SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 5.
Issue: 1.
Page N. 95-102
Year: 2021

Original Article www.wjahr.com

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DAUNORUBICIN AND CYTARABINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

A. Suneetha*, V. Mounika and S. Bindu Venkata Sravani

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi road, Guntur-522 002, Andhra Pradesh, India.

Received date: 22 October 2020 **Revised date:** 12 November 2020 **Accepted date:** 02 December 2020

***Corresponding author: A. Suneetha**

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi road, Guntur-522 002, Andhra Pradesh, India.

ABSTRACT

This present work describes a new validated Reverse Phase High Performance Liquid Chromatography (HPLC) method for the simultaneous determination of anti-cancer drugs, Daunorubicin and Cytarabine. A simultaneous determination method saves cost and time as both drugs can be injected into a single HPLC system without the need to change or re-equilibrate with a new mobile phase. The objective of the study is to develop a simultaneous estimation of two anti-cancer drugs in Pharmaceutical Dosage forms. The mobile phase consists of a mixture (50:50 v/v) of 0.1% Octane sulphonic acid: acetonitrile at a flow rate of 1 ml/min, with a PDA detector at 238 nm. Separation was achieved on a Xbridge C18 (150 x 4.6mm) maintained at 30°C temperature in a column oven. The method was linear between 22µg/ml – 110µg/ml for Daunorubicin and 50µg/ml - 250µg/ml for Cytarabine. The limit of detection was 2.98µg/ml for Daunorubicin, and 3.00µg/ml for Cytarabine and the limit of quantification was 10.00µg/mL for Daunorubicin and 9.98µg/mL for Cytarabine. The developed RP-HPLC method achieved good precision and accuracy. The developed and validated method was suitable to be used for routine analysis of Daunorubicin and Cytarabine.

KEYWORDS: Daunorubicin, Cytarabine, RP-HPLC, ICH-guidelines, Method development and Validation.

INTRODUCTION

Daunorubicin is also known as Daunomycin, is a chemotherapy medication used to treat cancer (Fig.1). Specifically, it is used for acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), and Kaposi's sarcoma. It is used by injection into a vein. Cytarabine (cytosine arabinoside, 1-b-D-arabinofuranosyl cytosine, ara-C) is a pyrimidine nucleoside analog which is predominantly used against acute myelogenous leukemia and non-Hodgkin's lymphoma (Fig. 2).

Daunorubicin and Cytarabine (I.V injection) is a liposomal combination of that is FDA approved for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC).[1-3]

Daunorubicin interacts with DNA by intercalation and inhibition of macromolecular biosynthesis. This inhibits the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription. Daunorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication. Cytarabine is a cell cycle phase-specific anti-neoplastic agent, affecting cells only during the S-phase of cell division. Cytarabine acts primarily through inhibition of DNA polymerase.

Literature review reveals estimation of by RP-HPLC.[4-6] and by Spectroscopy method,[7] individually. In combination, Dounorubicin and Cytarabine only one method was published,[8] but yet there is a need to develop new stability indicating RP-HPLC method with more sensitivity, accuracy and precision.

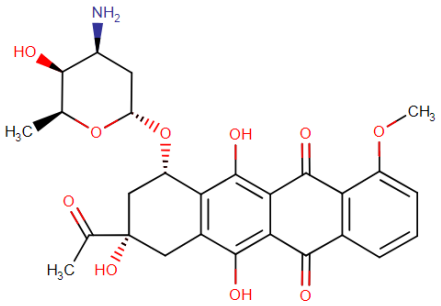

Fig. 1: structure of daunorubicin

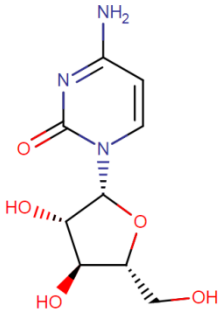

Fig. 2: Structure of cytarabine

MATERIALS AND METHODS

Chemicals and Reagents: Both Daunorubicin and Cytarabine (API) were obtained as a gift sample from Pharmatrain Pvt. Ltd., Hyderabad, India. The marketed formulation in the brand name Vyoxeos (Dauno-29 mg & Cyta-65 mg) procured from the local pharmacy. All the chemicals and reagents used in this work were HPLC grade water, acetonitrile, methanol, potassium dihydrogen orthophosphate buffer, orthophosphoric acid was obtained from Merck.

