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STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAUNORUBICIN AND CYTARABINE

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Keywords:

Daunorubicin, Cytarabine, RP-UPLC, Stability studies, Validation

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ABSTRACT: The main objective of the study was to develop an RP-UPLC method for the simultaneous determination and validation of the Daunorubicin and Cytarabine in the pharmaceutical dosage form. The technique was developed by using the Mobile phase containing Buffer 0.01N KH_2PO_4: Acetonitrile used in the ratio 50:50 v/v and X-Bridge C18 (2.1 × 100 mm, 1.6 µm) as a stationary phase at a flow rate of 0.3 ml/min. The buffer used in this method was 0.01N KH_2PO_4 (3.5 pH) buffer pH was accustomed by means of the solution of 0.1% OPA. The temperature was maintained throughout the method at 30°C, and the optimized wavelength chosen was 240.0 nm. The technique was developed and thoroughly validated based on the ICH (International Conference on Harmonization) guidelines. The validation process was done by precision, linearity, robustness studies, accuracy, Limit of Detection, Limit of quantification, and degradation studies. The robustness studies were observed by making variations in the mobile phase, temperature, and flow rate. The accuracy was observed at more than 96% recovery. The stability of drug samples was showed more than 98% recovery over different environmental and laboratory circumstances. The LOD and the LOQ values were within limits. The developed RP-UPLC method achieved good precision and accuracy. The developed and validated method was suitable for the analysis of Daunorubicin and Cytarabine.

INTRODUCTION: Daunorubicin **Fig. 1** is an anthracycline antibiotic that has antineoplastic activity and is used for the treatment of acute leukemia and acquired immune deficiency syndrome-related Kaposi sarcoma. It is chemically, (1S,3S)-3-Acetyl-3,5,12-trihydroxy-10- methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydro-1-tetracenyl 3-amino- 2,3,6-trideoxy-α-L-lyxo-hexopyranoside.

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It is one among six Topo II inhibitors which is prescribed as a highly antineoplastic drug in clinical use [2].

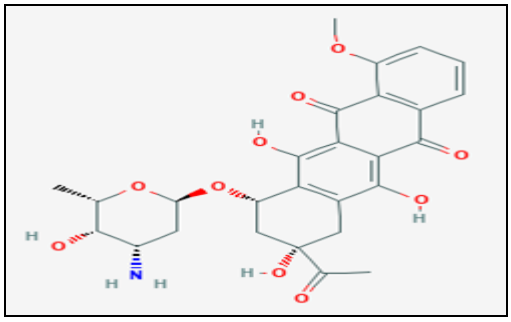


FIG. 1: DAUNORUBICIN

Cytarabine **Fig. 2** is a pyrimidine nucleoside analog used mainly in the treatment of leukemia,

especially acute non-lymphoblastic leukemia, acute myelogenous leukemia, and meningeal leukemia. It is chemically 4-amino-1-[(2R,3S,4S,5R)-3,4-dihyd-roxy-5-(hydroxymethyl)oxolan- 2-yl] pyrimidin-2-one. It has moderate emetogenicity, which has been managed with antiemetic drugs [1].

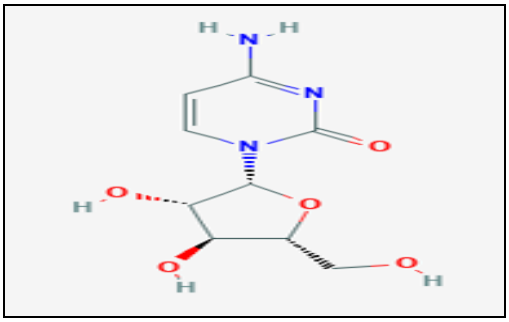


FIG. 2: CYTARABINE

A detailed literature survey revealed that there were RP-HPLC methods for the estimation of bulk and infusion formulation [3, 5]. Bioanalytical method development and validation for estimation of daunorubicin and cytarabine in blood plasma by using RP-HPLC [6] and by spectroscopy method [7] individually. In combination, Doxorubicin and Cytarabine only one method was published [8]. Liquid chromatographic methods for the estimation of daunorubicin in bulk and pharmaceutical formulation [9, 10, 11, 12].

