Section A-Research paper

SIMULTANEOUS ESTIMATION AND STABILITY INDICATING RPHPLC METHOD DEVELOPMENT AND VALIDATION FOR DAUNORUBICIN AND CYTARABINE IN SOLID DOSAGE FORM

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ABSTRACT

Daunorubicin (DRB) and cytarabine (CYT) are medications used to treat cancer agents used in chemotherapy, and a standardized and reliable technique for estimating their concentration in bulk tablet form was established utilizing RP-HPLC. Mobile phase (KH2PO4: Acetonitrile of 50:50) was passed through an STD AGILENT 150 x 4.6 mm,5 columns at a rate of 1.0 ml/min. The retention time of DRB equaled 2.255 minutes, whereas that of CYT equaled 2.963 minutes. The relative standard deviations (RSDs) of DRB and CYT being 0.9 and 0.6, correspondingly, the percentages of drugs recovered were 100.15 and 100.42, correspondingly. Regression models for both DRB and CYT yielded LOQ and LOD values of 0.21 and 0.64, and 0.45 and 1.35, correspondingly. Overall, the % yield was 100% for both CYT and DRB. The regression equation for DRB is y = 37361.x + 4337, but that for CYT is y = 41833.x + 24623.

Keywords: Daunorubicin, Cytarabine, RP-HPLC, Flow rate, Tablet dosage form

INTRODUCTION [1-6]

Daunorubicin is used to treatment of blood cancer and other neoplastic diseases and its molecular weight is 531.53 and its formula is C_{26} ($_{13}C$) $H_{26}D_3NO_{10.}$

Cytarabine is a pyrimidine nucleoside analogue with a molecular weight of about 246.19 that is prescribed primarily for those suffering from leukemia, particularly acute non-lymphoblastic leukemia and its molecular formula is $C_6(_{13}C)_3H_{13}N_3O_5$ and soluble in DMF, DMSO, and hot methanol

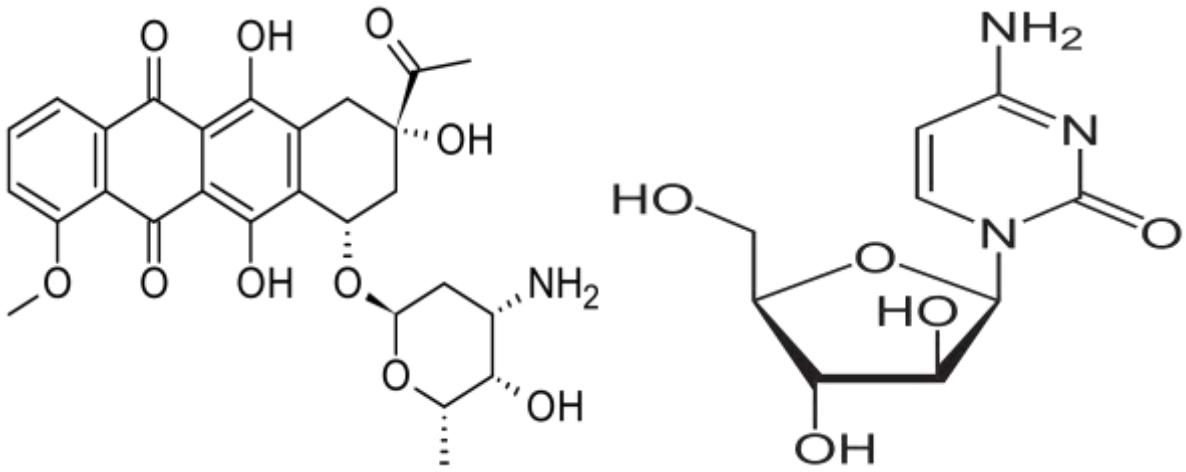


Figure 1 &2: Structure of Daunorubicin & Cytarabine

MATERIALS AND METHODS [7-21]

Chemicals & Reagents

Daunorubicin & Cytarabine purchased from of Rankem Pharmaceutical Technologies Pvt. Ltd., Hyderabad. Acetonitrile (ACN) and HPLC Water were bought from Rankem Fine Chemicals Ltd. Merck, India provided orthophosphoric acid (OPA) and NaOH pallets.

Equipment

The analysis was performed using a HPLC system equipped with a UV-visible detector (Agilent-1260 VWD and DAD detector). Open Lab E.Z. Chrom with Data storage A.01.05 was used to track the results.