Instrumentation: A HPLC system with waters 2695 separation module provided with an UV detector, autosampler injection with Empower-2 software. Electronic balance, ultrasonicator, hot air oven and pH meter were used.

Chromatographic Conditions: The chromatographic separations achieved on a Xbridge C18 column (150 × 4.6 mm, 5 µm particle size) as a stationary phase. The mobile phase was composed of 50:50 v/v of 0.1% Octane sulphonic acid pH 3 and acetonitrile at a flow rate of 1.0 ml/min and injection volume is 20 µl. The column oven temperature was maintained at 30 ºC, and the drugs were detected at 238 nm.

Preparation of 0.1% Octane sulphonic acid

Transferred about 1gm of Octane sulphonic acid into 1000 ml of HPLC water and pH was adjusted up to 3.0. Final solution was filtered through 0.44 µm whatmann membrane filter and sonicated it for 10 mins.

Preparation of mobile phase

Transferred 500 ml (50%) of above buffer and 500 ml (50%) of HPLC grade Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as a diluent.

Mixed Standard Solution

Accurately weighed quantity of 100 mg of Cytarabine and 44 mg of Daunorubicin working standard were transferred into a 100 ml clean dry volumetric flask, sufficient amount of solvent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution). Further pipetted out 1.5 ml of the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent.

Sample Solution

Accurately weighed powder equivalent to 100 mg of Cytarabine and 44 mg Daunorubicin sample was transferred into a 100 ml clean dry volumetric flask dissolved with diluent and sonicated and made volume up to the mark with the same solvent. Then it is filtered through 0.45-micron injection filter. Further pipette out 1.5ml and dilute to 100 ml with same solvent.

Procedure

20 µl of the standard, sample were injected into the chromatographic system and measured the areas for Cytarabine and Daunorubicin peaks and calculate the % assay by using the formulae.

Validation of the RP-HPLC Method: The proposed RP-HPLC method was validated as per ICH guidelines.[9-12]

System Suitability Parameters: The system suitability parameters were determined by preparing standard solutions of Daunorubicin (50 µg/mL) and Cytarabine (22 µg/mL), and the solutions were injected six times and the parameters like retention time, peak tailing, resolution and USP plate count were determined.

Specificity: As per ICH guidelines "Specificity" can be defined as the ability of the method to specifically separate the particular API or analyte in the presence of other components.

Linearity: The stock solution of Daunorubicin and Cytarabine was prepared by using diluent. From that, various working standard solutions were prepared in the range of 22µg/ml to 110µg/ml, 50µg/ml to 250µg/ml and injected into the HPLC system. The calibration plot (peak area *vs.* concentration) was generated by replicate analysis (n=5) at all concentration levels. The linear relationship was evaluated using the least square method within Microsoft excel program.

Accuracy: The accuracy method was carried out using one set of different standard addition methods at different concentration levels 50%, 100% and 150% and then comparing the theoretical value and found value.

Precision: The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (50µg/mL Daunorubicine, 22µg/mL Cytarabine). The precision of the assay also determined in terms of intraday and interday variation in the peak area of a set of drug solutions on three different days. The peak area of a set of drug solutions was calculated in terms of relative standard deviation (RSD).

Detection Limit and Quantification Limit: Detection limit and quantification limit established based on the calibration curve parameters, according to the following formulas.
LOD = 3.3 SD/slope and LOQ = 10 SD/slope
or
Detection limit = 3.3σ/s, Quantification limit = 10 σ/s,

Where σ is the standard deviation of Y-intercept of the regression line and S is the slope of the curve.

Robustness: The Robustness of the proposed method carried out by small but deliberate changes in method parameters such as flow rate (±0.1), column temperature (±5), mobile phase ratio (±5%). The percentage recovery and RSD of peak area were evaluated.

Forced Degradation Tests: The stability indicating of the method can be demonstrated by applying stress conditions.[13,14] using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the both drugs were studied for peak purity, that indicating the method effectively separated the degradation products from the pure active ingredient.

Acid Degradation Studies: To 1 ml of Daunorubicin and Cytarabine stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N NaOH and madeup to final volume to obtain (50 µg/mL and 22 µg/mL) solution. Cool the solution to room temperature and filtered with 0.22 µm membrane filter. A sample of 10 µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution of Daunorubicin and Cytarabine 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCl and madeup to final volume to obtain (50 µg/mL and 22 µg/mL) solution. Cool the solution to room temperature and filtered with 0.22 µm membrane filter. The sample of 10 µl was injected into the system, and the chromatograms were recorded to an assessment of sample stability.

