RESEARCH ARTICLE

A Simple Reverse Phase Ultra Performance Liquid Chromatography Validated Method for Concurrent Estimation of Daunorubicin and Cytarabine in Drug Substances and Drug Product

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ABSTRACT

A fast, precise, accurate, and steadiness indicating isocratic liquid chromatographic technique was created for the synchronous assurance of the daunorubicin and cytarabine in bulk and formulation. To optimize a column CHS C18 100 × 2.1 mm, 1.8 µm, mobile phase, including buffer 0.1% orthophosphoric acid, acetonitrile pick in the proportion 70:30 v/v, was pumped through the column at a flow rate of 0.3 mL/min at 240 nm, initiate to be an efficient method for elution of drug with good peak shapes, as well as, retention times. The retention time of daunorubicin and cytarabine were initiated to be 0.556 and 0.743 minutes. The % recovery was got at 100.07 and 99.88% for daunorubicin and cytarabine separately. The limit of detection (LoD) and limit of quantitation (LoQ) values got from the relapse formula of daunorubicin and cytarabine were 0.16, 0.5, and 0.64, 1.93, correspondingly. The relapse equation of daunorubicin is y = 2974.3x + 648.32, and y = 4896.5x + 4851.5 of cytarabine.

Keywords: Cytarabine, Daunorubicin, Forced degradation study, LoD, LoQ, RP-UPLC.

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INTRODUCTION

The daunorubicin is widely utilized in the treatment of acute non-lymphocytic leukemia.[1] Daunorubicin is (8S,10S)-8-acetyl-10-{[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy}-6,8,11-trihydroxy-1-methoxy-5,7,8,9,10,12-hexahydrotetracene-5,12-dione.

Daunorubicin movement has been ascribed majorly to their intercalation between the base pairs of native Deoxyribonucleic acid (DNA).[2] Daunorubicin represses topoisomerase II action by settling the DNA-topoisomerase II complex, avoiding the relegation bit of the ligation-relegation reaction that topoisomerase II catalyzes,[3] mixture with antineoplastic and antiviral properties. Cytarabine-13C3 is a specific inhibitor of DNA synthesis but does not restrain RNA combination.[4] Cytarabine is chemically 4-amino-1-[(2R, 3S, 4S, 5R)-3, 4-dihydroxy-5-(hydroxyl methyl) oxolan-2-yl]-1, 2-dihydropyrimidin-2-one. Cytarabine, a manufactured pyrimidine nucleoside, is changed over intracellular, essentially by deoxycytidine kinase, to dynamic cytarabine triphosphate.[1,2] Activity occurs basically as the consequence of inhibition of DNA polymerase via competition with deoxycytidine triphosphate, resulting in the restraint of DNA synthesis.[5] Acute myeloid leukemia (AML) treatment has dependably been a test to the treating physician. Constant endeavors are being made to improve treatment outcomes in AML. CPX-351 is a pharmacologic progression toward this path. It is a liposomal fixed medication mix of cytarabine and daunorubicin. Early investigations show that it will assume a major role in AML treatment.[6]

From the literature survey, we found that only two reverse phase – high performance liquid chromatography (RP-HPLC) techniques are there in a combination form.[7,8] Resolution of daunorubicin by liquid chromatography-mass spectrometry/

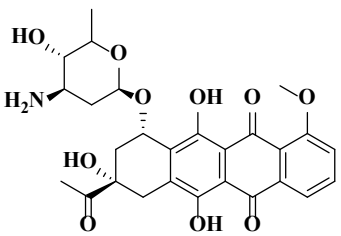

Figure 1: Daunorubicin structure

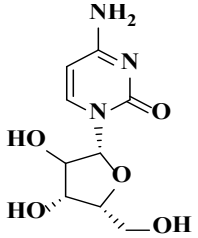

Figure 2: Cytarabine structure

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mass spectrometry (LC-MS/MS),[9] RP-HPLC[10]; cytarabine by RP-HPLC,[11,12] and by spectroscopy method.[13] There were no techniques were accounted for the simultaneous estimation of daunorubicin and cytarabine by RP-UPLC. Thus, an aim was made to develop an easy, sensitive, precise, and accurate analytical RP-UPLC method for the evaluation of daunorubicin and cytarabine in bulk and formulation.