Preparation of standard stock solution

After careful weighing, 11 mg of DRB and 25 mg of CYT were added to a 50 ml flask. Dissolving the addition of a few milliliters of mobile phase using sonication. A sufficient amount of the same solvent was added to bring the volume up to the specified level. The final solution containedabout220µg/ml and500µg/ml of DRB and CYT respectively

Preparation of standard working solution

Pippete out 1 ml of standard stock solution and relocated to 10ml of volumetric flask and add the diluents in the ratio of ¾ then (22µg/ml and 50µg/ml of Daun and Cyta.

Preparation of sample standard solution

Five tablets were weighed and average weight correspondent to 1 tablet make into the fine powder and transfer into 100 ml volumetric flask containing ¾ diluents and sonicate for 20 min and filtered through HPLC filters

System suitability

In these the resolution factor (R_s), usp plate count (N), peak tailing (TF) can be obtained by taking Cyta(50ppm) and Daun (22ppm), the solution was injected 6 times. the %RSD of six injections should <2

Precision:

After properly weighing 11 mg of Daun and 25mg of cytara working standard, both were introduced to 50 mL sterile, dried volumetric flasks, mixed and dissolved in dilutant using a sonicator, and then the volume was brought up to the appropriate level using the same dilutant (Stock solution). 1ml of both solutions were taken in 10 ml flasks and volume was raised up using diluents. The area of six separate injections of the reference solution was determined using HPLC. The %RSD of the region from five duplicate injections was determined to be well within the allowed range.

Accuracy:

Pippete out 1 ml of standard stock solution and translocated to 10ml flask and add the diluents in the ratio of ¾ then (22µg/ml and 50µg/ml of Daun and Cyta. 50%, 100% and 150% Injections were made of the standard solution. Individual recovery and mean recoveries, as well as the amounts detected and added, were computed for both Daun and cytara.

Linearity:

The approach was linear across 5.5-33 µg/ml for Daun and 12.5-75.0µg/ml for Cytara. The linearity of the suggested approach was confirmed by estimating the r^2, intercept, and slope from the calibration curve.

Robustness

A technique is considered robust if it can withstand minor, manipulative changes in its parameters without compromising its performance. Deliberate shifts in chromatographic parameters, such as wavelength (± 2 nm), temp. (± 2 ºC), and flow rate (± 10%) were made to assess their impact.

Forced Degradation Studies

Studies of degradation were conducted according to ICH recommendations. The goal of this research was to identify drug degradants that may be used to determine degradation routes and drug stability. Degradation tests were performed in acidic, basic, neutral, and oxidative settings to test the selectivity of the suggested methodology.

Acid degradation

Exactly 10 mg of each pure medication was taken out separately and translocated to separate dry, clean, round-bottom flasks. They were placed in a water bath at 600C and vortexed for 4 hours after adding 30 ml of 0.1 N HCl. Set to ambient temperature before further processing. Final concentrations of 22 µg/ml for DRB and 50 µg/ml for CYT were obtained using mobile phase after the samples were neutralized with a diluted 0.1 N NaOH solution. Against a mobile phase blank, the samples were loaded into the HPLC apparatus (after adjusting the mobile phase ratio). The degradation profile was studied after the experiment was performed numerous times with the same dose of HCl (0.1N).

Alkali degradation

The two pure medicines, each weighing 10 mg, were taken in separate dry, clean, round-bottom flasks. Thirty milliliters of 0.1N NaOH was poured. It was then placed in a water bath at 600C for 4 hours to allow the refluxing process to take place. Cooled to room temperature before further processing. Final concentrations of 22 µg/ml for DRB and 50 µg/ml for CYT were obtained using mobile phase after the samples were neutralized with a diluted 0.1 N HCl solution. Against a mobile phase blank, the samples were loaded into the HPLC apparatus (after adjusting the mobile phase ratio). The degradation profile was studied after the experiment was performed numerous times with the same dose of NaOH (0.1N).

