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Research

Analytical Method Development and Validation for Simultaneous Estimation of Epirubicin and Docetaxel in Bulk and Pharmaceutical Dosage form by Using RP-HPLC

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Abstract

A simple, rapid, and reliable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Epirubicin and Docetaxel in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a Phenomenex Luna C18 column (4.6 x 150 mm, 5 µm) at an optimized temperature of 35ºC. The mobile phase consisted of methanol and water in a 70:30 v/v ratio, with a flow rate of 1 ml/min, and detection was performed at 230 nm. The injection volume was 10 µL, and the total run time was 10 minutes. The method was validated as per standard guidelines, demonstrating excellent linearity, precision, accuracy, and sensitivity for both drugs. The results confirmed that the method was specific, reproducible, and suitable for the quality control analysis of Epirubicin and Docetaxel in bulk and dosage forms. This RP-HPLC method offers a fast and efficient approach for the simultaneous determination of these drugs, making it an ideal tool for routine analysis in pharmaceutical industries.

Keywords: RP-HPLC, Epirubicin, Docetaxel, Phenomenex Luna C18 column, simultaneous estimation, validation.

INTRODUCTION

EPIRUBICIN

The anthracycline anticancer drug epirubicin (10-(4-amino-5-hydroxy-6-methyloxan-2-yl)oxy-6,8,11-trihydroxy-8-(2hydroxy acetyl)- 1-methoxy-9,10-dihydro-7H-tetracene-5,12-dione) is used for the therapy for lymphomas, gastric cancer, lung cancer, ovarian cancer, and node-positive breast cancer.A molecule generated

by specific strains of Streptomyces peucetius is chemically transformed to produce epirubicin [1]. It inhibits the production of DNA and RNA by intercalating into nucleotide base pairs to form a complex with DNA[2].It is preferred over doxorubicin because it is less hazardous at equimolar doses, possibly as a result of the hydroxyl group at the 4' carbon having a distinctspatialorientation.of the sugar moiety causes the opposite chirality, which speeds up its removal and lessens its toxicity[3].It can be administered intravenously, but because of the abrupt increase in blood levels and their quick removal, it frequently reaches subtherapeutic levels requires regular dosage, which can have detrimental effects. In order to achieve controlled medication release, which would not only prevent adverse effects but also aid in lowering dose and dosing frequency, we developed a polymeric nanoparticle delivery method. The complicated pharmacokinetics of nanotechnology-based delivery systems necessitates strict quality control of these dosage forms in order to preserve their efficacy and safety.

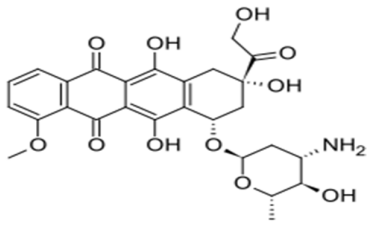

Fig 1: Structure of Epirubicin

DOCETAXEL

A mitotic inhibitor called docetaxel is used to treat many cancers[4]. This covers cancers of the ovaries, breast, lungs, Kaposi sarcoma, cervix, and pancreas[5,6]. Additionally, docetaxel is utilized to stop rest enosis. In 1967, Monroe E. Wall and Mansukh C. Wani isolated it from the bark of the Pacific yew tree, Taxus brevifolia, and termed it taxol as part of a US National Cancer Institute program at the Research Triangle Institute[7,8]. In the UK, docetaxel is licensed for Kaposi's sarcoma, ovarian, breast and lung, bladder, prostate, melanoma, and other solid tumor malignancies[9].The FDA approved its usage in the US to treat non-small cell lung cancer, pancreatic cancer, and breast cancer. The medication is marketed under a number of names in India. It is administered by venous injection. Docetaxel is linked to albumin in a more recent version that is marketed under the Abraxane name.

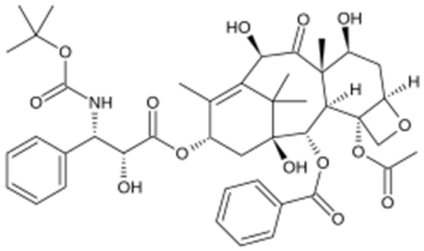

Fig 2: Structure of Docetaxel

Numerous analytical techniques for Docetaxel and Epirubicin analysis have been documented. Only stability-indicating liquid chromatography techniques have been published among them. The stability indicating HPLC method, assay method, bio-analytical method, UV spectrophotometer, and UV-derivative spectrophotometer are among the techniques for docetaxel. For estimating Docetaxel commercial formulations, it is now difficult to design an RP-HPLC method that is simpler in terms of mobile phase wide linearity range with a strong correlation coefficient value, high precision, rapidity, and economic stability indicating approach.