As per my knowledge, there is no existed technique for these samples with UPLC till now. The planned method is economical, simple, precise, stability-indicating, and appropriate for routine investigation of Daunorubicin and Cytarabine in the collective pharmaceutical dosage form. The guidelines entitled "Stability testing of novel drug samples and products" by The International Conference on Harmonization (ICH) need that stress testing be performed to clarify the natural stability distinctiveness of the dynamic substance [13]. The method was effectively validated in pursuance of ICH guidelines [14, 15].

MATERIALS AND METHODS:

Materials: Daunorubicin and Cytarabine pure drugs (API), Combination Daunorubicin and Cytarabine injections (VYXEOS), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: The analysis was eluted on an Acquity UPLC SYSTEM used was of column X-Bridge C18 (2.1 × 100mm, 1.6µm) with quaternary pumps, ACQUITY TUV detector, and Auto sampler integrated with the software Empower 2.0. It was equipped with UV-VIS spectrophotometer PG Instruments T60 having particular bandwidth of 2 mm and 10 mm and coordinated quartz cells incorporated with the software UV win 6. It was used for measuring absorbencies of Daunorubicin and Cytarabine solutions.

Chromatographic Conditions: The chromato-graphic separations achieved on column X-Bridge C18 (2.1 × 100 mm, 1.6 µm) with quaternary pumps as a stationary phase. The mobile phase was composed of 50:50 v/v 0.01N KH_2PO_4: Acetonitrile at a flow rate was maintained 0.3 ml/min and the total injection volume was taken 1.0 ml every time. The total run time was 3 min, and all establishments were performed at 30 °C, and the drugs were detected at 240 nm.

Standard Solutions:

Diluent: The diluents Acetonitrile and Water in use in the ratio of 50:50 were selected based on the solubility of drug samples.

Preparation of Standard Stock Solutions: Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25 ml volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 min. Flask was made up with diluents and labeled as Standard stock solution (440 µg/ml of Daunorubicin and 1000µg/ml Cytarabine).

Preparation of Standard Working Solutions (100% Solution): 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent (44 µg/ml of Daunorubicin and 100 µg/ml of Cytarabine).

Preparation of Sample Stock Solutions: 10 vials equivalent to 44 mg Daunorubicin & 100 mg Cytarabine was transferred into a 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 25 min; further the volume was made up with diluent and filtered by HPLC filters (440 µg/ml of Daunorubicin and 1000 µg/ml of Cytarabine).

Preparation of Sample Working Solutions (100% Solution): 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluents (44 µg/ml of Daunorubicin and 100 µg/ml of Cytarabine).

Method Validation Procedure: The current process was effectively validated as per ICH guidelines [13, 14, 15]. The different validation parameters which were performed are the following: system suitability, linearity, precision, accuracy, specificity, and limit of detection (LOD), the limit of quantification (LOQ), robustness, and degradation studies.

System Suitability Parameters: The system suitability parameters were determined by preparing standard solutions of Daunorubicin (44ppm) and Cytarabine (100ppm), and the solutions were injected six times, and the parameters like peak tailing, resolution, and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard Stock Solutions: Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 min. Flask was made up with diluents and labeled as Standard stock solution (440 µg/ml of Daunorubicin and 1000 µg/ml Cytarabine).

Preparation of Standard Working Solutions (100% Solution): 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent (44 µg/ml of Daunorubicin and 100µg/ml of Cytarabine).

Linearity:

25% Standard Solution: 0.25 ml each from two standard stock solutions was pipetted out and made up to 10 ml (11µg/ml of Daunorubicin and 25 µg/ml of Cytarabine).

50% Standard Solution: 0.5 ml each from two standard stock solutions was pipetted out and made up to 10 ml (22 µg/ml of Daunorubicin and 50 µg/ml of Cytarabine).

75% Standard Solution: 0.75 ml each from two standard stock solutions was pipetted out and made up to 10 ml (33 µg/ml of Daunorubicin and 75 µg/ml of Cytarabine).

100% Standard Solution: 1.0 ml each from two standard stock solutions was pipetted out and made up to 10 ml (44 µg/ml of Daunorubicin and 100 µg/ml of Cytarabine).

125% Standard Solution: 1.25 ml each from two standard stock solutions was pipetted out and made up to 10 ml (55µg/ml of Daunorubicin and 125µg/ml of Cytarabine).