MATERIALS AND METHODS

Samples of daunorubicin and cytarabine are acquired from Spectrum Pharma Labs, Hyderabad, India. HPLC grade distilled water, acetonitrile, methanol, potassium dihyrogen orthophosphate buffer, orthophosphoric acid, and trimethylamine, all are from Merck, Mumbai, India. Waters ultra performance liquid chromatography (UPLC) Acquity system equipped with a tunable ultraviolet (TUV) detector with Empower 2 software. The isocratic analytical system was run through CHS C18 100 × 2.1 mm, 1.8 mm column. The mobile phase includes 0.1% orthophosphoric acid buffer and acetonitrile is taken in the proportion 70:30 v/v, was pumped through the column at a flow rate of 0.3 mL/min. The temperature was kept at 30ºC. Optimized wavelength fix at 240 nm.

Preparation of Standard Stock Solutions

Exactly weighed and exchanged 11 mg of daunorubicin, and 25 mg of cytarabine working standards into a 25 mL clean dry volumetric flagon, included 10 mL of diluent, sonicated for 10 minutes and filled up to the final volume with diluents. 1 mL from every stock solution was pipette out and taken into a 10 mL volumetric carafe, and fill up to the mark with diluents (44 **µg**/mL daunorubicin and 100 µg/mL cytarabine).

Preparation of Sample Stock Solutions

1 vial equivalent to 44 mg daunorubicin and; 100 mg cytarabine was poured into a 100 mL volumetric carafe, 50 mL of diluents was included and sonicated for 25 minutes, further, the volume was filled up with diluent and separated by HPLC channels. 1 mL of filtered sample stock solution was poured to a 10 mL volumetric flagon and completed up with diluent (44 µg/mL daunorubicin and 100 µg/mL cytarabine).

Preparation of Diluent

Acetonitrile and water are taken in the ratio of 50:50 v/v.

Buffer [0.1% Orthophosphoric acid (OPA)]

1 mL of orthophosphoric acid solution in a 1,000 mL of volumetric flagon includes about 100 mL of Milli-Q water, and the last volume is arranged up to 1,000 mL with Milli-Q water.

Method Development

At first, liquid chromatography partition was attempted to create utilizing a variety of ratios of methanol and water, and acetonitrile and water, as a portable stage, in which, the medication did not react successfully, and the system suitability parameters not acknowledged the range. The natural substance of the portable stage was also examined to optimize the partition of mixed medications. To improve the tailing factor, the pH of the mobile phase becomes a significant factor. CHS C18 100 × 2.1 mm, 1.8 mm with an isocratic mobile phase comprised of 0.1% orthophosphoric acid buffer and acetonitrile blend in the ratio of 70:30 v/v at a flow rate of 0.3 mL/min. The column temperature was kept up at 30ºC, and the recognition was done utilizing a TUV detector at 240 nm was elected as the stationary stage to develop resolution, and the tailing of both peaks was diminished considerably and brought close to one. The retention times were found to about 0.556 and 0.743 minutes for daunorubicin and cytarabine, respectively. The method was validated in terms of precision, accuracy, and linearity as per International Council for Harmonization (ICH) rule. The system was validated in terms of accuracy, precision, the steadiness of the solution, LoD, LoQ, robustness, and which linearity was studied, as per ICH **g**uidelines.