MATERIALS AND METHODOLOGY

S. No	Chemical	Brand names
1	Epirubicin	Procured from Sun pharma, provided by Sura Pharma labs
2	Docetaxel	Procured from Sun pharma, provided by Sura Pharma labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)

Preparation of standard solution

Accurately weigh and transfer 10 mg of Epirubicin and Docetaxel working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 2.25ml of the above Epirubicin and 0.45ml of the Docetaxel stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and water in proportion 70:30 v/v respectively.

Preparation of mobile phase

Accurately measured 700ml (70%) of HPLC Methanol and 300ml of Water (30%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Optimized Chromatogram

Mobile phase : Methanol: water (70:30v/v)
Column : Phenomenex Luna C18 (4.6 x 150mm, 5µm)
Flow rate : 1 ml/min
Wavelength : 258 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 7 minutes

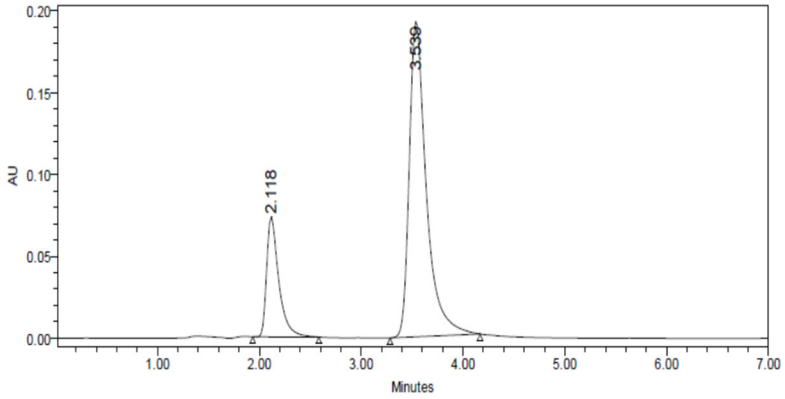

Fig 3: Optimized Chromatogram

Table 1: Peak Results for Optimized Chromatogram

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Epirubicin	2.118	658748	67824	3.25	1.06	6852
2	Docetaxel	3.539	8695825	841253	4.65	1.18	8548

RESULTS AND DISCUSSIONS

SYSTEM SUITABILITY

Table 2: Results of system suitability for Epirubicin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Epirubicin	2.117	658658	67854	6895	1.06
2	Epirubicin	2.118	657893	67582	6847	1.07
3	Epirubicin	2.116	658985	67895	6875	1.06
4	Epirubicin	2.109	659863	67852	6845	1.06
5	Epirubicin	2.102	658784	67456	6865	1.07
Mean			658836.6			
Std. Dev			707.2067			
% RSD			0.107342			

Table 2: Results of system suitability for Docetaxel

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Docetaxel	3.547	8658485	845258	8542	1.18	4.65
2	Docetaxel	3.539	8695847	847584	8574	1.19	4.66
3	Docetaxel	3.547	8657474	847612	8569	1.18	4.65
4	Docetaxel	3.565	8625698	846985	8532	1.18	4.65
5	Docetaxel	3.537	8675842	847526	8541	1.19	4.66
Mean			8662669				
Std. Dev			25911.66				
% RSD			0.299119				

ASSAY:

$$\frac{Sample\ area \times Weight\ of\ STD \times Dilution\ of\ sample \times Purity \times Wt.of\ sample}{Standard\ area \times Dilution\ of\ STD \times Weight\ of\ sample \times 100 \times Label\ claim} \times 100$$