Thermal Degradation

The two pure medicines, each weighing 10 mg, were taken in separate dry, clean, round-bottom flasks. 30ml of HPLC water was poured. It was then placed in a water bath at 600C for 6 hours to allow the refluxing process to take place. Cooled to room temperature before further processing. Final concentrations of 22 µg/ml for DRB and 50 µg/ml for CYT were obtained using mobile phase. Against a mobile phase blank, the samples were loaded into the HPLC apparatus

Photolytic degradation studies:

About 10 milligrams of each pure medication were placed in two separate, sterile, and air-dried Petri dishes. A UV cabinet of 254 nm wavelength was used to store it for a 24 hrs uninterrupted. 1 mg of the drug that had been irradiated with UV light was carefully weighed and then placed in a dry, sterile volumetric flask. The UV-exposed drug was first diluted using mobile phase. Final concentrations of 22 µg/ml for DRB and 50 µg/ml for CYT

were obtained. Against a mobile phase blank, the samples were loaded into the HPLC apparatus and chromatogram were recorded.

Oxidation with (3%) H2O2 studies:

The two pure medicines, each weighing 10 mg, were taken in separate dry, clean, volumetric flasks. 30ml of 3% H_2O_2 water and a small volume of MeOH were added. It was then placed under shade for 24 hrs. Final concentrations of 22 µg/ml for DRB and 50 µg/ml for CYT were obtained using mobile phase. Against a mobile phase blank, the samples were loaded into the HPLC apparatus

RESULTS AND DISCUSSIONS

Optimized chromatographic conditions	
Mobile phase	0.01N KH2PO4 : ACN (50: 50)
Flow rate	1ml/min
Column	Agilent c18 (4..6 x150mm,5µm)
Detector Wavelength	240nm
Runtime	10 Min
Column Temperature	30^0c
Injection Volume	10µL

Table No: 1 Optimized chromatogram of Daunorubicin & Cytarabine

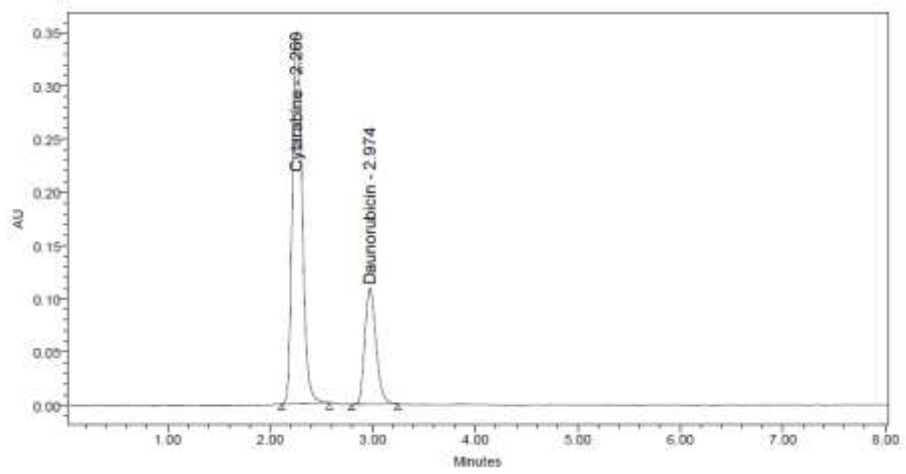


Figure: 3 Optimized chromatograms of Daunorubicin & Cytarabine

System suitability

According to ICH criteria, all system suitability metrics fell into the acceptable range. N must always be greater than 2000, TF must not exceed 2, R_s must be over 2. So, all system's appropriate parameters have been proven to be valid, and they were all in acceptable bounds.

Validation:

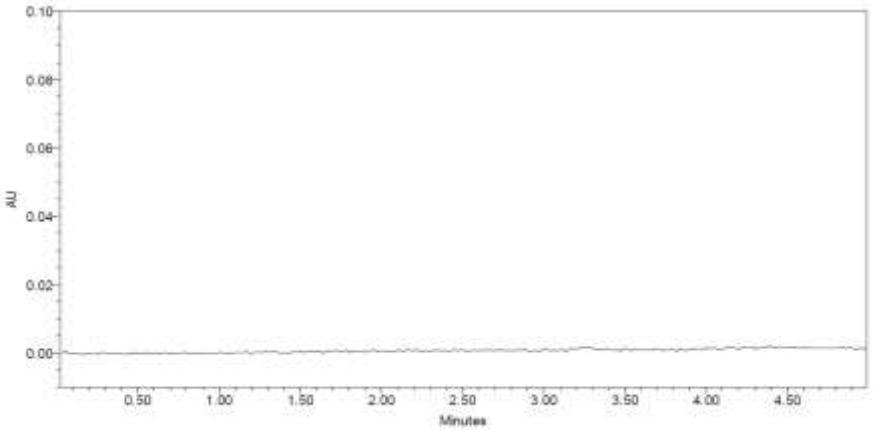


Figure No: 4 Chromatogram of Blank

Linearity

Two sets of six injections each of DRB (5.5-33 µg/ml) and CYT (12.5-75 µg/ml) were delivered. Comparing Cytarabine with Daunorubicin, the regression equation for Cytarabine was y = 41833x + 24623, whereas Daunorubicin was y = 37361x + 4337. The estimated correlation coefficient for the two agents was 0.999.