Method Validation

System Suitability

The system suitability parameters were resolute by preparing standard solutions of daunorubicin 44 µg/mL and cytarabine 100 µg/mL. The solutions were infused multiple times and the factors, like peak tailing, resolution, and united states pharmacopeia (USP) plate count were resolute. The % relative standard deviation (RSD) for the region of six standard infusions results should not be > 2%. The results were noted in Table 1.

Specificity

Specificity was resolute by standard solutions of 44 µg/mL daunorubicin and 100 µg/mL cytarabine, blank, and placebo solutions. There should not asset meddling peaks in the blank and placebo at maintenance times of these medications in this technique, so this system was said to be specific.

Linearity

By suitable aliquots of the standard daunorubicin and cytarabine, prepared six working solutions ranging between 11-66 and 25-150 µg/mL, each trial linearity point was performed in triplicate, indicated by optimized chromatographic conditions.

Table 1: System suitability variables

Parameter	*Daunorubicin*	*Cytarabine*
Retention time (min)	0.556	0.743
Theoretical plates (N)	2,458.9	3,592.7
Tailing factor (T)	1.5	1.6
Resolution	-	3.8

Table 2: Results for linearity

Parameter	*Daunorubicin*	*Cytarabine*
Y intercept	2,974.3	4,896.5
Correlation coefficient r2	0.999	0.999
Regression equation	y = 2,974.3x + 648.32	y = 4,896.5x + 4,851.5
Linearity range	11–66 µg/mL	25–150 µg/mL
LoD	0.16	0.64
LoQ	0.5	1.93

Table 3: Results for accuracy

Daunorubicin				*Cytarabine*		
Recovery level (%)	*Amount added (µg/mL)*	*Amount found (µg/mL)*	*% recovery*	*Amount added (µg/mL)*	*Amount found (µg/mL)*	*% recovery*
50	22	22.03	100.14	50	50.05	100.11
100	44	44.04	100.09	100	99.95	99.95
150	66	65.99	99.99	150	149.37	99.58
Mean recovery			100.07%	Mean recovery		99.88%
% RSD			0.27	% RSD		0.47

Table 4: Results of precision

Drug	*Interday precision (% RSD)*	*Method precision (% RSD)*
Daunorubicin	0.41	0.29
Cytarabine	0.36	1.04

Table 5: Results for LoD and LoQ

S. No.	*Drug*	*LoD (µg/mL)*	*LoQ (µg/mL)*
1	Daunorubicin	0.16	0.5
2	Cytarabine	0.64	1.93

Table 6: Results of robustness conditions

S. No.	*Condition*	*Plus*	*Minus*
1	Flow rate	0.27 mL/min	0.33 mL/min
2	Mobile phase	75B:25A	65B:35A
3	Temperature	35ºC	25ºC

Calibration curves were plotted with observed peak areas against concentration, followed by the assurance of relapse formula and computation of the correlation coefficient on curves for daunorubicin and cytarabine. The results were noted in Table 2.

Accuracy

Accuracy was carried out by % recovery studies of daunorubicin and cytarabine at three different concentration levels (50, 100, and 150%). Percentage recovery was intended from the quantity include and the amount recovered. The percentage recovery was within the approval criteria, this indicates the accuracy of the technique (acceptance criteria: percentage of recovery between 98 to 102). The results were noted in Table 3.

Precision

The repeatability of the system was seen by calculating the % RSD of six replicate infusions of 100% concentration (44 µg/mL of daunorubicin and 100 µg/mL of cytarabine) on an identical day, and for intermediate precision, % RSD was intended from repeated studies on variable days. The results were noted in Table 4.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The LoD and LoQ were deliberate from the slope(s) of the calibration plot and the standard deviation (SD) of the pinnacle territories, utilizing the equation LoD = 3.3 σ/s and LoQ = 10 σ/s. The outcome was noted in Table 5.

