2.	Doxorubicinone	50 %	100 %	99.39	98-102 %	0.6	<2
		100%	100 %	99.47	98-102 %	0.9	<2
		150%	100 %	99.63	98-102 %	0.6	<2
		200%	100 %	99.53	98-102 %	0.1	<2
		300%	100 %	99.33	98-102 %	0.9	<2
3.	Doxorubicin	50 %	100 %	99.02	98-102 %	2.0	<2
		100%	100 %	99.58	98-102 %	0.7	<2
		150%	100 %	99.05	98-102 %	1.0	<2
		200%	100 %	99.08	98-102 %	0.2	<2
		300%	100 %	99.47	98-102 %	1.1	<2

Precision

System precision was achieved by injecting the sample solution of EPI and its specified impurities (DXR & DXR-one) six times consecutively to calculate the SD & % RSD. In the same way the intermediate precision was also performed by injecting the sample solution of only EPI drug six times consecutively interday and intraday. The results of the obtained results were summarized in the Tables 4 & 5.

Table 4: System Precision data of EPI, DXR and DXR-one

Sr. No.	Epirubicin % Assay (n=6)	Doxorubicinone % Assay (n=6)	Doxorubicin % Assay (n=6)
1.	97.90	01.588	0.512
2.	97.94	1.518	0.542
3.	97.25	1.549	0.511
4.	97.89	1.529	0.521
5.	97.95	1.547	0.503
6.	97.91	1.523	0.516
Average	97.60	1.534	0.534
SD	0.3475	0.0127	0.0280
% RSD	0.4	0.8	1.9

Table 5. Intermediate Precision data of EPI

Sr. No.	Analyst –I/Column-I/System-I % Assay (n=6)	Analyst –II/Column-II/System-II % Assay (n=6)
1.	97.55	97.6
2.	97.94	97.5
3.	97.25	94.3
4.	97.89	97.8
5.	97.17	96.7
6.	97.91	97.7
Avg.	97.6	96.9
SD	0.3475	1.36
%RSD	0.4	1.4

Detection Limit & Quantification Limit

The Detection Limit & Quantification Limit were calculated for the developed method for determining the lowest detectable amount and lowest quantifiable amount of the EPI & its impurities DXR & DXR-one. The formulae of LOD and LOQ is given below.

$$\text{LOD} = \frac{10 \times SD}{Slope\ of\ the\ Caliberation\ curve}$$

The results of Detection Limit & Quantification Limit are shown in the Table 6.

Table 6: Detection Limit & Quantification Limit data

Name of the Drug	Detection Limit (µg/ml)	Quantification Limit (µg/ml)
Epirubicin	0.0144	0.0479
Doxorubicinone	0.0142	0.0474
Doxorubicin	0.0149	0.0496

Robustness

The robustness study was performed by slightly changing the optimized conditions and the %RSD was calculated. The optimized conditions viz., Buffer pH, flow rate, column temperature, sample temperature and mobile phase composition were changed slightly and robustness study was carried out. The results of the robustness data are mentioned in the Table 7.

Table 7: Robustness data of the developed method

Parameter	Optimised conditions	Robust changes	Resolution between EPI & DXR	% RSD	Acceptance criteria
Flow Rate	1.0 ml/min	0.9 ml/min	3.8	0.1	<2
		1.1 ml/min	3.3	0.3	<2
Column Temperature	25ºC	20ºC	3.3	1.5	<2
		30ºC	3.2	0.5	<2
Sample Temperature	25ºC	20ºC	3.9	0.3	<2
		30ºC	3.8	0.2	<2
pH	2.5	2.3	3.7	0.3	<2
		2.7	4.2	0.3	<2
Mobile phase composition	Buffer	Less organic	4.5	0.3	<2
		More organic	2.9	0.3	<2

Forced degradation studies

The blank, placebo, sample solutions were prepared and injected into the system under acid, base, water hydrolysis, thermal and photolytic stress conditions [26-30]. Sample solutions were prepared by dissolving the drug substances in the diluent and treated with 0.1M aqueous hydrochloric acid, 0.1M aqueous sodium hydroxide and 30% aqueous hydrogen peroxide at 60°C for 60 minutes. Thermal degradation was carried out by placing the sample of the drug in the controlled temperature oven at 80°C for seven days. Photolytic study was conducted by exposing the sample to photolytic degradation at 1.2 million lux hours. The %degradation, peak purity and mass balance were calculated. The degradation peaks found were well resolved. The forced degradation studies data is given in the Table 8.