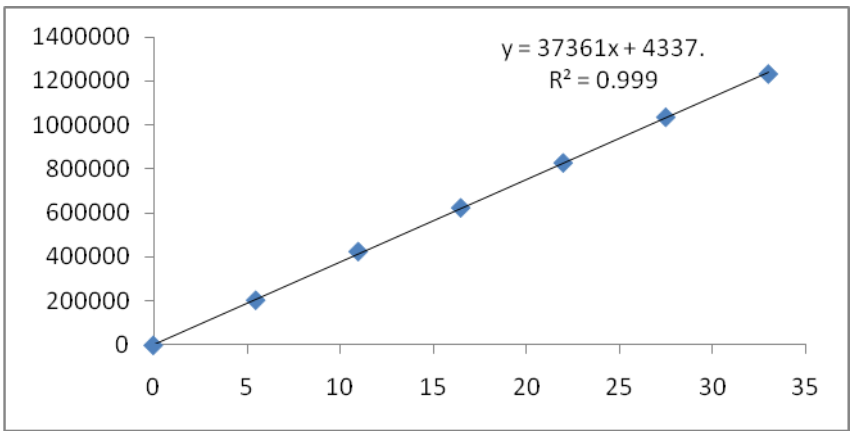


Figure No: 5 Calibration curve of DRB

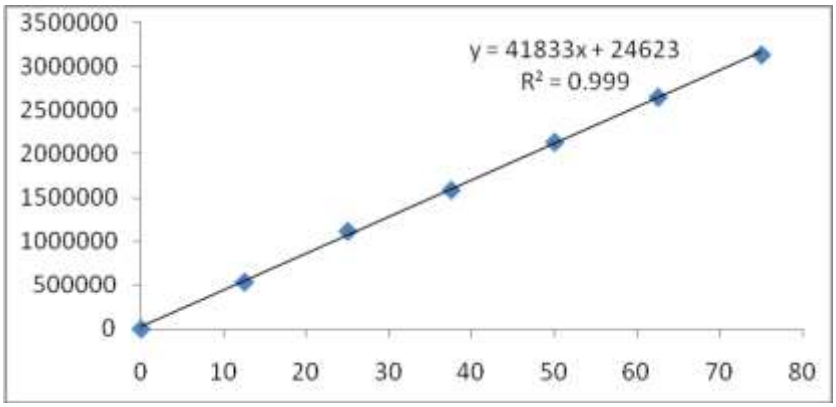


Figure No: 6 Calibration curve of Cyta

System Precision

Six injections were made using working standard solution, and the corresponding areas were analyzed. Two medications' average area SD and % RSDs were estimated. The RSDs for DRB and CYT were 0.9% and 0.6%, correspondingly. The system's precision was below 2, hence the system precision was valid.

Accuracy

% Level	Qty. Spiked (µg/mL)	Qty. recovered (µg/mL)	% Recovery	Mean %Recovery
50%	11	10.99	99.94	100.15%
	11	11.02	100.16	
	11	10.98	99.85	
100%	22	21.90	99.52	
	22	22.19	100.88	
	22	21.97	99.84	
150%	33	32.9	99.6	
	33	33.1	100.3	
	33	33.4	101.2	

Table No : 2 Accuracy results of Daunorubicin

% Level	**Qty Spiked (µg/mL)**	**Qty recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	25	25.06	100.24	100.16%
	25	24.98	99.92	
	25	25.26	101.05	
100%	50	50.18	100.36	
	50	49.88	99.75	
	50	50.15	100.31	
150%	75	74.89	99.85	
	75	75.03	100.05	
	75	74.90	99.87	

Table No: 3Accuracy Results of Cytarabine

Discussion: The Std addition method was used to prepare 3 levels of Accuracy samples. For each degree of accuracy, triplicate injections were administered, and percent recovery for Daun and Cyta was 100.15 percent and 100.16 percent, respectively.

