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**Research Article**

# Stability Indicating Method Development and Validation for Determination of Daunorubicin and Cytarabine in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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## ABSTRACT

A Simple, Accurate and Precise method was developed for the simultaneous estimation of Daunorubicin and Cytarabine in bulk and pharmaceutical dosage form by RP- HPLC technique. Chromatogram was run through standard Zorbax C18 (150mm x 4.6 mm, 5µ particle size). Mobile phase containing buffer 0.01N KH2PO4: Acetonitrile taken in the ratio of 60:40 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.01N KH2PO4 buffer. Temperature was set to 30ºC. Optimized wavelength selected was 240.0 nm. Retention time of Cytarabine and Daunorubicin were found to be 2.325 min and 3.208 nm. % RSD of Daunorubicin and Cytarabine were found to be 0.6 and 0.6 respectively. %Recovery was obtained as 100.30% and 100.33% for Daunorubicin and Cytarabine respectively. LOD, LOQ values obtained from regression equation of Daunorubicin and Cytarabine were 0.09, 0.28 and 0.32, 0.97 respectively. The regression equation of Daunorubicin and Cytarabine is y=39589X+28710 and y=32928X+12457 respectively Retention times were decreased and that run was decreased, so the method was simple and economical that can be adopted in regular Quality control test in Industries.

**Key Words:** Daunorubicin, Cytarabine, RP-HPLC

## INTRODUCTION

Daunorubicin is also known as Daunomycin, is chemotherapy medication and a very toxic anthracycline aminoglycoside anti-neoplastic isolated from streptomyces paucities used to treat cancer. Specially, it is used for acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML). It is administered into vein by injection. Cytarabine is a pyrimidine nucleoside and an antimetabolite antineoplastic agent that inhibits the synthesis of DNA. It is mainly used in treatment of acute non-lymphoblastic leukemia. Daunorubicin and Cytarabine (IV injection) is a liposomal combination of that is FDA approved for the treatments of adults with newly diagnosed therapy -

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related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC).

Daunorubicin has antimitotic and cytotoxic activity that forms complexes with DNA by intercalation between base pairs, and it inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the relegation portion of the ligation-religation reaction that topoisomerase II catalyzes. Cytarabine acts through direct DNA damage incorporation into DNA by inhibiting the DNA polymerase.

Literature review reveals estimation of Daunorubicin and Cytarabine by RP-HPLC and spectroscopy method individually. In combination, Daunorubicin and Cytarabine only one method was published but there is a need to develop new stability indicating RP-HPLC method with more sensitivity, accuracy and precision.

**Objective:** Following are the objectives of the present work-

1. To develop a new stability indicating HPLC method for simultaneous estimation of Daunorubicin and Cytarabine and to develop the validated method according to ICH guidelines
2. To apply the validated method for the simultaneous estimation of Daunorubicin and Cytarabine in pharmaceutical formulation.

## MATERIALS AND METHODS

**Chemicals and Reagents:** Daunorubicin and Cytarabine drugs (API) received from spectrum labs, Hyderabad, India. The marketed combination of Daunorubicin (44 mg/$m^2$) and Cytarabine (100 mg/$m^2$) injection (VYXEOS), received from local market. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho- phosphoric acid. All the above chemicals and solvents are from Rankem.

**Instruments and Chromatographic conditions:** A WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and auto sampler integrated with Empower 2 software provided with UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 software was used for measuring absorbance of Daunorubicin and Cytarabine solutions. Electronic Balance- Denver, $P^H$ meter - BVK enterprises, India, Ultrasonicator- BVK enterprises. The chromatographic separation achieved on a Zorbax C18 column (4.6×150mm, 5µm particle size) as a stationary phase. The mobile phase was composed of 60;40 v/v of 0.01 N potassium dihydrogen ortho phosphate and acetonitrile at a flow rate of 1 ml/min and injection volume is 10µL. The column oven temperature was maintained at $30^0$C, and the drugs were detected at 245 nm.

**Methods**

**Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

**Buffer:**

**0.1%OPA Buffer:** 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

**0.1N Potassium dihydrogen Ortho phosphate:** Accurately weighed 1.36 gm of potassium dihydrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of triethylamine then $p^H$ adjusted to 3.8 with dilute Orthophosphoric acid solution.

**Standard Preparation:** Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4$^{th}$ of diluents was added to these flasks and sonicated for 10 min. Flask were made up with diluents and labeled as standard stock solution (440µg/ml of Daunorubicin and 1000µg/ml of Cytarabine). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (44µg/ml/ml of Daunorubicin and 100µg/ml of Cytarabine)

**Sample Preparation:** 1 vial equivalent to 44 mg Daunorubicin and 100mg Cytarabine was transferred into a 100ml volumetric flask, 50ml of diluents were added and sonicated for 25 min, further volume was made up with diluent and filtered by HPLC filters (440µg/ml of Daunorubicin and 1000µg/ml of Cytarabine). 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (44µg/ml of daunorubicin and 100µg/ml of Cytarabine)

**Method Validation**

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

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

**Precision:** From a single volumetric flask of working standard solution six injections were given. A study was carried out for repeatability under same operating conditions and also for intermediate precision with the same analyst on the different day for six sample preparations. Average area, standard deviation and
%RSD were calculated for two drugs in both methods and the results obtained are presented in table. As the limit of precision was less than"2" in both methods, the system precision was passed in these methods.