Table 3: Showing Assay Results

S. No	Name of Compound	Label Claim	Amount Taken	% Purity
1	Epirubicin	20 mg	17.6	99.8%
2	Docetaxel	15 mg	8.9	99.5%

LINEARITY

Table 4: Results for Linearity of Epirubicin

Concentration µg/ml	60	80	120	140	180
Area	395687	523568	659748	791286	927987

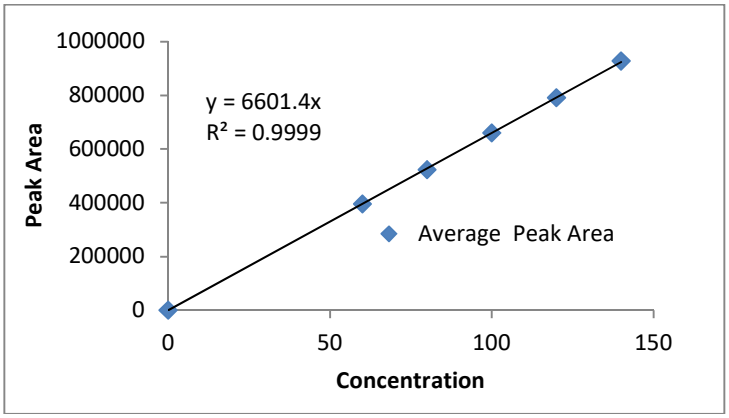

Fig 4: Calibration Curve of Epirubicin

Table 5: Results for Linearity of Docetaxel

Concentration µg/ml	30	40	50	60	70
Area	5648983	7379854	9195825	10965984	1285656

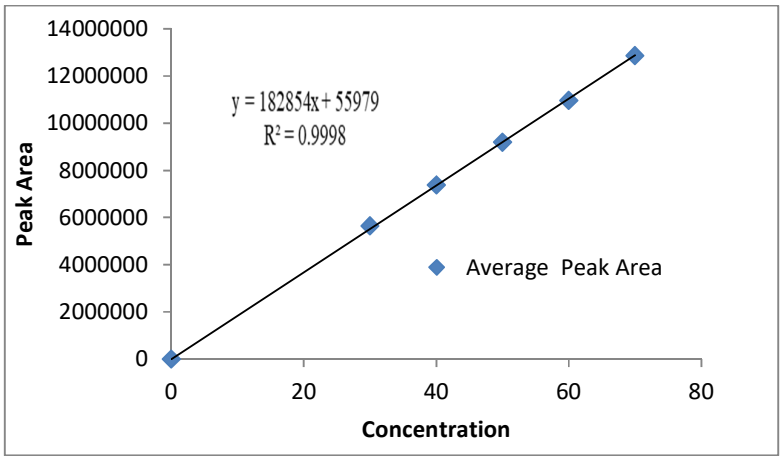

Fig 5: Calibration Curve of Docetaxel

PRECISION

1. **Repeatability**

HPLC Injection Replicates	AUC of Epirubicin	AUC of Docetaxel
1	658658	8658485
2	657893	8695847
3	658985	8657474
4	659863	8625698
5	658784	8675842
Mean	658836.6	8662669
Std.Dev	707.2067	8662669
%RSD	0.107342	0.299119

2. **Intermediate Precision**

HPLC Injection Replicates	DAY- 1		DAY- 2	
	Epirubicin	Docetaxel	Epirubicin	Docetaxel
1	648598	8589785	665985	8758452
2	648579	8547893	665982	8759865
3	659852	8698547	678598	8745895
4	648968	8547584	665467	8865412
5	658745	8745875	678549	8758462
6	649845	8659873	668475	8856983
Mean	652431.2	8631593	670509.3	8790845
Std.Dev	5350.652	82648.75	6333.921	54795.44
%RSD	0.82011	0.957515	0.944643	0.623324

ACCURACY

Table-: The accuracy results for Epirubicin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	334903	50	50.346	100.692%	100.36%
100%	665145	100	100.255	100.255%	
150%	995669.7	150	150.205	100.136%	

Table 6: The accuracy results for Docetaxel

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	515824.67	25	25.148	100.592%	100.44%
100%	975214.7	50	50.272	100.544%	
150%	1429742	75	75.130	100.173%	

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: Epirubicin:= 0.96µg/ml

Docetaxel:= 2.5µg/ml

LIMIT OF QUANTITATION

The quantitation limit of anindividual analytical procedure isthe lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: Epirubicin:= 2.88µg/ml

Docetaxel:= 7.5µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Epirubicin and Docetaxel.

Table 7: Results for Robustness of Epirubicin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	658748	2.118	6852	1.06
Less Flow rate of 0.9 mL/min	725416	2.330	6985	1.05
More Flow rate of 1.1 mL/min	648514	1.950	6548	1.02
Less organic phase	635254	2.290	6354	1.03
More organic phase	625898	1.998	6487	1.04

Table 8: Results for Robustness of Docetaxel

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	8695825	3.539	8548	1.18
Less Flow rate of 0.9 mL/min	9145487	3.885	8785	1.17
More Flow rate of 1.1 mL/min	8524583	3.263	8256	1.16
Less organic phase	8245147	4.435	8461	1.14
More organic phase	8365876	3.009	8199	1.15

SUMMARY

Parameter	Details / Findings
Drug(s) Analyzed	Epirubicin and Docetaxel
Column	Phenomenex Luna C18 (4.6 × 150 mm, 5 µm)
Mobile Phase	Methanol : Water (70:30 v/v)

Flow Rate	1.0 mL/min
Wavelength	258 nm
Column Temperature	Ambient
Injection Volume	10 µL
Run Time	7 minutes
Linearity	$R^2 > 0.999$ (excellent correlation)
Accuracy	Recovery: 98–102%
Precision	%RSD < 2% (repeatability and intermediate precision within limits)
Specificity	No interference from excipients, peaks well resolved
LOD & LOQ	Low values indicating high sensitivity
Robustness	Small variations in chromatographic conditions did not significantly affect results
System Suitability	Resolution > 2, Tailing factor < 2, Theoretical plates within acceptable limits
Peak Characteristics	Well-resolved, symmetric peaks for both drugs

CONCLUSION

The developed RP-HPLC method is simple, rapid, accurate, precise, robust, and cost-effective for the simultaneous estimation of Epirubicin and Docetaxel in bulk and pharmaceutical dosage forms. The optimized chromatographic conditions ensured well-resolved symmetric peaks with a short run time (7 min), making it suitable for routine quality control, stability testing, and formulation analysis.

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