Limit of Detection & Quantification

Mol	**LOD**	**LOQ**
Dauno	0.21	0.64
Cytara	0.45	1.35

Table No: 4 Results of LOD & LOQ of Daunorubicin & Cytarabine

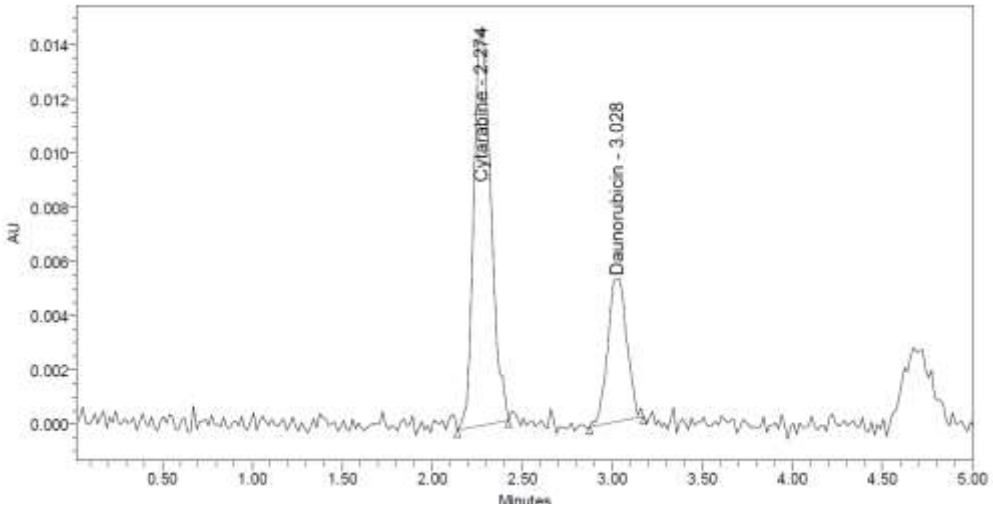


Figure No: 7 chromatogram of Limit of detection Daunorubicin & Cytarabine

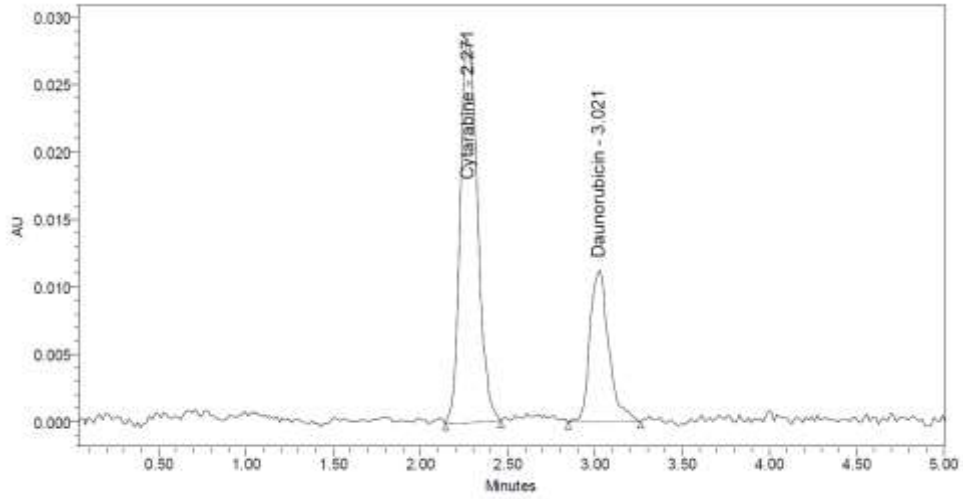


Figure No: 7 chromatogram of Limit of Quantification of Daunorubicin & Cytarabine

Robustness

S.no	Condition		%RSD of Daunorubicin	%RSD of Cytarabine
1	Flow rate	0.9ml/min	0.8	0.8
		1.1ml/min	0.1	1.5
2	Mobile phase	55B:45A	0.7	0.8

		45B:55A	0.2	0.8
3	Temperature	25°C	0.5	0.5
		35°C	0.6	1.2

Table No: 5 Results of Robustness of Daunorubicin & Cytarabine

The estimated % Assay for the DRB and CYT were 100.12 %& 100.42%respectively

Degradation Studies: The formulated was put through degradation testing, and then degradants were subjected to analysis. All of the samples were found to be degraded below the set thresholds as determined by their assay.