Solution stability

Instability of the solutions can cause inaccurate measurements as well as influences the storage conditions, which may affect the product quality. Stability studies of the solutions direct suitable storage and quality control methods for long term analysis. Related substances i.e. impurities DXR &DXR-one solutions were prepared by mixing with diluents and put in the refrigerator at 2 to 8°C. The solution stability was examined by injecting the standard and the sample solutions of DXR & DXR-one. Stability of the solutions was also evaluated over the unspecified impurities, unknown signal maximum and total impurities. At the room temperature stability of the sample solutions was steady. The values of the DXR and DXR-one were compared with LOQ concentrations and noticed that the solutions were stable. The stability studies were carried out at temperatures which were set at 10°C and 2-8°C & at 25°C with the time interval between 3 hours to 24 hours. The solution stability data in shown in the Tables 9 & 10.

Table 8: Force degradation study results of EPI, DXR & DXR-one

Sr. No.	Condition	0.1N HCl for 60 min at 60°C	0.1N NaOH for 60 min at 60°C	30% H_2O_2 for 60 min at 60°C	Thermal degradation at 105°C for 2 hours	Photo stability at 1.2 million lux hours.
1.	% Assay of EPI	97.14	97.60	86.39	91.87	96.98
2.	% Assay of DXR-one	1.17	0.009	0.013	3.672	0.027
3.	% Assay of DXR	0.133	0.132	0.122	0.079	0.083
4.	Unspecified at 0.85 RRT	0.055	0.073	0.436	0.129	0.005
5.	SMUI	0.437	0.426	1.446	0.249	0.187
6.	Total Impurity (Net Degradation)	1.856	0.640	3.934	4.301	0.365
7.	Mass balance	99.00	98.24	90.32	96.2	97.3
8.	Purity Angle	0.31	0.069	0.056	0.642	0.106
9.	Purity Threshold	0.227	0.254	0.254	0.783	0.272
10.	Peak purity	PASS	PASS	PASS	PASS	PASS
11.	Acceptance criteria	Mass balance should be between	Mass balance should be between 90 % and 110 %	Mass balance should be between 90	Mass balance should be between 90 % and 110 %	Mass balance should be between 90 % and 110 %

Table 9: Solution stability of sample solution at 10°C and 2-8°C

Condition	Doxorubicinone		Doxorubicin		Unspecified impurity		Unknown signal maximum		Total impurities	
	10°C	2-8°C	10°C	2-8°C	10°C	2-8°C	10°C	2-8°C	10°C	2-8°C
Initial	1.596	NA	1.198	NA	0	NA	0.375	NA	3.225	NA
3 hours	1.630	1.597	1.117	1.117	0	0	0.348	0.229	3.172	2.994
Difference	0.034	0.002	0.081	0.081	0	0	0.03	0.146	0.05	0.232
7 hours	1.590	1.590	0.997	0.997	0	0	0.358	0.262	3.011	2.897
Difference	0.006	0.006	0.201	0.201	0	0	0.018	0.113	0.215	0.329
12 hours	1.604	1.604	1.067	1.067	0	0	0.324	0.324	3.048	3.048
Difference	0.008	0.008	0.131	0.131	0	0	0.051	0.051	0.178	0.178
16 hours	1.589	1.589	1.138	1.138	0	0	0.348	0.348	3.128	3.128
Difference	0.007	0.007	0.06	0.06	0	0	0.028	0.028	0.098	0.098
20 hours	1.601	1.601	1.173	1.173	0	0	0.368	0.368	3.194	3.194
Difference	0.005	0.005	0.025	0.025	0	0	0.007	0.007	0.031	0.031
24 hours	1.603	1.603	1.164	1.164	0	0	0.367	0.367	3.194	3.194
Difference	0.007	0.007	0.034	0.034	0	0	0.008	0.008	0.032	0.032

Table 10: Solution stability of sample solution at 25^{0}C

Condition	Doxorubicinone	Doxorubicin	Unspecified impurity	Unknown signal maximum	Total impurities
	25°C	25°C	25°C	25°C	25°C
Initial	1.340	0.856	0	0.321	2.624
5 hours	1.345	0.860	0	0.317	2.628
Difference	0.006	0.005	0	0.005	0.004
9 hours	1.348	0.850	0	0.304	2.610
Difference	0.008	0.006	0	0.017	0.014
14 hours	1.352	0.844	0	0.273	2.578
Difference	0.012	0.012	0	0.048	0.046
18 hours	1.349	0.832	0	0.188	2.57
Difference	0.009	0.024	0	0.133	0.054
24 hours	1.345	0.794	0	0.158	2.395
Difference	0.005	0.062	0	0.163	0.229