150% Standard Solution: 1.5 ml each from two standard stock solutions was pipettede out and made up to 10 ml (66 µg/ml of Daunorubicin and 150 µg/ml of Cytarabine).

Accuracy:

Preparation of Standard Stock Solutions: Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25 ml volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 min. Flask was made up with diluents and labeled as Standard stock solution (440 µg/ml of Daunorubicin and 1000 µg/ml Cytarabine).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.

Robustness: Small, deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made, but there were no recognized changes in the result and are within range as per ICH Guide lines. Robustness conditions like flow rate (±0.1), mobile phase minus, mobile phase plus, temperature minus (±10) were maintained, and samples were injected in a duplicate manner. System suitability parameters were not much affected, and all the parameters were passed. %RSD was within the limit.

LOD Sample Preparation: 0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flasks and made up with diluents. From the above solutions, 0.1ml each of Daunorubicin and Cytarabine solutions respectively was transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ Sample Preparation: 0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flask and made up with diluent. From the above solutions, 0.3 ml each of Daunorubicin, Cytarabine and solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluent.

Degradation Studies:

Oxidation: To 1 ml of stock solution of Daunorubicin and Cytarabine, 1 ml of 10% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60 °C. For UPLC study, the resultant solution was diluted to obtain 44 µg/ml and 100 µg/ml solution, and 0.30 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Acid Degradation Studies: To 1 ml of stocks solution Daunorubicin and Cytarabine, 1 ml of 1N Hydrochloric acid was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 44 µg/ml and 100 µg/ml solution, and 0.30 µl solutions were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution Daunorubicin and Cytarabine, 1 ml of 1N sodium hydroxide was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 44 µg/ml & 100 µg/ml solution, and 0.30 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: The standard drug solution was placed in an oven at 105 °C for 1 h to study dry heat degradation. For the UPLC study, the resultant solution was diluted to 44 µg/ml & 100 µg/ml solution, and 0.30 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability Studies: The photochemical stability of the drug was also studied by exposing the stock solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m^2 in a photostability chamber. For the UPLC study, the resultant solution was diluted to obtain 44 µg/ml & 100 µg/ml solution, and 0.30 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1 h at a temperature of 60º. For UPLC study, the resultant solution was diluted to 44 µg/ml and 100 µg/ml solution, and 0.30 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample**.**

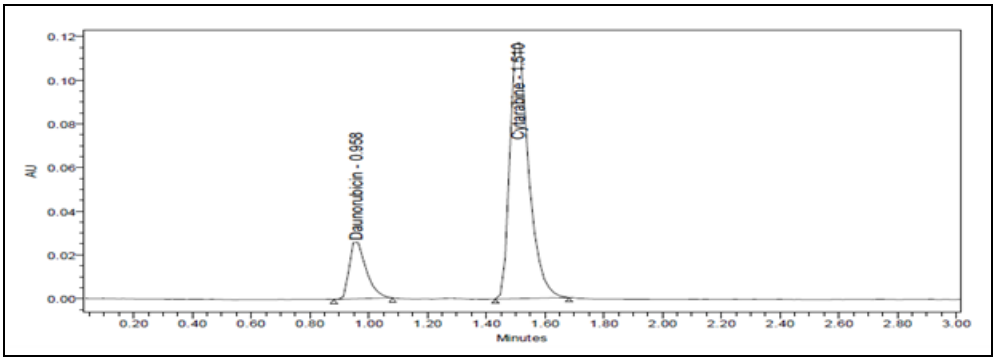
RESULTS AND DISCUSSION: A simple, speedy, and perfect method was developed and validated for the drug samples of Daunorubicin and Cytarabine. There is no official method for this combination so far in the RP-UPLC method development and validation. However, few methods have been reported in either of one or two in this combination with some other drugs.

In comparison with literature, the mobile phase consisting of water: acetonitrile in the ratio of 55:45 with a flow rate of 1 ml/min [3]. A mobile phase consisting of Methanol: phosphate Buffer (3pH) (60:40% v/v) at a flow rate of 1mL/min [4]. The mobile phase consists of a mixture (55:45 v/v) of 0.1% OPA: acetonitrile at a flow rate of 0.8 ml/min [5]. The isocratically using 20 mM KH_2PO_4:

Acetonitrile (pH 2.5) in the ratio of 20:80 v/v as mobile phase [6].