Peroxide Degradation Studies: To 1 ml of stock solution of Daunorubicin and Cytarabine 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 30 min at 60ºC.

For HPLC study, the resultant solution was diluted to obtain (50 µg/mL and 22 µg/mL) solution. Cool the solution to room temperature and filtered with 0.22 µm membrane filter. A sample of 10 µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Thermal Degradation Studies: The 1 ml of standard drug solution was placed in the oven at 105 ºC for 6 h to study dry heat degradation. For HPLC study, the resultant solution was madeup to final volume to obtain (50 µg/mL and 22 µg/mL) solution.

Cool the solution to room temperature and filtered through a 0.22 µm membrane filter. A sample of 10 µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Photo Degradation Studies: The photostability of the drug was studied by exposing the stock solution to UV light for 7 days or 200 Watt-hours/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain (50 µg/mL and 22 µg/mL) solution and filtered with 0.22 µm membrane filter. A sample of 10 µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

RESULTS AND DISCUSSION

Method validation was performed according to ICH Q2 guidelines. In the blank chromatogram, there were no peaks observed at the retention times of Daunorubicine and Cytarabine.

System Suitability: System suitability was performed to evaluate the parameters like tailing factor, theoretical plates, resolution and % RSD for replicate injections. The results were within limits and were given in Table 1 and shown in Fig. 3.

Specificity: Retention times of Daunorubicin and Cytarabine were 4.144 min and 2.461 min for standard and4.144 min and 2.461 min for sample respectively. Which were shown in Fig. 4, 5, 6.

We did not find any interfering peaks in blank at retention times of these drugs in this method. So, this method was said to be specific.

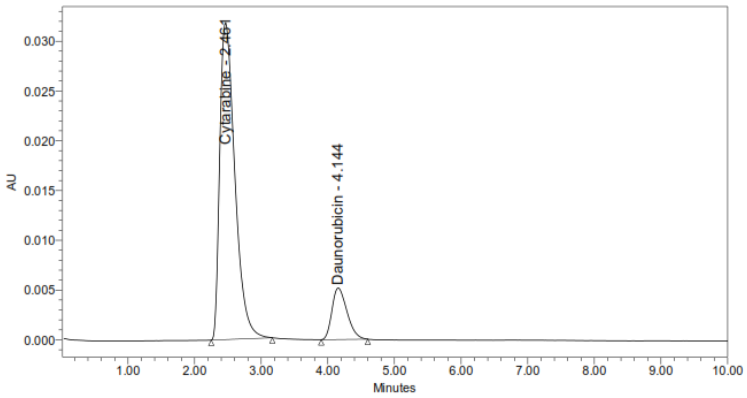

Figure 3: Chromatogram for system suitability.

Table 1: Results of system suitability parameters.

S. No.	Name	RT (min)	Area (µV)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Cytarabine	2.461	494276	32593		1.40	5397.09
2	Daunorubicin	4.144	76298	5263	4.09	1.32	6776.27

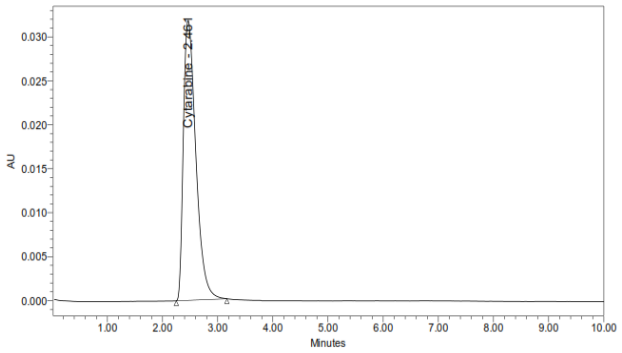

Figure 4: Chromatogram for Cytarabine.

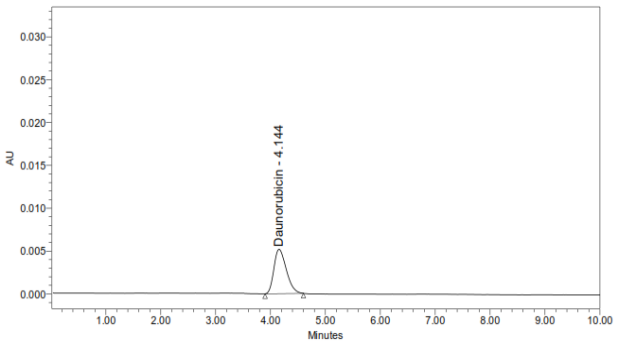

Figure 5: Chromatogram for Daunorubicin.