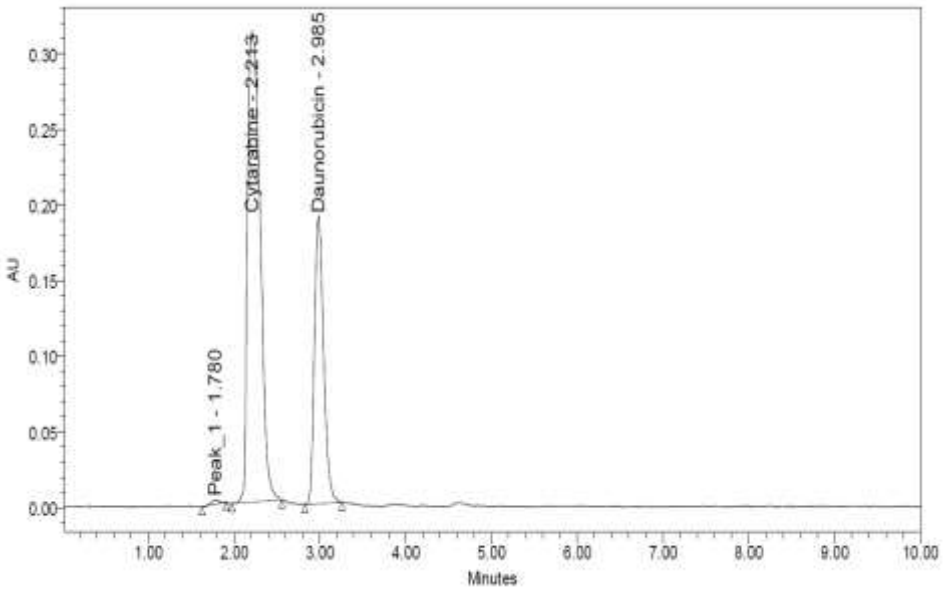
S.No	Degradation Condition	Percentage Drug Ungraded	Percentage Drug Degraded
1	Acid	94.12	5.88
2	Alkali	95.51	4.49
3	Oxidation	95.31	4.69
4	Thermal	97.42	2.58
5	UV	98.60	1.40
6	Water	98.60	1.40

Table No :6 Degradation results of Daunorubicin

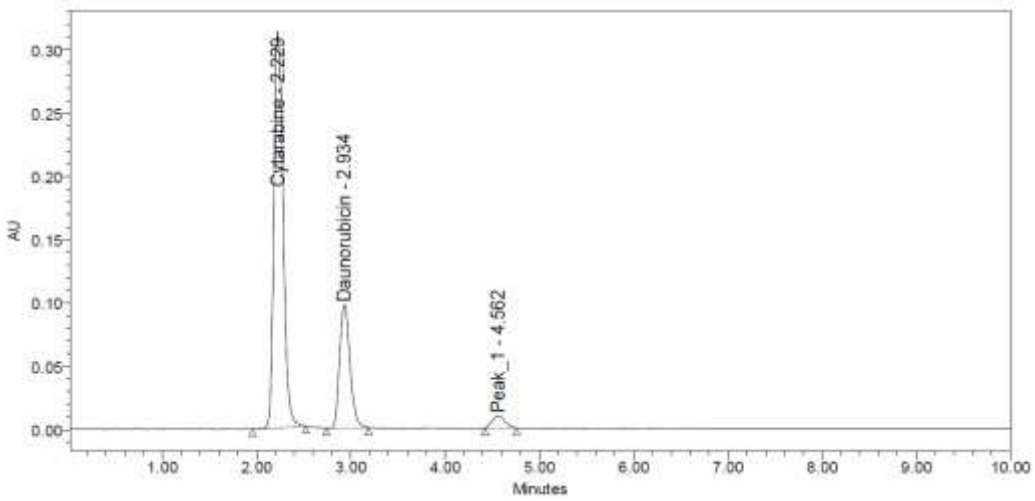
S.No	Stress Condition	Percentage Drug Ungraded	Percentage Drug Degraded
1	Acid	93.75	6.25
2	Alkali	95.58	4.42
3	Oxidation	95.87	4.13
4	Thermal	98.18	1.82