In the proposed method a simple mobile phase consisting of buffer and acetonitrile was used which elute the Daunorubicin and Cytarabine with lower retention time. The retention times 0.958 min and 1.510 for Daunorubicin and Cytarabine, respectively. The calibration curve was linear over the concentration range of 15-90 ppm and 10-60 ppm, respectively. The LOD values were 0.14, and 0.33 and LOQ values were found to be 0.41 and 1.0 for Daunorubicin and Cytarabine, respectively. The high percentage of revival and low percentage coefficient of difference authenticate the appropriateness of the method, and the forced degradation studies show that the developed method was stability-indicating. Hence, it was completed that the RP-UPLC method developed was highly suitable for the usual study, and all the parameters result in data was shown in method validation systematically.

Method Development: Three trials were executed for the method development, and the finest peaks with the least fronting factor were eminent for Daunorubicin and Cytarabine with RT = 0.958 min, RT = 1.510 min. accordingly. The resultant chromatogram is revealed in **Fig. 3**.



	Peak name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Daunorubicin	0.958	103849	4909.7		1.5
2	Cytarabine	1.510	525039	2694.3	5.0	1.3

FIG. 3: STANDARD CHROMATOGRAM OF DAUNORUBICIN AND CYTARABINE

Method Validation: The method was validated as per ICH guidelines [13, 15]. The different validation parameters which were determined are the following: system suitability test, precision, accuracy, linearity and specificity, the limit of detection, the limit of quantification, robustness, degradation studies, and the stability-indicating capability.

System Suitability Test: Six repeat injections of a standard solution of Daunorubicin and Cytarabine were injected, and the chromatograms are recorded. The system was appropriate for examination if the % relative standard deviation (%RSD) of area counts in six repeat injections must be not more than 2.0%. USP tailing factor for Daunorubicin and Cytarabine peak should be not more than 2.0. The USP resolution factor between the peaks corresponding to Daunorubicin and Cytarabine should be more than 2.0. The results are revealed in **Table 1**.

TABLE 1: SYSTEM SUITABILITY PARAMETERS OF DAUNORUBICIN AND CYTARABINE

S. no.	DAUNORUBICIN				CYTARABINE			
	RT	Area	USP Plate Count	USP Tailing	RT	Area	USP Plate Count	USP Tailing
1	0.966	104207	5445	1.38	1.551	496110	2914	1.37
2	0.966	103485	5460	1.37	1.551	494208	3097	1.35
3	0.967	104340	5404	1.37	1.551	497020	3297	1.34
4	0.967	104755	5379	1.36	1.553	494250	3488	1.35
5	0.969	102651	5233	1.39	1.558	501089	2874	1.37
6	0.969	103274	5350	1.37	1.561	500098	3073	1.30
Mean		103785				497129		
Std. Dev		782.9				2910.9		
% RSD		0.8				0.6		

Precision: The standard Daunorubicin and Cytarabine solutions were injected six times and measured the area for all six injections in UPLC. The % RSD for the area of six repeat injections was established to be within the specified limits. The data was presented in **Table 2**.

TABLE 2: PRECISION STUDY OF DAUNORUBICIN AND CYTARABINE

S. no.	Daunorubicin			Cytarabine		
	Peak area	%Assay	Inter day Precision	Peak area	%Assay	Inter day Precision
1	103838	99.65	103398	492723	98.92	451949
2	103225	99.06	105155	500146	100.41	459018
3	103409	99.24	102473	496550	99.68	457349
4	104038	99.84	103028	495758	99.52	456419
5	103249	99.09	103268	495001	99.37	453657
6	103294	99.13	102673	499675	100.31	453511
AVG	103509	99.33	103333	496642	99.70	455317
SD	344.3	0.33	958.8	2839.7	0.6	2698.2
%RSD	0.3	0.3	0.9	0.6	0.6	0.6

Acceptance Criteria: The % RSD should not be more than 2%

Intermediate Precision (Day-Day Precision):

TABLE 3: INTERMEDIATE PRECISION TABLE OF DAUNORUBICIN AND CYTARABINE

S. no.	Area of Daunorubicin	Area of Cytarabine
1	103398	451949
2	105155	459018
3	102473	457349
4	103028	456419
5	103268	453657
6	102673	453511
Mean	103333	455317
S.D	958.8	2698.2
%RSD	0.9	0.6

Accuracy: Accuracy of the readings was computed by % recovery of three different concentrations of Daunorubicin and Cytarabine at 50%, 100%, and 150%, and also standard addition technique was carried out for some samples.