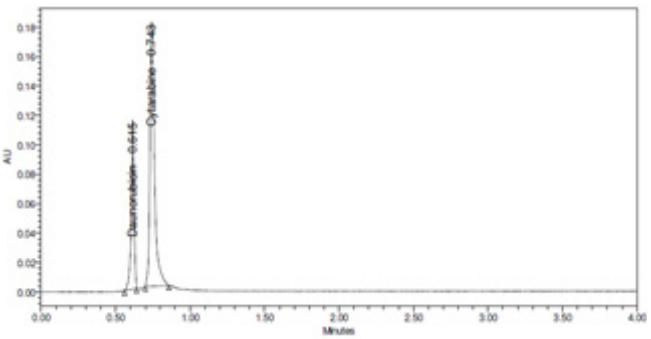

Figure 3: Acid degradation

Robustness

Robustness of the technique was seen by revising the chromatographic conditions, like flow rate, mobile phase ratio, and temperature, are prepared, but there was no known change in the outcome, and all are within limit, as per ICH rule. Robustness conditions, like flow minus (FM) (0.27 mL/min), flow plus (FP) (0.33 mL/min), 75:25 mobile phase minus 55:35 mobile phases plus, temperature minus (25ºC), and temperature plus (35ºC) were maintained, and samples were infused in a copy manner. The method suitability parameter was approved. % RSD was within the range. The result was noted in Table 6.

Degradation Studies

Acid Degradation

To 1 mL of stock solution daunorubicin and cytarabine, 1 mL of 1N hydrochloric acid was included and refluxed for 30 minutes at 60ºC. The resultant solution was diluted to get 44 and 100 µg/mL solutions, and 1 µL solution was implanted to the method, and the chromatograms were recorded to review the stability of the sample. The outcome was noted in Figure 3.

Oxidative Degradation

To 1 mL of stock solution of daunorubicin and cytarabine, 1 mL of 20% hydrogen peroxide (H_2O_2) was included separately. The solutions were kept for 30 minutes at 60ºC. For the UPLC study, the resultant arrangement was weakened to get 44 and 100 µg/mL solution, and 1 µL was imbued into the method, and the chromatograms were recorded to review the steadiness of the sample. The outcome was noted in Figure 4.

Alkali Degradation

To 1 mL of stock solution daunorubicin and cytarabine, 1 mL of 1N sodium hydroxide was included and refluxed for 30 minutes at 60ºC. The resultant solution was diluted to get