**Linearity:** The linearity expresses the proportional relationship between concentration and responses. This was evaluated at six concentration levels in the range between 11-66 µg/ml and 25- 150µg/ml for Daunorubicin and Cytarabine respectively. A calibration curve was plotted by considering concentration against corresponding peak area, and using least square regression analysis, the correlation coefficient was determined.

**Accuracy:** Preparation of Standard stock solutions: Accurately weighed 11mg of Daunorubicin, 25mg of Cytarabine and transferred to two separate 25ml volumetric flasks, 3/4$^{th}$ of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (440µg/ml of Daunorubicin and 1000 µg/ml of 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:** 1.0ml 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 150% Spiked Solution:** 1.5ml 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.

**Robustness:** Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guidelines.
Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit.

**LOD sample Preparation**: 0.25ml of Standard stock solution was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Daunorubicin and Cytarabine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

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

**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%.

**Assay of Daunorubicin and Cytarabine:** Bearing the label claims Daunorubicin 44mg, Cytarabine 100mg. Assay was performed with the above formulation. Average % Assay for Daunorubicin and Cytarabine obtained was 100.80% and 100.77% respectively.

## RESULTS AND DISCUSSION

**Optimization of Chromatographic Conditions**: To develop and establish a suitable RP-HPLC method for estimation of Daunorubicin and Cytarabine in bulk and pharmaceutical dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 60% Potassium dihydrogen ortho phosphate:40% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 240 nm detector wave length and at an injection volume of 10µL using zorbax C18 4.6 x 150mm, 5µm with run time of 6min. The proposed method was optimized to give sharp peak with good resolution, minimum tailing effect and theoretical plate count for Daunorubicin and Cytarabine, the optimized chromatogram was obtained as shown in (Figure- 3).

**Validation:** Linearity was established for Daunorubicin (11- 66µg/ml) and Cytarabine (25-150µg/ml) at six linear concentrations each were injected in a duplicate manner and average areas

were determined and linearity equations obtained for Daunorubicin was y= 32928$x$ + 28710 and of Cytarabine was $y$ = 32928$x$ + 12457, correlation coefficient ($R^2$) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Fig-3,4). Retention time of Daunorubicin is 3.202 min and Cytarabine is 2.322 min where no interfering peaks in blank and placebo were found in this method. So this method holds its specificity.

Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean% recovery was obtained as 100.30% for Daunorubicin and100.33% for Cytarabine was shown in (Table-2,3).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Daunorubicin and Cytarabine was obtained as 0.6% and 0.6% respectively. % RSD for Repeatability was obtained as 0.4% and 1.1% for Daunorubicin and Cytarabine respectively, Low % RSD values indicates that the method developed was precise as shown in (Table-4). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Daunorubicin and Cytarabine. The detection limit value was obtained as 0.09 and 0.32 for Daunorubicin and Cytarabine respectively. The Quantitation limit was found to be 0.28 and 0.97 respectively as given in (Table-5). Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in duplicate manner (Table-6). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table-7). Daunorubicin and Cytarabine pure drugs (API) were obtained from Spectrum labs, bearing the label claim 44mg/$m^2$ and 100mg/$m^2$ of Daunorubicin and Cytarabine. Assay was performed with the above formulation. Average % Assay obtained was 100.80% and 100.77% for Daunorubicin and Cytarabine respectively the result was shown in (Table-7) and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-4, 5) respectively.

**Degradation Studies:** Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 6.8).

**Oxidation:** To 1ml of stock solution of Daunorubicin and Cytarabine. 1ml of 20% hydrogen peroxide (H2O2) was added separately. The solution were kept for 30min at $60^0$C. For HPLC study, the resultant solution was diluted to obtain 44µg/ml solution and the chromatogram were recorded to assess the stability of sample.

**Acid degradation studies:** To 1ml of stock solution Daunorubicin and Cytarabine, 1ml of 2N HCl was added and refluxed for 30 min at $60^0$ c. The resultant solution was diluted to obtain 44µg/ml and 100µg/ml solution and 10µL solutions were injected into the system and the system and the chromatograms were recorded to access the stability of sample.

**Alkali degradation studies:** To 1ml of stock solution Daunorubicin and Cytarabine, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at $60^0$c. The resultant solution was diluted to obtain 44µg/ml and 100µg/ml solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry heat degradation studies:** The standard drug solution was placed in oven at $105^0$C for 1hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 44µg/ml and 100µg/ml solution and 10µ 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 440µg/ml Daunorubicin and 1000µg/ml Cytarabine solution to UV light by keeping the beaker in UV chamber for 1day or 200-watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 44µg/ml and 100µg/ml solutions and 10µL were injected into the system and the chromatograms were injected into the system and 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 1hr at a temperature of $60^0$C. For HPLC study, the resultant solution was diluted to 44µg/ml and 100µg/ml solution and 10 µ were injected into the system and the chromatograms were recorded to assess the stability of the sample.

## CONCLUSION

Chromatographic conditions used are stationary phase Zorbax C18 (150mm×4.6mm, 5µm) Mobile phase 0.01N KH2PO4: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1ml/min, detection wave length was 240 nm, column temperature was set to 30ºC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied

by injecting the standard six times and results were well under the acceptance criteria.

Linearity study was carried out between 25% to150 % levels, $R^2$ value was found to be as 0.999. Precision was found to be 0.6% and 0.6% for Daunorubicin and Cytarabine respectively, for repeatability 0.4% and 1.1% for Daunorubicin and Cytarabine respectively. LOD and LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.09, 0.28 and 0.32, 0.97 respectively. By using above method assay of marketed formulation was carried out and % Assay for Daunorubicin and Cytarabine obtained was 100.80% and 100.77% respectively.