The results acquired, including the means of the recovery and standard deviations, were displayed in **Table 4.**

Acceptance Criteria: The % Recovery for Daunorubicin and Cytarabine at each stage should be between 99 to 101%.

TABLE 4: ACCURACY DATA OF DAUNORUBICIN AND CYTARABINE

Sample	Daunorubicin			Cytarabine		
% concentration	50%	100%	150%	50%	100%	150%
Trail-I	98.55	99.92	99.60	100.71	99.08	100.50
Trail-II	99.12	99.04	100.21	99.95	100.01	100.31
Trail-III	98.90	99.80	99.18	100.53	99.95	99.85
AVG (%Recovery)	98.9	99.59	99.66	100.39	99.68	100.22
SD	0.29	0.48	0.52	0.396	0.5178	0.3363
%RSD	0.29	0.48	0.52	0.39	0.52	0.34

Recovery Studies: To estimate the accuracy and precision of the proposed method, recovery studies were carried out. A fixed amount of sample was taken, and reference drugs were added at 50%, 100%, and 150% levels. The results were analyzed and the results were within limits.

Linearity and Calibration Curve: The stock solution of Daunorubicin and Cytarabine was prepared using diluents. From it, various working standard solutions were prepared and injected into the HPLC system. The calibration plot (peak area *vs.* concentration) was generated by replicate analysis (n=6) at all concentration levels. The linear relationship was evaluated using the least square method within the Microsoft Excel program. From these, the mean peak areas were computed and shown in **Table 5**. A plot of concentration vs. peak areas was constructed and shown in **Fig. 4** and **5** for Daunorubicin and Cytarabine, respectively.

The regression of the plot was calculated by least square regression method. The slope and intercept value for calibration curve for Daunorubicin and Cytarabine was $y = 2174.2x + 655.46$ $R^2 = 0.999$, $R^2 = 0.999$ and $y = 4896.x + 2531.1$ ($R^2 = 0.9994$) respectively.

TABLE 5: LINEARITY MEANS PEAK AREA VALUES

S. no.	Concentration of Daunorubicin (µg/ml)	Response	Concentration of Cytarabine (µg/ml)	Response
1	0	0	0	0
2	11	23183	25	126910
3	22	48836	50	251164
4	33	73870	75	371185
5	44	98733	100	491169
6	55	120501	125	602795
7	66	141708	150	745339