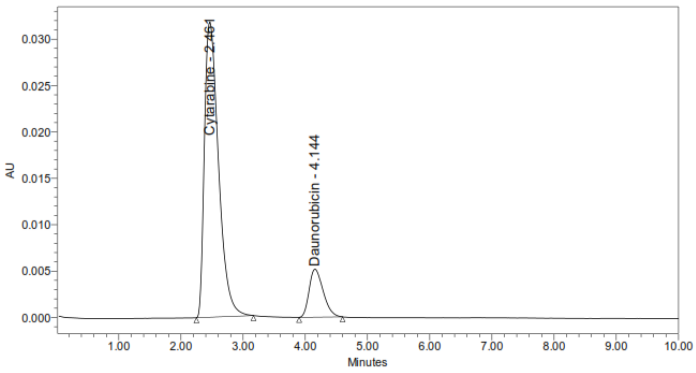

Figure 6: Chromatogram for Cytarabine and Daunorubicin.

Linearity: The linearity of the measurement was evaluated by analyzing different concentrations (50% to 250%) of the standard solutions of Daunorubicin and Cytarabine. The calibration curve was constructed by plotting concentration against mean peak area, and the regression equation was computed. The coefficient of correlation (R^2) for Daunorubicin and Cytarabine were 0.999. The summary of the parameters is given in Table 2 and shown in Fig. 7, 8.

Table 2: Area of different concentration of Cytarabine and Daunorubicin.

S. No.	Cytarabine		Daunorubicin	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	50	167253	22	26286
2	100	331583	44	51274
3	150	489537	66	76528
4	200	663425	88	104363
5	250	830462	110	130287

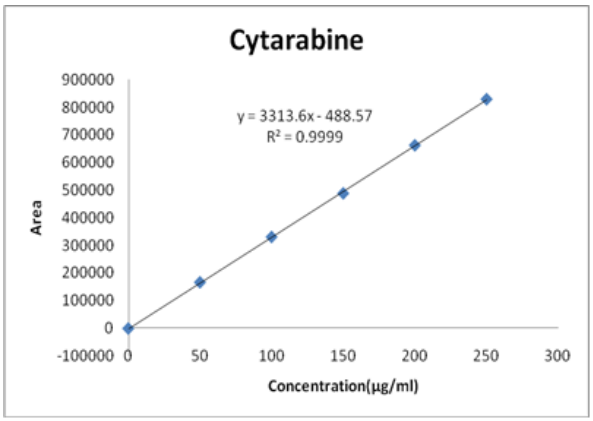

Figure 7: Calibration graph for Cytarabine.

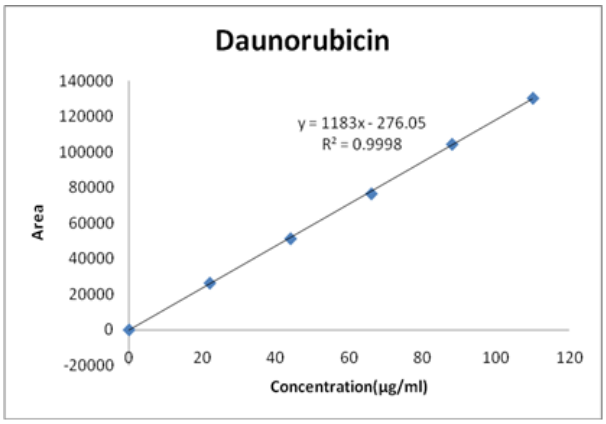

Figure 8: Calibration graph for Daunorubicin.

Accuracy: To determine the accuracy of the proposed method, recovery studies were conducted at three different levels, 50, 100 and 150% and were calculated. Accuracy was calculated as the percentage of recovery, and the results were shown in Table 3,4.

Table 3: Accuracy (recovery) data for Cytarabine.

%Concentration (at specification Level)	Area	Concentration of pre analyzed sample (µg)	Amount Added (µg)	Amount Found (µg)	% Recovery	Mean Recovery
50%	248273.7	100	50	50.13	100.25	100.42
100%	497256.3	100	100	100.40	100.40	
150%	747586.3	100	150	150.94	100.63	

Table 4: Accuracy (recovery) data for Daunorubicin.