Green Assessment of the developed and optimized method

Green Analytical Chemistry (GAC) is guided by twelve fundamental principles that prioritize sustainability, efficiency, and environmental responsibility in chemical analysis [31-40]. These principles advocate for reduction or elimination of hazardous substances, promoting safer alternatives that minimize risk to human health and the environment. GAC emphasizes the use of renewable resources, energy efficient techniques and the reduction of waste generation throughout the analytical process. It encourages the development and application methods that consume fewer resources, including water and energy, while striving for high analytical performance. GAC also promotes transparency and proclamation of analytical methodologies to facilitate their adoption and improvement across scientific disciplines. By maintaining these principles, GAC also advances analytical capabilities, contributes to the greater extent to the sustainable development goals and responsible stewardship of natural resources. To evaluate and assess the developed method is environment safe and operator friendly Analytical chemists using AGREE and GAPI tools. The tools are crucial in analytical chemistry due to concerns about environmental safety and sustainability. Analytical procedures in research and quality control often contribute significantly to long-term environmental impacts. Therefore, assessing the ecological footprint of new analytical methods has become essential. Over the past decade, AGREE and GAPI metrics have emerged as prominent evaluation criteria. AGREE [31,32] metrics utilize a pictogram divided into 12 areas, each representing a principle of GAC, with a central score ranging from 0 to 1. A higher score towards 1 indicates greater environmental friendliness. The current study has obtained a score of 0.71 for the developed method to separate and identify EPI from its impurities DXR & DXR-one. The orange colour at 7th & 8th areas are depicting the generation of large volume of analytical waste and single analyte analysis at a time. The AGREE metrics in the form of the pictograms is shown in the Figure 4.

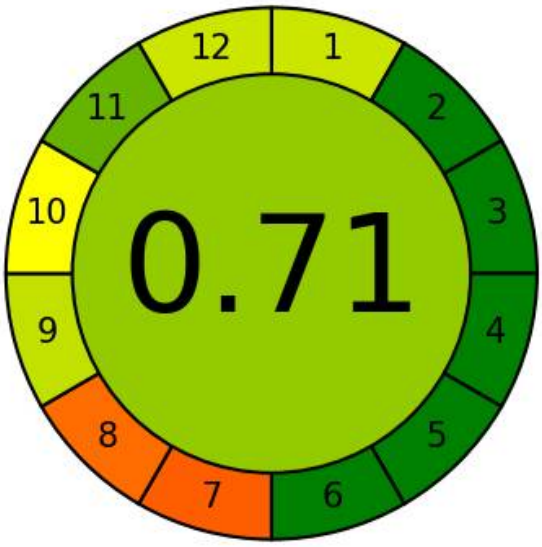


Figure 4: AGREE Metrics of the developed method for EPI drug

GAPI [33,34], is another crucial evaluation tool, which also employs a pictogram with five pentagons representing key steps in the analytical methodology. Sample approach, sample preparation, regents and compounds used, instrumentation, and general method type. These pentagons are further divided into 15 areas, evaluation is done on the basis of three colours (red, yellow, green) to assess the nature of the analytical procedure and various stages from sample collection to result interpretation. Fig. 5 illustrates the GAPI metrics for the developed method to separate and identify EPI drug, red color areas are highlighting & representing the key steps such as offline sample collection, sample transportation, the use of acetonitrile solvent and waste more than 10 mL.

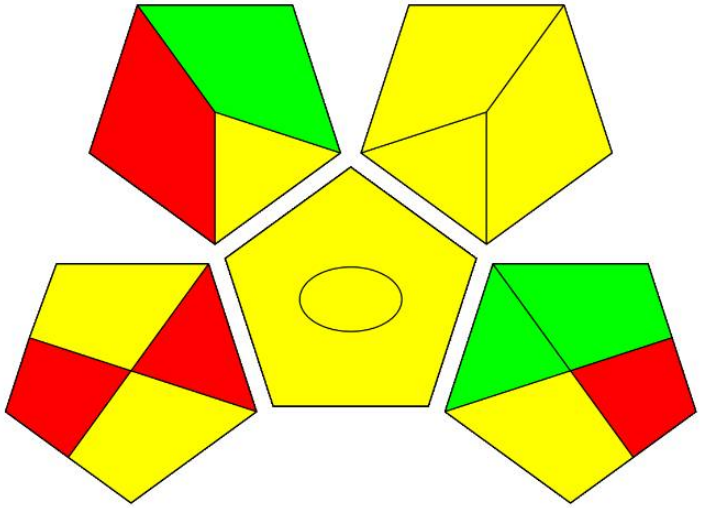


Figure 5: GAPI Metrics of the developed method for EPI drug

CONCLUSION

A stability study was carried out with an efficient HPLC method for the qualitative quantitative estimation of related substances of Epirubicin. The ICH recommendations were followed in the validation of the current investigation to demonstrated the linearity, accuracy, precision and specificity of the analytical HPLC approach, which allows for the separation of the active medication from its breakdown products. It was also determined that the suggested approach exhibits resilience concerning the temperature of the column, the mobile phase composition, and the flow velocity. Owing to these qualities, the method exhibits stability, suggesting that it is appropriate for its intended use. As such, it could be useful for routine analyses of the related components of Epirubicin in marketed formulation.

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