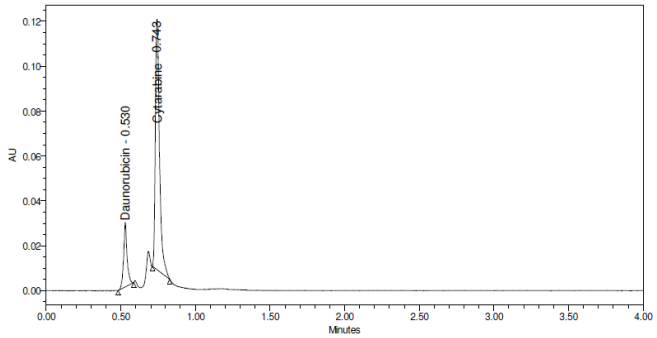

Figure 4: Peroxide degradation

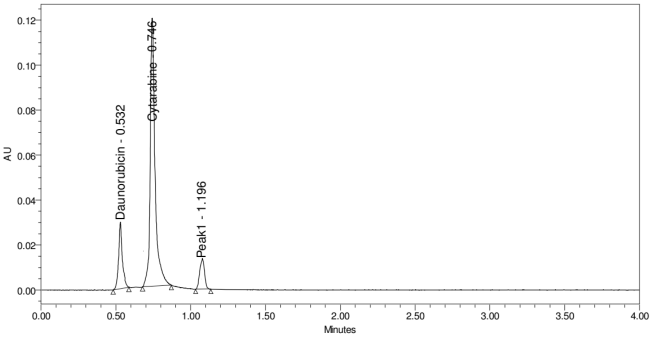

Figure 5: Alkaline degradation

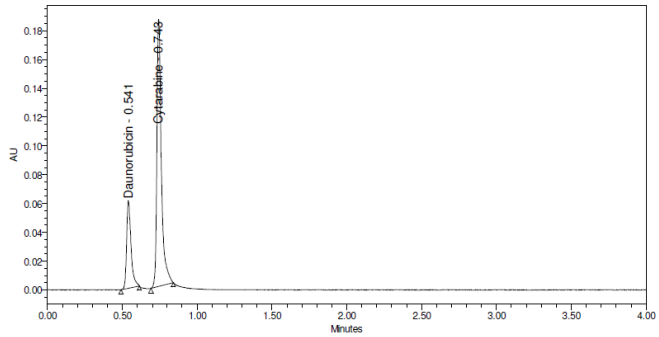

Figure 6: Thermal degradation

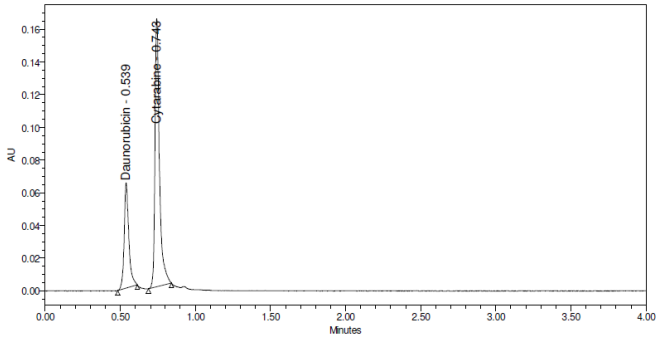

Figure 7: Photo degradation

44 and 100 µg/mL solution, and 1 µL was infused into the technique, and the chromatograms were recorded to review the solidity of the sample. The outcome was noted in Figure 5.

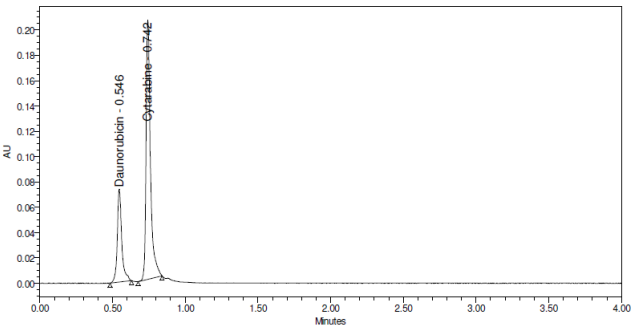

Figure 8: Water degradation

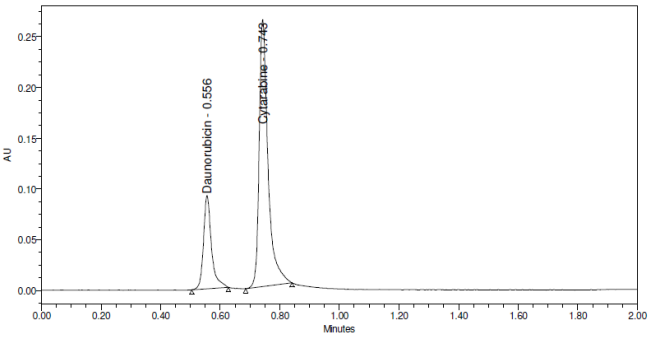

Figure 9: Optimized chromatogram of daunorubicin and cytarabine

Thermal Degradation

The standard mediation of solute was located in an oven at 105ºC for 6 hours to study dry heat degradation. For the UPLC study, the resultant arrangement was weakened to 44 and 100 µg/mL solution, and 1 µL was infused into the method, and the chromatograms were recorded to review the solidity of the sample. The outcome is noted in Figure 6.