5	UV	99.18	0.82
6	Water	99.33	0.67

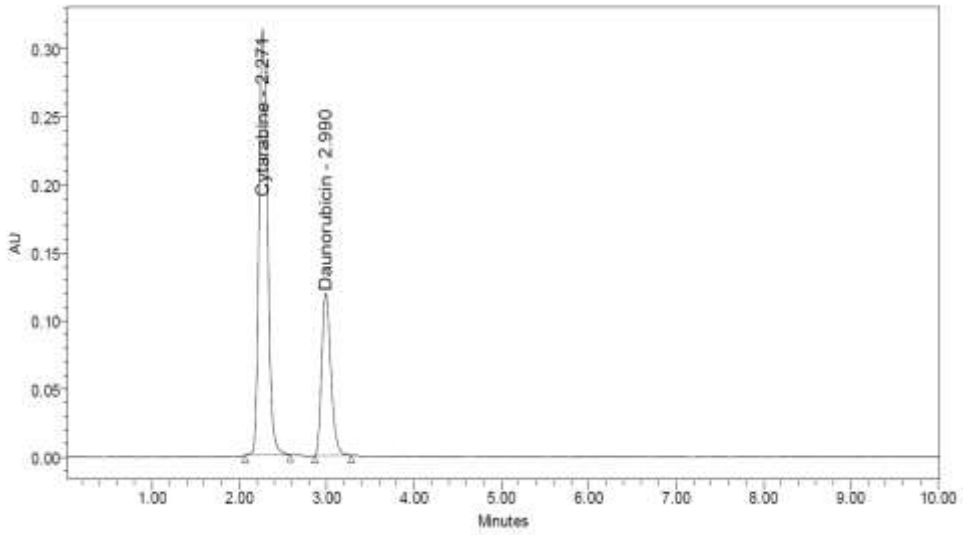
Table No :7 Degradation results of Cytarabine



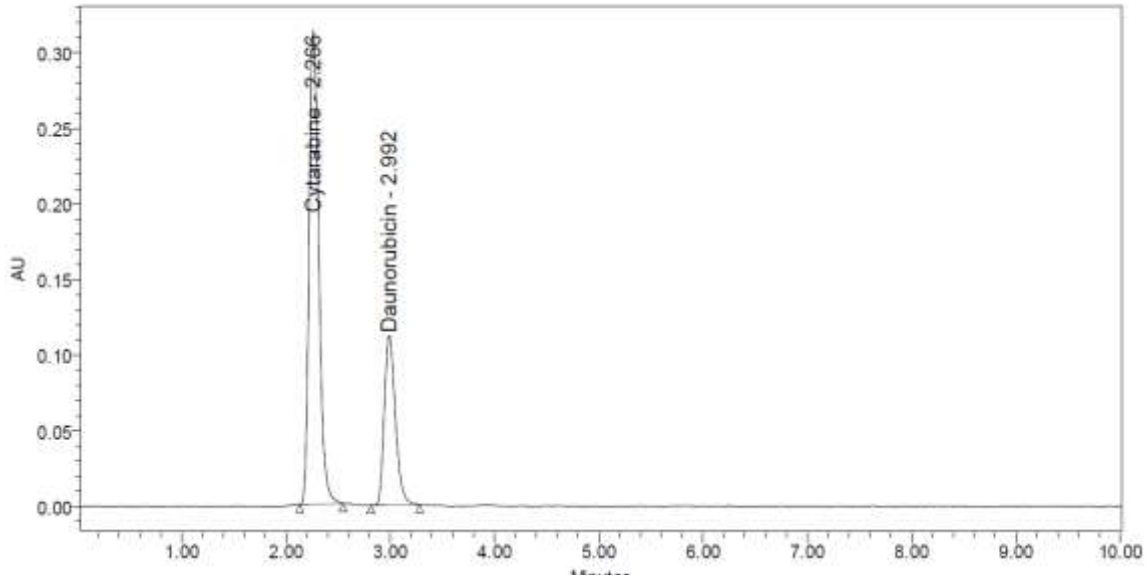
A) Acid chromatogram Daunorubicin & Cytarabine