Degradation studies of Daunorubicin and Cytarabine were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Daunorubicin and Cytarabine. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

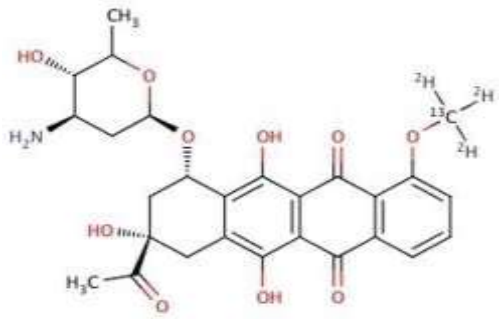

**Figure-1: Chemical Structure of Daunorubicin**

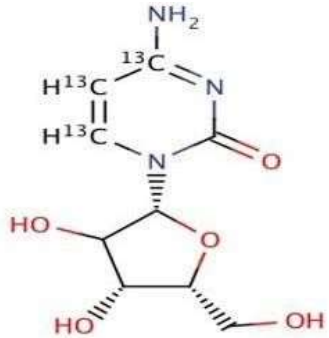

**Figure-2: Chemical Structure of Cytarabine**

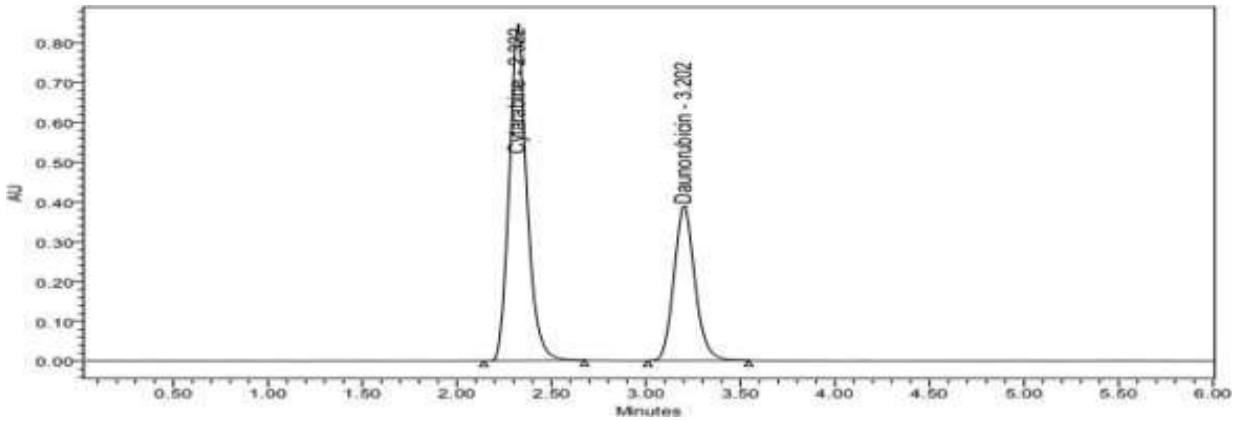


**Figure-3: Optimized Chromatogram of Daunorubicin and Cytarabine**

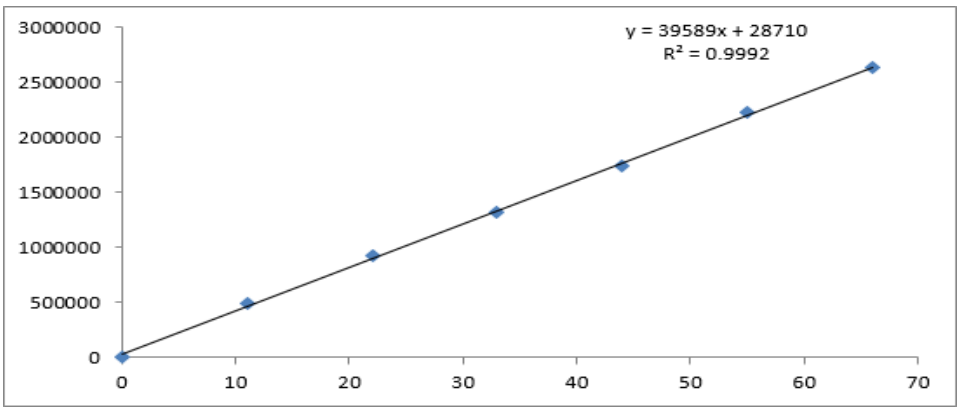


**Figure-4: Linearity Curve of Daunorubicin**

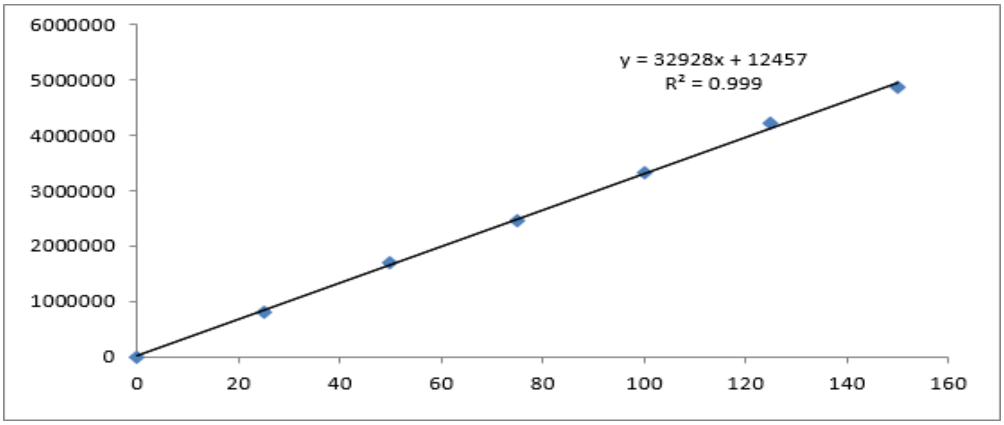


**Figure-5: Linearity Curve of Cytarabine**

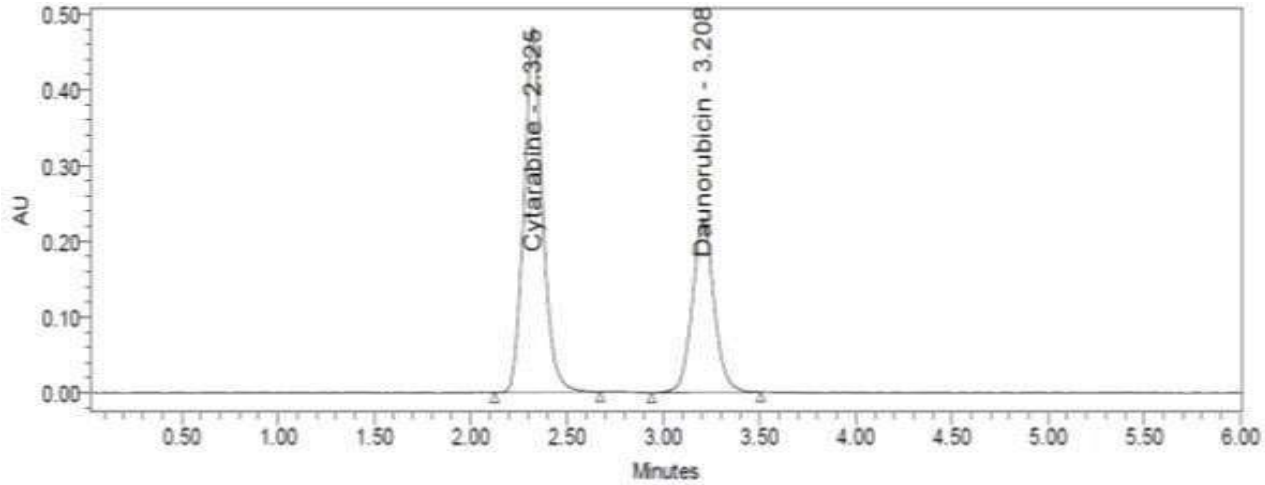