Photo Degradation

The photochemical stability of the drug was also studied by exposing the 440 and 1,000 µg/mL solution to UV light, by keeping the beaker in the UV cavity for 7 days. For the UPLC study, the resultant arrangement was weakened to get 44 and 100 µg/mL solutions, and 1 µL was infused into the system, and the chromatograms were recorded to review the stability of the sample. The outcome is noted in Figure 7.

Neutral Degradation Studies

Stress testing in constant conditions was considered by refluxing the compound in water for 6 hours at a temperature of 60ºC. For UPLC consider, the resultant arrangement was diluted to (44 ppm and 100 ppm) solution, and 1 µL were imbued into the technique, and the chromatograms were recorded to review the steadiness of the example. The outcome is noted in Figure 8.

RESULTS AND DISCUSSION

After various trails with mobile phases of various compositions, and mobile phases, including 0.1% orthophosphoric acid buffer

Table 7: Results for stability studies of daunorubi cin and cytarabine

Variables	*Peak area*		*% of degradation*	
Drug substance	*Daunorubicin*	*Cytarabine*	*Daunorubicin*	*Cytarabine*
Acid degradation	130,629	440,934	6.07	7.67
Alkaline degradation	132,713	451,043	4.57	5.55
Peroxide degradation	130,161	462,800	6.41	3.09
Thermal degradation	134,064	465,135	3.6	2.6
Photo degradation	137,670	468,684	1.01	1.86
Water degradation	138,687	476,518	0.28	0.22