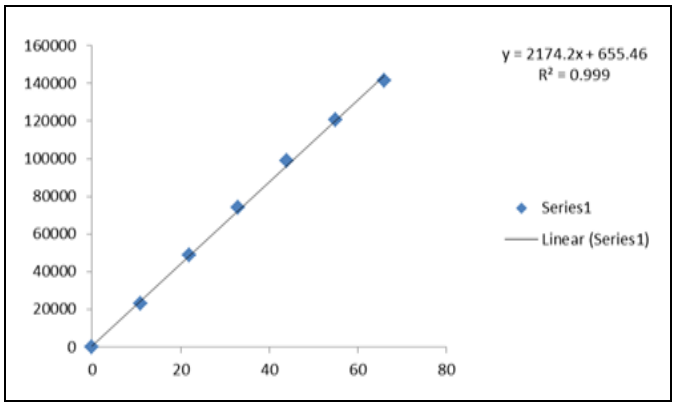


FIG. 4: LINEARITY GRAPH DAUNORUBICIN

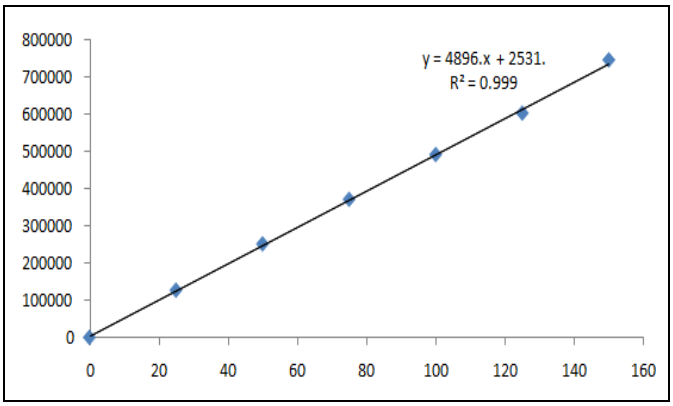


FIG. 5: LINEARITY GRAPH CYTARABINE

Where, X-Axis = Concentration, Y-Axis = Peak area

Specificity: The specificity of the RP-UPLC method is furnished, where complete separations of Daunorubicin and Cytarabine were distinguished in presence of other inert excipients used in tablets. In addition, there was no deterrence at the retention time of in the chromatogram of placebo solution. In the case of peak purity analysis with the ACQUITY TUV detector, the purity gradient was always not greater than the purity threshold for the analytes. This shown that the peaks of the analyte were pure, and excipients in the formulation do not interfere the analyte. The data were listed in **Table 6.**

TABLE 6: SPECIFICITY STUDIES OF DAUNORUBICIN AND CYTARABINE

S. no.	Name	No. of Injections	Daunorubicin	Cytarabine
			Area	Area
1	Blank	1	-	-
2	Placebo	1	-	-
3	Standard	1	104207	496110
4	Sample	1	103849	525039

Limit of Detection and Limit of Quantification: Limit of Detection (LOD) is the least concentration of an analyte in a sample that can be identified but not quantified. LOD is indicated as a concentration at a précised signal to noise ratio. The LOD will depend on the process of examination, along with the type of instrument. In the chromatography, the detection limit is the injected quantity that consequences in a peak with a height at least thrice or twice as high as the baseline noise level. LOD was computed by using the formula

$$LOD = 3.3(SDS)$$

The LOD was found to be 0.14 and 0.33 for Daunorubicin and Cytarabine, respectively.

Limit of quantification (LOQ) is the least concentration of an analyte in a sample that can be estimated with tolerable precision, accuracy, and reliability by a specified method under affirmed experimental conditions. LOQ is uttered as a concentration at a specified signal to noise ratio. In chromatography, the limit of quantification is the injected amount that consequences in a peak with a height ten times as high as the foundation line noise level. LOQ is calculated by using the formula

$$LOQ = 10(SDS)$$

The LOQ was originated to be 0.41 and 1.0 for Daunorubicin and Cytarabine, respectively. The data were listed in **Table 7.**

TABLE 7: LOD AND LOQ RESULTS

Molecule	LOD	LOQ
Daunorubicin	0.14	0.41
Cytarabine	0.33	1.00

Robustness: Robustness is denoted by making speculate changes in the chromatographic conditions like change in temperature, mobile phase composition, and flow rate were assessed for the impact on the present method. It was founded from the chromatograms that the results were not more than the limits.

This represents that the method developed is robust and shown in **Table 8**.

TABLE 8: ROBUSTNESS STUDY OFDAUNORUBICIN AND CYTARABINE

Parameter		Daunorubicin	Cytarabine
Temperature	25 °C	98052	511269
±5 °C	35 °C	102115	488780
Flow	0.2 ml	111224	545607
rate±0.1ml	0.4 ml	91619	414347
Mobile Phase	45:55	108897	499362
change ±5 pH	55:45	99474	512640

Assay: Bearing the label, claims 44 mg Daunorubicin & 100 mg Cytarabine. An assay was performed with the above formulation. The average % assay for Daunorubicin and Cytarabine obtained was 99.33% and 99.70%, respectively. The data were listed in **Table 9.**

TABLE 9: ASSAY RESULTS

Drug	Labeled amount (mg/tab)	% of Assay
Daunorubicin	44	99.33
Cytarabine	100	99.70

Degradation Studies: Since, no interference of blank and degradants, the RP-UPLC 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 drug Daunorubicin and Cytarabine were found to be more degraded when exposed to peroxide, base, and acidic conditions and least degraded when exposed to necessary water and photolysis degradation. The data were listed in **Table 10**.