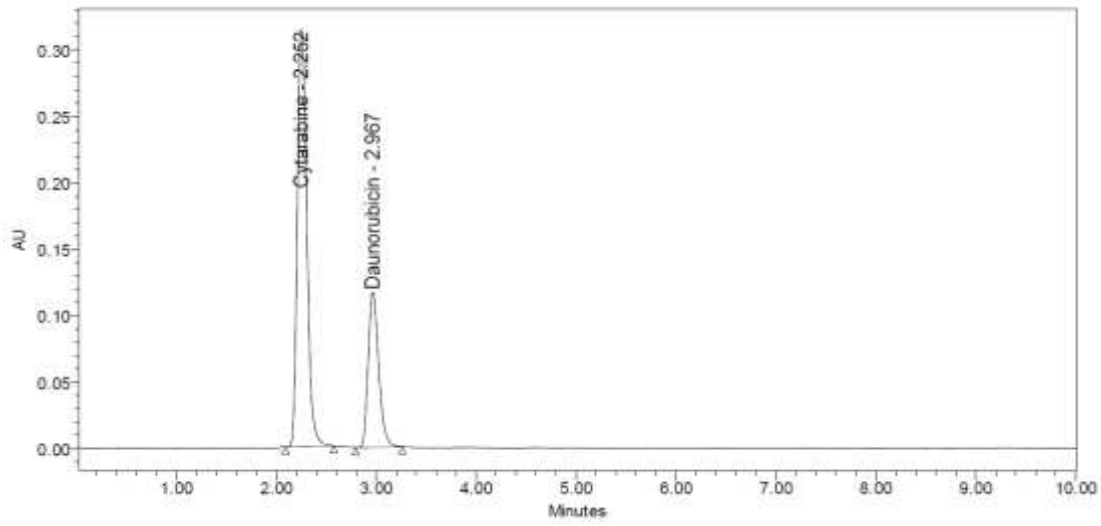
B) Base chromatogram Daunorubicin & Cytarabine



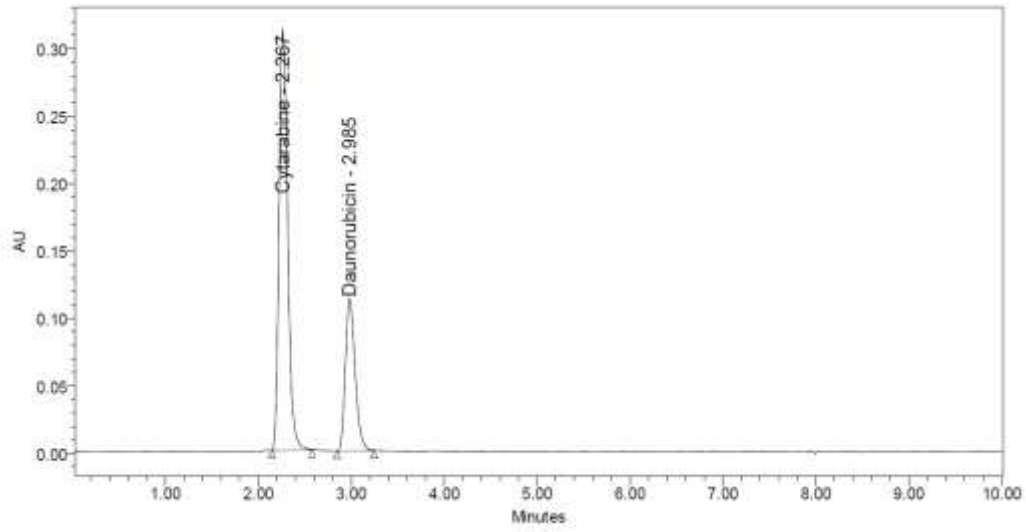
C) Peroxide chromatogram Daunorubicin & Cytarabine



D) **Thermal chromatogram Daunorubicin & Cytarabine**



E) U.V chromatogram Daunorubicin & Cytarabine



F) Oxidation chromatogramDaunorubicin & Cytarabine

Figure :6 (A)Acid chromatogram (B)Base chromatogram (C)Perioxide chromatogram (D)Thermal chromatogram (E)UV chromatogram (F)Oxidation chromatogram

Discussion: Acid and base degradation experiments show that changing the pH of solvent sytem has an impact on the retention times of the compounds. However, no variation in retention time was observed when an acidic or basic sample was neutralized using a 2N base or acid solution, correspondingly.

CONCLUSION

The quantification of DRB and CYT in bulk and therapeutic dose form has been improved with the designing and validation of a specific and sensitive stability indicating RP-HPLC approach. According to sample analysis data showing a very high peak purity, it may be deduced that the proposed technique is unique for the quantitative determination of Daunorubicin and Cytarabine, as shown by the lack of a co-eluting peak alongside the peaks of Daunorubicin and Cytarabine.

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