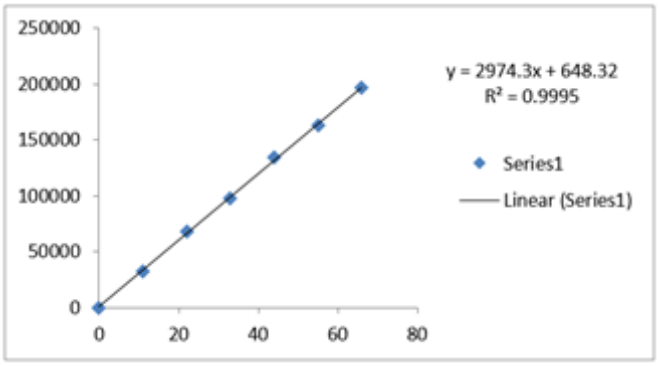

Figure 10: Calibration curve of daunorubicin

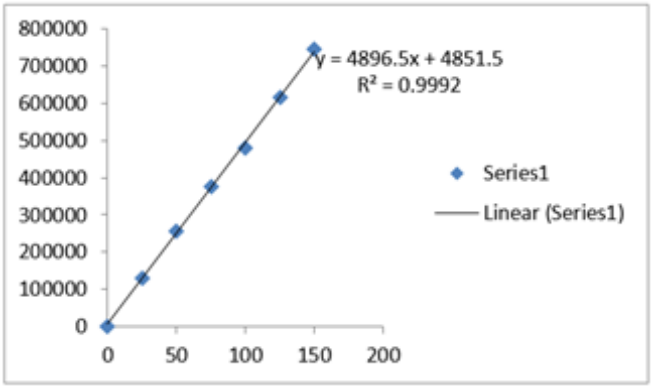

Figure 11: Calibration curve of cytarabine

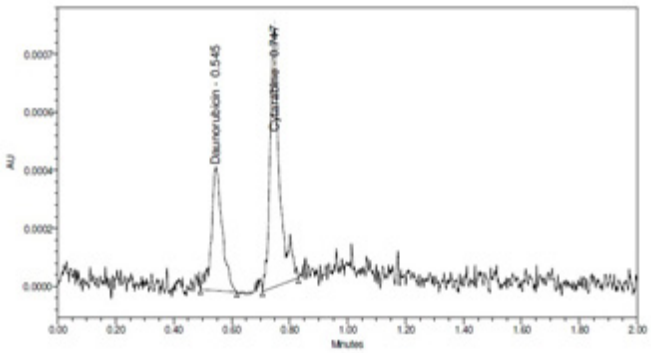

Figure 12: LoD of daunorubicin and cytarabine

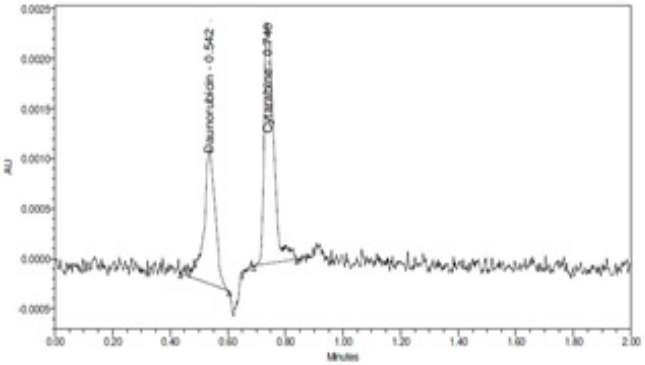

Figure 13: LoQ of daunorubicin and cytarabine

and acetonitrile took in the proportion of 70:30 v/v, was chosen as a mobile phase because of better resolution more number of theoretical plates and symmetric peaks. Daunorubicin and cytarabine were initiates to indicate considerable absorbance at 240 nm when decided spectro-photometrically, and thus, it was selected as the optimized wavelength. An optimized chromatogram demonstrating the separation of Daunorubicin and Cytarabine (Figure 9).

System Suitability

According to ICH rule, plate count should be more than 2,000, the tailing factor should be less than 2, and resolution must be more than 2. All the system suitable factors were approved and were within the range.

Linearity

Concentration range of 11–66 µg/mL for daunorubicin and 25 to 150 µg/mL of cytarabine was initiate to be linear with correlation coefficients 0.999, were within range. The outcome was noted in Figures 4 and 5.

Accuracy

The % of accuracy was an RSD for accuracy, at every level, is well within the range. Over the entire % recovery start to be 100.07 and 99.88%, daunorubicin and cytarabine, correspondingly, and overall the percentage relative standard deviation was initiate to be 0.27 and 0.47%, daunorubicin and cytarabine, correspondingly, for all the levels, was within the range.

Precision

The percentage relative standard deviation of six outcomes was inside the range. The zones of all the imbued were taken and standard deviation, % RSD was conscious, and the outcome was noted in Table 4.

Limit of Detection (LoD)

The LoD of target assay ppm of daunorubicin and cytarabine

by utilizing equation method 0.16 and 0.64 µg/mL. The grades were noted in Figure 12.

Limit of Quantification (LoQ)

The LoQ of the target assay conc. of daunorubicin and cytarabine, by utilizing equation method 0.5 and 1.93 µg/mL, were within the range. The grades were observed in Figure 13.

Robustness

In all the variable conditions of flow rate, mobile phase ratio and temperature, %RSD of peak area, tailing factor, and theoretical plate's exhibit were found within the range (Table 6).

Forced Degradation Study

Degradation studies demonstrated the particularity of the created system in the occurrence of degradation products. Stability was done in a mixture of two medications and purity of medication pinnacles was proved by purity angles. Their mixed drug items were bare to acid, alkali, oxidative, thermal, photo, and water stress conditions. Then, initiate to be no degradable material presence and proved that the proposed system was stable towards acid, alkali, peroxide, thermal, photo, and water conditions, within the ranges (Table 7).

CONCLUSION

Stress testing is the major criterion of the drug optimization method and the pharmaceutical industries have a lot of interest in this area. The after-effects of stress testing attempt as per the ICH rules uncover that the strategy is explicit and dependability showing. The proposed technique can separate these compounds from their stability products in the formulation, and thus, can be connected to the study of routine quality control (QC) tests and tests obtained from degradation studies.

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