% Concentration (at specification Level)	Concentration of pre analysed sample (µg)	Area	Amount Added (µg)	Amount Found (µg)	% Recovery	Mean Recovery
50%	44	38450.7	22	22.16	100.74	100.55
100%	44	76713.3	44	44.11	100.26	
150%	44	115511.0	66	66.42	100.64	

Precision: Precision was carried out in terms of system precision, repeatability, and intermediate accuracy. These are assessed by using six replicates at a concentration of 50 µg/mL of Daunorubicin and 22 µg/mL of Cytarabine. The data was given in Table 5& 6. The % RSD was found to be <2, indicating the repeatability of the method is good.

Table 5: Results of Precision for Cytarabine & Daunorubicin.

Injection	Peak Area for Cytarabine	Peak Area for Daunorubicin
Injection-1	493762	76493
Injection-2	496733	76325
Injection-3	495728	76408
Injection-4	497362	76384
Injection-5	497582	76354
Injection-6	497526	76423

Average	496448.8	76397.8
Standard Deviation	1489.8	58.7
%RSD	0.3	0.1

Table 6: Results of Intermediate precision for Cytarabine and Daunorubicin.

Injection	Peak Area for Cytarabine	Peak Area for Daunorubicin
Injection-1	497387	76345
Injection-2	496638	76487
Injection-3	497243	76847
Injection-4	496739	76534
Injection-5	498863	76948
Injection-6	493647	76354
Average	496752.8	76585.8
Standard Deviation	1717.8	254.4
%RSD	0.3	0.3

LOD and LOQ: Estimation of the limit of detection (LOD) and limit of quantification (LOQ) considered the acceptable signal-to-noise ratios 3: 1 and 10: 1, respectively. LOD and LOQ of Daunorubicin and Cytarabine were determined 2.465µg/mL, 4.156µg/mL and 2.461 µg/mL, 4.144 µg/mL respectively. Which were given in Table 7, 8 and shown in Fig. 9 and 10.

Table 7: Results of LOD.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Cytarabine	58	174	3.00
Daunorubicin	58	173	2.98

Table 8: Results of LOQ.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Cytarabine	58	579	9.98
Daunorubicin	58	578	10.00

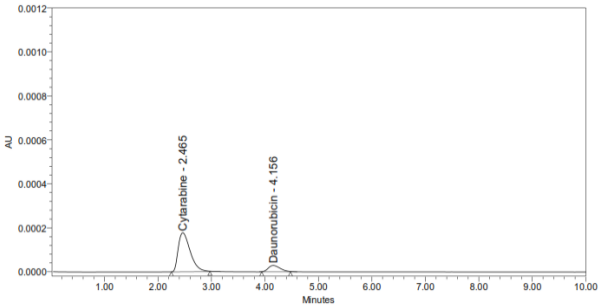

Figure 9: Chromatogram showing LOD.

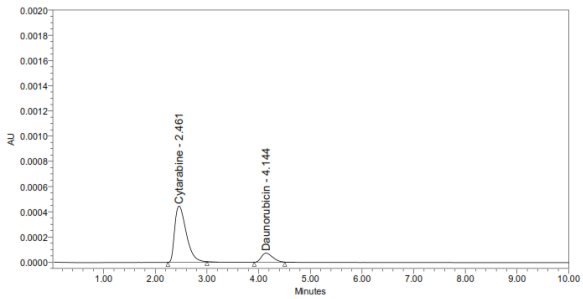

Figure 10: Chromatogram of showing LOQ.

Robustness: The robustness of the method was evaluated by the method conditions such as, flow rate (± 0.1) and the column temperature (±5 ºC), solvent composition (± 5%) were altered, and the influence of these changes on the assay, peak tailing, number of theoretical plates and peak area were evaluated. The % RSD of peak areas was found to be well within the limit of 2.0%, and results were shown in Table 9-12.

Table 9: Results for variation in flow for Cytarabine.

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.40	5286.39
2	1.0	1.40	5397.09
3	1.1	1.40	5354.87

Table 10: Results for variation in flow for Daunorubicin.

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	4.21	1.34	6687.39
2	1.0	4.09	1.32	6776.27
3	1.1	3.98	1.32	6753.29

Table 11: Results for variation in mobile phase composition for Cytarabine.

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Tailing	USP Plate Count
1	10% less	1.40	5487.33
2	*Actual	1.40	5397.09
3	10% more	1.40	5398.49

Table 12: Results for variation in mobile phase composition for Daunorubicin.