TABLE 10: DEGRADATION STUDIES OF DAUNORUBICIN AND CYTARABINE

Sample name	Total purity	Daunorubicin			Cytarabine		
		% of Purity	% of Degradation	Purity of peak area	% of Purity	% of Degradation	Purity of peak area
Acid	100	90.64	9.36	104207	96.59	3.41	496110
Base	100	91.22	8.78	103485	96.93	3.07	494208
Peroxide	100	91.62	8.38	104340	97.48	2.52	497020
Thermal	100	97.92	2.08	104755	97.57	2.43	494250
UV	100	98.77	1.23	102651	98.19	1.81	501089
Water	100	98.77	1.23	103274	99.56	0.44	500098

CONCLUSION: The current study describes a new and simple, reliable, economic elution RP-UPLC method for the simultaneous estimation of Daunorubicin and Cytarabine. The forced degradation studies were conducted for the three drugs by using several degradation conditions like oxidation, acidic, alkali, thermal, and photolytic conditions, and the proposed method was effectively employed from the resolution of employed sample peaks. To the best of our knowledge, no such detailed and stability indicating method has been presented for the assay of this drug mixture. The developed method finished use of UPLC as a tool for peak integrity and purity confirmation. Therefore the proposed study method can be used for quantification Daunorubicin and Cytarabine in bulk and pharmaceutical dosage form.

Finally, this method was carefully validated; as a result, it can be suggested for routine analysis and for testing quality through stability studies of the drugs.

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REFERENCES:

1. Kirthi C, Azra A, Mounika R, Aamir AS, Aparna Y and Sanjeev S: A study on the adverse effects of anticancer drugs in an oncology center of a tertiary care hospital. Int J Pharm PharmSci 2014; 6(2): 580-3.

2. Shefali A, Shilpi A and Shailey S: Anticancer activities of thiosemicarbazides/ thiosemicarbazones: A review. Int J Pharm Pharm Sci 2014; 6(9): 34-41.
3. Chengalva P, Peddavengari LL and Kuchana M: A validated analytical method for the simultaneous estimation of cytarabine and daunorubicin in bulk and infusion formulation by reverse phase high performance liquid chromatography. Asian J Pharm Clin Res 2019; 12(8): 128-31.
4. Kushagra R: Method development and validation of RP-HPLC method for simultaneous estimation of daunorubicin and cytarabine in synthetic mixture; World Journal of Pharmacy and Pharmaceutical Sciences 2018; 7(5): 1301-10.
5. Sujana K and Satyanarayana V: Validated stability indicating RP-HPLC method for simultaneous estimation of daunorubicin and cytarabine in bulk and its pharmaceutical dosage form. Int J of Pharmaceutical Sciences and Research 2019; 10(4): 1895-01.
6. Agrawal A and Sharma M: Bioanalytical method development and validation for estimation of daunorubicin and cytarabine in blood plasma by using RP-HPLC. J of Drug Delivery and Therapeutics 2019; 9(4): 366-73.
7. Murthy V, Rohini A, Pravallika KE, Prameela RA and and Rahaman SA: Visible spectrophotometric method for Cytarabine in bulk and pharmaceutical dosage forms. Scholars Research Library 2013; 5(4): 51-55.
8. Kushagra R: Method development and validation of RP-HPLC method for simultaneous estimation of Daunorubicin and Cytarabine in the synthetic mixture. World Journal of Pharmacy and Pharmaceutical Sciences 2018; 1301-10.
9. Suman P, Rao TS, Reddy KV. Development of stability-indicating RP–HPLC method for estimation of daunorubicin-A chemotherapic drug in bulk and pharmaceutical formulations. World J Pharm Res 2017; 6: 1158-74.
10. Lukawska M, Oszczapowicz L, Jelinska A, Piontek JC, Zajac M and Dobrowolski, L: Development and validation of RP-HPLC method for determination of novel derivatives of daunorubicin. Chem Anal 2009; 54: 907-17.
11. Anna J, Marianna Z, Judyta C, Kinga G, Bartosz T and Anna K: Validation of a stability indicating LC-UV Method for [(N-Morpholine)methylene] daunorubicin hydrochloride. Chromatographia 2008; 67: 107-11.
12. Zhang H, Xian-Rong Q and Zhang Q: HPLC determination of daunorubicin hydrochloride in its liposomes. Chin J Pharm Anal 2002; 22: 282-4.
13. ICH, Q1A, Stability Testing of New Drug Substances and Products, in: Proceedings of the International Conference on Harmonisation, Geneva, October, 1993.
14. ICH, Q2A, Hamonised Tripartite Guideline, Test On Validation of Analytical Procedures, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March, 1994.
15. ICH, Q2B, Hamonised Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March 1996.

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