**Figure-6: Standard Chromatogram of Daunorubicin and Cytarabine**

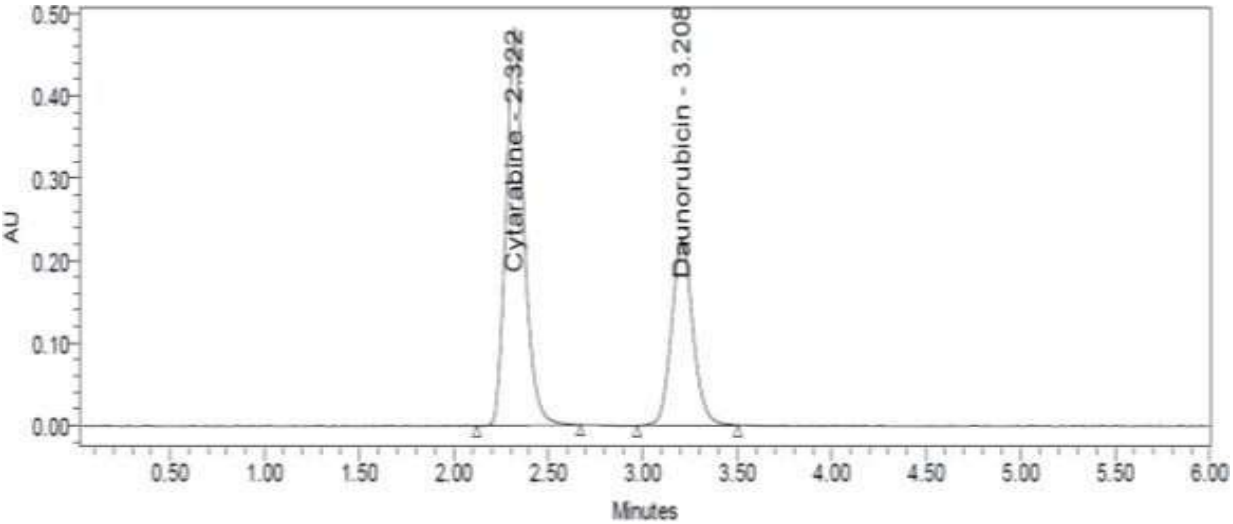


**Figure-7: A Sample Chromatogram of Daunorubicin and Cytarabine Pharmaceutical Dosage Form**

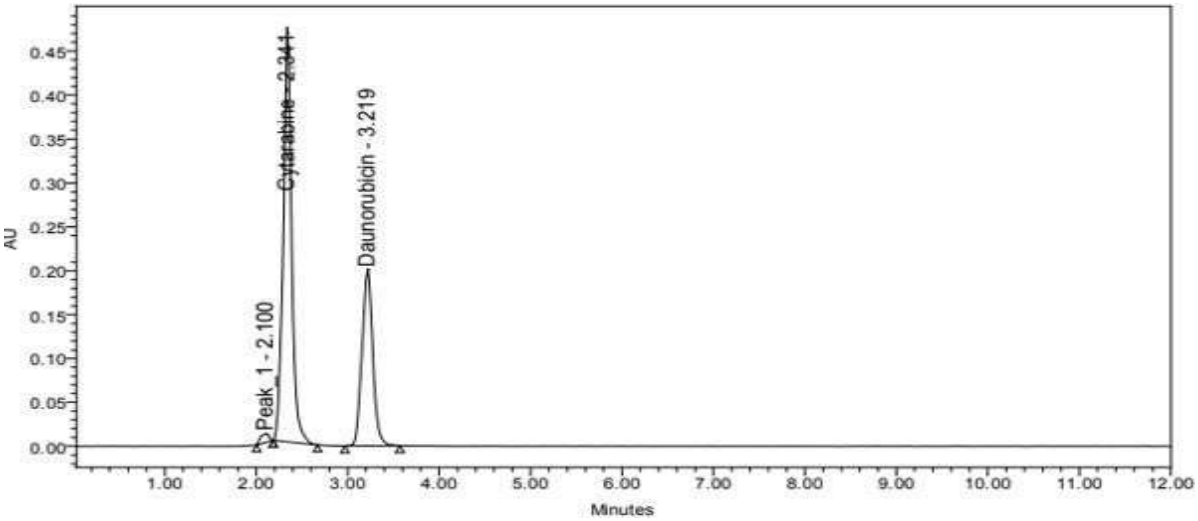


**Figure-8: Acid Chromatogram of Daunorubicin and Cytarabine**

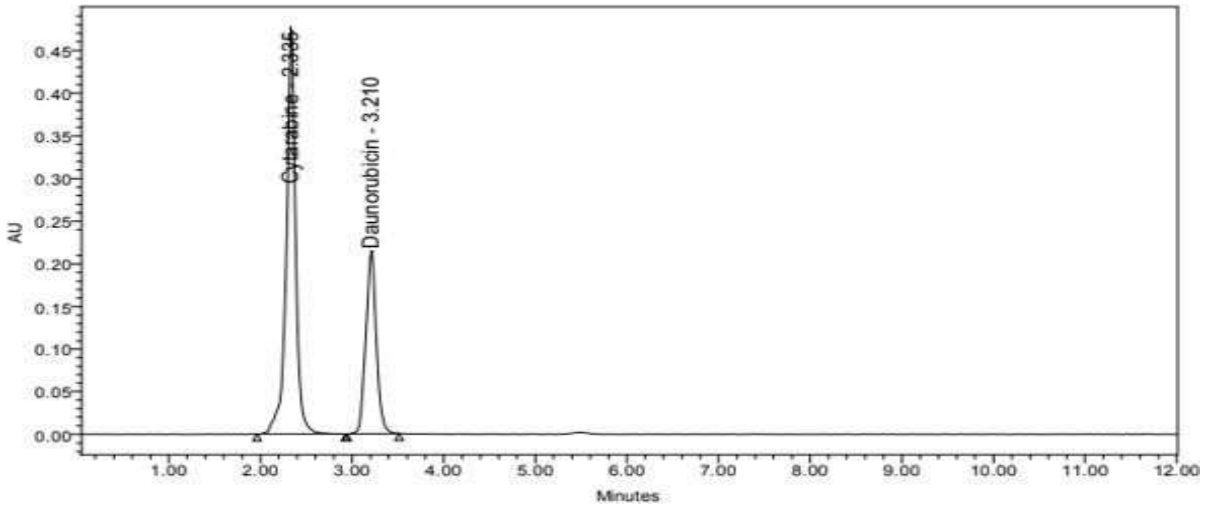


**Figure-9: Base Chromatogram of Daunorubicin and Cytarabine**

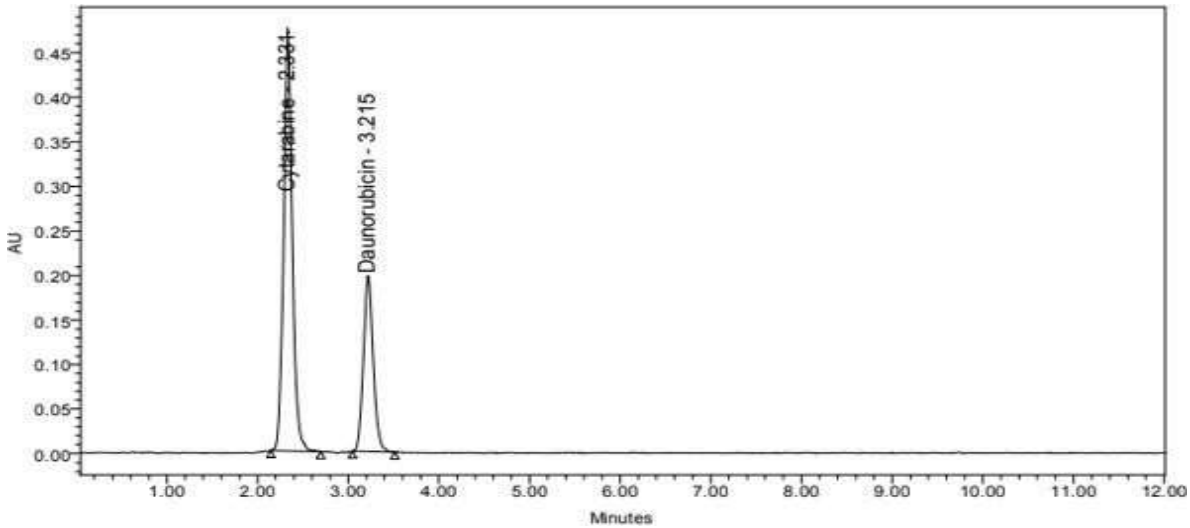


**Figure-10: Peroxide Chromatogram of Daunorubicin and Cytarabine**

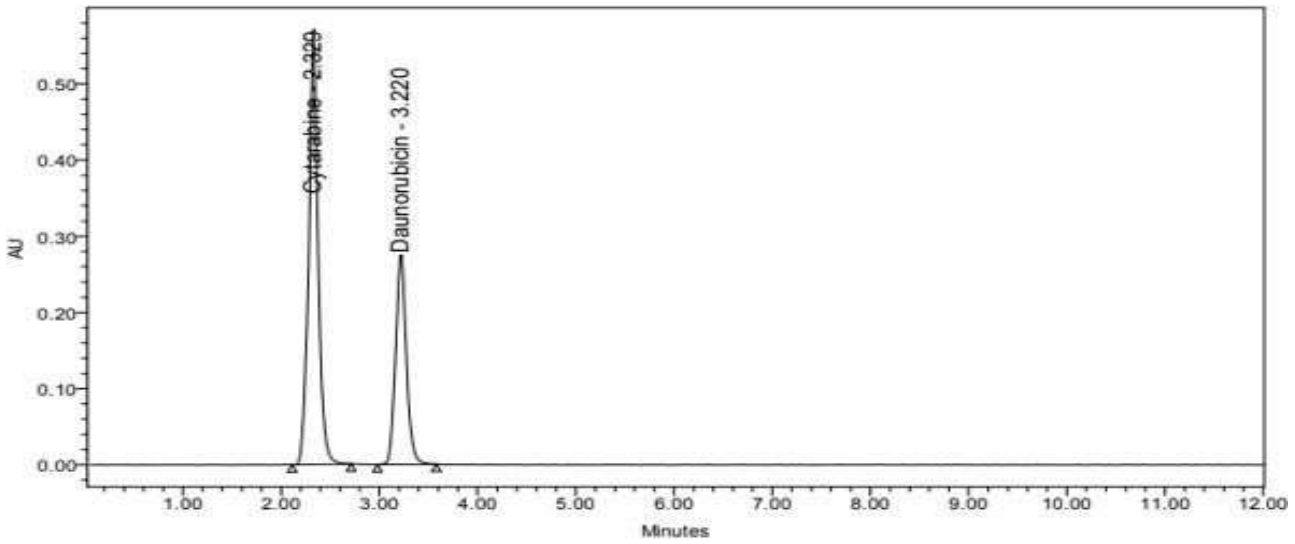


**Figure-11: Thermal Chromatogram of Daunorubicin and Cytarabine**

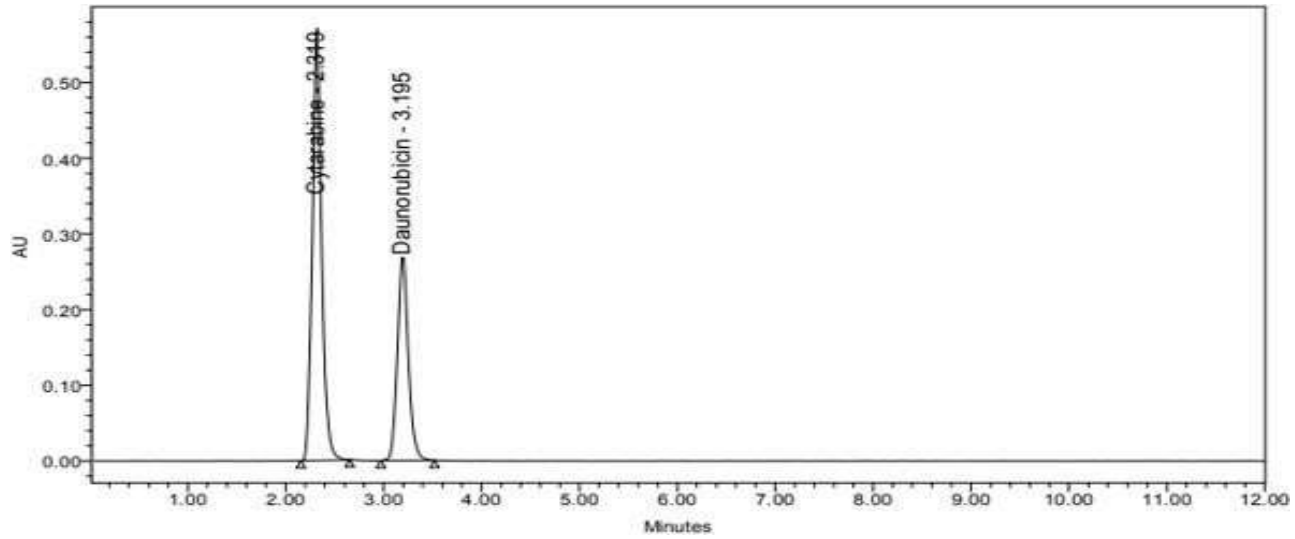


**Figure-12: UV Chromatogram of Daunorubicin and Cytarabine**

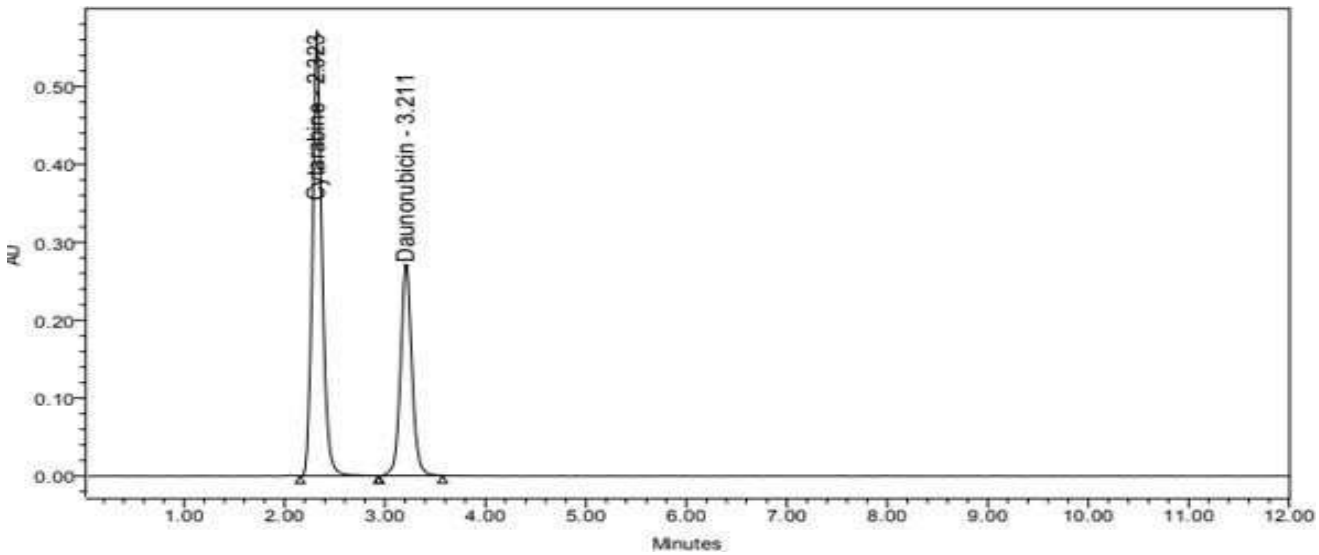