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	10% less	4.18	1.32	6573.97
2	*Actual	4.09	1.32	6776.27
3	10% more	3.91	1.31	6877.56

Table 13: Results of Assay for Cytarabine and Daunorubicin.

	Label Claim (mg)	% Assay
Cytarabine	100	100.48
Daunorubicin	44	100.76

Degradation Studies: Since no interference of blank and degradants, the HPLC results showed that the three active ingredients Daunorubicin and Cytarabine purity angle was less than the purity threshold and hence the proposed method was the specific and revealed its stability-indicating power. The results were summarized in Table 14.

The drug Daunorubicin and Cytarabine were found to be more degraded when exposed to peroxide and acidic conditions and least degraded when exposed to necessary, thermal and photolysis degradation.

Table 14: Results for Stability of Cytarabine and Daunorubicin.

Sample Name	Cytarabine		Daunorubicin	
	Area	% Degraded	Area	% Degraded
Standard	494304		76362.7	
Acid	486373	1.60	73452	3.81
Base	473456	4.22	73173	4.18
Peroxide	478476	3.20	72794	4.67
Thermal	471874	4.54	72364	5.24
Photo	476773	3.55	73956	3.15

CONCLUSION

A simple, specific and reliable reverse phase HPLC method was developed for the estimation of Daunorubicin and Cytarabine in their pharmaceutical dosage form. The method was validated over a concentration range 7.25µg/mL and 50.0 µg/mL for Daunorubicin and 8.2 µg/mL and 66 µg/mL for Cytarabine. The two compounds were subjected to forced degradation applying several stress conditions. The proposed method successfully separated the two compounds with degradants. The proposed method was specific and stability-indicating. Hence the developed method can be adapted to regular quality control analysis.

REFERENCES

1. Tripathi KD: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd,Edition 7th, 2013.
2. https://www.drugbank.ca/drugs/DB00694.
3. https://www.drugbank.ca/drugs/DB00987.
4. Suman P, Sivarao T and Reddy VSK: Development and validation of a stability-indicating RP-HPLC method for estimation of Daunorubicin - a

chemotherapic drug in bulk and pharmaceutical formulations. World Journal of Pharmaceutical Research, 2017; 1158-1174.
5. Murthy V, Rohini A, Pravallika KE, Prameela R A and Rahaman SA: Development and validation of RP-HPLC method for estimation of Cytarabine in bulk and pharmaceutical dosage forms. International Journal of Pharmaceutical Sciences and Research, 2013; 4573-4576.
6. Bhatnagar A, Loura S and Chaudhary M: A stability indicating RP-HPLC method for determination of anticancer agents Cytarabine in the lyophilized dosage form. Eurasian Journal of Analytical Chemistry, 2012; 160-167.
7. Murthy V, Rohini A, Pravallika KE, Prameela RA and Rahaman SA: Development and validation of a novel UV-Visible spectrophotometric method for Cytarabine in bulk and pharmaceutical dosage forms. Scholars Research Library, 2013; 51-55.
8. Kushagra R: Method development and validation of RP- HPLC method for simultaneous estimation of Daunorubicin and Cytarabine in the synthetic World Journal of Pharmacy and Pharmaceutical Sciences, 2018; 1301-1310.
9. Sharmila Donepudi & Suneetha Achanti., Simultaneous Estimation of Saxagliptin and Dapagliflozin in Human Plasma by Validated HPLC-UV Method. Turkish Journal of Pharmaceutical Sciences, 2019; 16(2): 227-233.
10. ICH harmonized tripartite guideline, validation of analytical procedures: text and methodology Q2 (R1), 27 October 1994; 1-13.
11. Sujana K: Validation and calibration of analytical instruments. Journal of Biomedical Sciences and Research, 2010; 2: 89-99.
12. ICH harmonized tripartite guideline, stability testing of new drug substances and products, Q1A (R2), 2003; 1-18.

A. Suneetha, V. Mounika & Shaik Mahammad Sajid., Development and Validation of Stability indicating RP-HPLC Method for the simultaneous Estimation of Sitagliptin and Ertugliflozin in bulk and Tablet Dosage Forms. Asian J. Pharm. Ana, 2020; 10(2): 81-85.

13. A. Suneetha, N. Sharmila and M. Purnima., Stability Indicating RP-HPLC Method For The Determination & Validation of Loperamide Hydrochloride & Simethicone In Pharmaceutical Dosage Form. World Journal of Pharmacy and Pharmaceutical Sciences, 2017; 6(9): 955-971.