**Figure-13: Water Chromatogram of Daunorubicin and Cytarabine**

**Table-1: Optimized Chromatographic Conditions**

| Parameter | Condition |
|---|---|
| **RP-HPLC** | WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detectors |
| **Mobile Phase** | 60% KH2PO4 (0.1%):40% Acetonitrile |
| **Flow rate** | 1 ml/min |
| **Column** | Zorbax C18 150 ×4.6mm,5µ |
| **Detector Wavelength** | 240 nm |
| **Column Temperature** | $30^0$C |
| **Injection Volume** | 10µL |
| **Run Time** | 6 min |
| **Diluent** | Water and Acetonitrile in the ratio 50:50 |
| **Retention Time** | Daunorubicin 3.202 and Cytarabine 2.322 |
| **Theoretical Plates** | Daunorubicin3975 and Cytarabine2325 |

**Table-2: Accuracy results of Daunorubicin**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean % Recovery |
|---|---|---|---|---|
| **50%** | 22 | 22.15 | 100.67 | 100.30% |
| | 22 | 21.85 | 99.30 | |
| | 22 | 22.30 | 101.37 | |
| **100%** | 44 | 43.56 | 99.0 | |
| | 44 | 44.32 | 100.74 | |
| | 44 | 44.64 | 101.46 | |
| **150%** | 66 | 65.7 | 99.6 | |
| | 66 | 66.4 | 100.6 | |
| | 66 | 66.0 | 100.1 | |

**Table-3: Accuracy results of Cytarabine**

| S.no | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovered (µg/mL) | Mean %Recovery |
|---|---|---|---|---|
| **50%** | 50 | 49.84 | 99.67 | 100.33 |
| | 50 | 50.03 | 100.07 | |
| | 50 | 49.53 | 99.05 | |
| **100%** | 100 | 100.06 | 100.06 | |
| | 100 | 101.79 | 101.79 | |
| | 100 | 99.59 | 99.59 | |
| **150%** | 150 | 149.08 | 99.39 | |
| | 150 | 152.93 | 101.96 | |
| | 150 | 152.15 | 101.43 | |

**Table-4: Precision Results of Daunorubicin**

| S.no | Daunorubicin | | Cytarabine | |
|---|---|---|---|---|
| | Repeatability | Intermediate Precision | Repeatability | Intermediate Precision |
| **1** | 1825904 | 2815558 | 3233036 | 3243605 |
| **2** | 1810193 | 2790232 | 3243876 | 3250641 |
| **3** | 1823013 | 2768307 | 3199644 | 3229351 |
| **4** | 1836260 | 2749854 | 3277448 | 3237347 |
| **5** | 1805298 | 2764120 | 3286286 | 3138451 |
| **6** | 1821776 | 2760814 | 3292509 | 3191182 |
| **Mean** | 2829645 | 2774814 | 3255467 | 3215096 |
| **S.D** | 11160.7 | 23972.9 | 36218.2 | 42943.8 |
| **%RSD** | 0.4 | 0.9 | 1.1 | 1.3 |

**Table-5: LOD and LOQ values of Daunorubicin and Cytarabine**

| Molecule | LOD | LOQ |
|---|---|---|
| **Daunorubicin** | 0.09 | 0.28 |
| **Cytarabine** | 0.32 | 0.97 |

**Table-6: Robustness Data of Daunorubicin and Cytarabine**

| S.no. | Condition | %RSD of Daunorubicin | %RSD of Cytarabine |
|---|---|---|---|
| 1 | Flow rate (-) 0.9 ml/min | 0.6 | 0.8 |
| 2 | Flow rate (+) 1.1 ml/min | 0.3 | 1.3 |
| 3 | Mobile phase (-) 65B: 35A | 0.8 | 0.6 |
| 4 | Mobile phase (+) 55B: 45A | 1.1 | 1.0 |
| 5 | Temperature (-) 25°C | 1.2 | 0.8 |
| 6 | Temperature (+) 35°C | 0.1 | 0.9 |

**Table-7: System Suitability Parameters Results of Daunorubicin and Cytarabine**

| S.no | Cytarabine | | | Daunorubicin | | | |
|---|---|---|---|---|---|---|---|
| Injection | Rt (min) | USP Plate Count | Tailing | Rt (min) | USP Plate Count | Tailing | Resolution |
| **1** | 2.325 | 2746 | 1.11 | 3.208 | 3942 | 1.06 | 4.5 |
| **2** | 2.325 | 2819 | 1.14 | 3.204 | 3884 | 1.07 | 4.5 |
| **3** | 2.326 | 2803 | 1.12 | 3.209 | 3973 | 1.07 | 4.5 |
| **4** | 2.326 | 2813 | 1.13 | 3.208 | 3935 | 1.06 | 4.5 |
| **5** | 2.327 | 2803 | 1.13 | 3.202 | 3975 | 1.09 | 4.5 |
| **6** | 2.328 | 2797 | 1.14 | 3.207 | 4011 | 1.07 | 4.5 |

**Table-8: Assay results of Daunorubicin and Cytarabine**

| S.no | %Assay of Daunorubicin | %Assay of Cytarabine |
|---|---|---|
| 1 | 101.11 | 100.07 |
| 2 | 100.24 | 100.41 |
| 3 | 100.95 | 99.04 |
| 4 | 101.68 | 101.45 |
| 5 | 99.96 | 101.72 |
| 6 | 100.88 | 101.92 |
| **AVG** | 100.80 | 100.77 |
| **STDDEV** | 0.62 | 1.1 |
| **%RSD** | 0.6 | 1.1 |

**Table - 9. Degradation Data of Daunorubicin and Cytarabine**

| S.NO | Degradation Condition | Daunorubicin | | Cytarabine | |
|---|---|---|---|---|---|
| | | %Drug Degraded | %Drug undegraded | %Drug Degraded | %Drug undegraded |
| 1 | Acid | 5.56 | 94.44 | 4.27 | 95.73 |
| 2 | Alkali | 4.51 | 95.49 | 3.07 | 96.93 |
| 3 | Oxidation | 4.20 | 95.80 | 2.79 | 97.21 |
| 4 | Thermal | 3.73 | 96.27 | 3.85 | 96.15 |
| 5 | UV | 1.04 | 98.96 | 0.37 | 99.63 |
| 6 | Water | 1.04 | 98.96 | 0.94 | 99.06 |

**REFERENCES**


1. B.K Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd edition Goel publication, Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg 13-14, (2004)
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences, Vol.2, Issue 2, Pg 191-196 (2012)
4. Malvia R, Bansal V, Pal O.P and sharma P.K. A Review of High Performance Liquid Chromatography. Journal of Global Pharma Technology (2010)
5. Douglas A Skoog, F.James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760
6. Dr.S.Ravi Shankar, Text book of Pharmaceutical analysis, Fourth edition, Pg 13.1-13.2
7. David G.Watson Pharmaceutical Analysis, A text book for Pharamcy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Edition., Pg 221-232.
8. Remingtonn's The sciences and Practise of Pharamcy, 20th Edition (2000)
9. Connors Ka.A Textbook of Pharmaceutical Analysis, woley inter sciences Inc; Delhi, 3rd Edition, Pg 373-421,(1994)
10. Gurdeep R.Chatwal, Sham K.Anand, Instrumental Methods of Chemical Analysis, Pg 2.566-2.638 (2007)
11. David G.Watson Pharmaceutical Analysis, A textbook for pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Edition., Pg-267-311
12. Nasal A, Siluk D and Kaliszan R Chromatographic Retention Parameters in Medicinal Chemistry and Pharmacology, Pubmed Vol.10, Issue 5 Pg.no-381-426, March (2003)
13. Ashok Kumar, Lalith Kishore, Navpreet Kaur, Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutical sciences, Vol 2, Issue 3, Jul-Sep (2012)
14. Kaushal C, Srivatsava B, A Process of Method Development and Validation of HPLC method. International Research Journal of Pharmaceutical and Applied Sciences, Vol @, Issue 2, 519-545, (2010)
15. Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil, Development and validation of HPLC method. International Research Journal of Pharmaceutical and Applied sciences, Vol 2,Issue 4, Jul-Aug (2012)