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# DEVELOPMENT AND VALIDATION OF SIMPLE, PRECISE AND ACCURATE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAUNORUBICIN AND CYTARABINE IN BULK AND FORMULATION

**Dr. G. Tulja Rani[1]*, Dr. Jabeen Farhana[2] and S. Yousif Ahmed[3]**

[1]Principal, Professor, Pharmaceutical Analysis, Malla Reddy Pharmacy College, Maisammaguda, Dhullapally, Secunderabad, Telangana 500014.

[2]Professor, Pharmaceutical Analysis, Malla Reddy Pharmacy College, Maisammaguda, Dhullapally, Secunderabad, Telangana 500014.

[3]Associate Professor, Pharmaceutical Analysis, Malla Reddy Pharmacy College, Maisammaguda, Dhullapally, Secunderabad, Telangana 500014.

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***Corresponding Author**
**Dr. G. Tulja Rani**
Principal, Professor, Pharmaceutical Analysis, Malla Reddy Pharmacy College, Maisammaguda, Dhullapally, Secunderabad, Telangana 500014.

## ABSTRACT

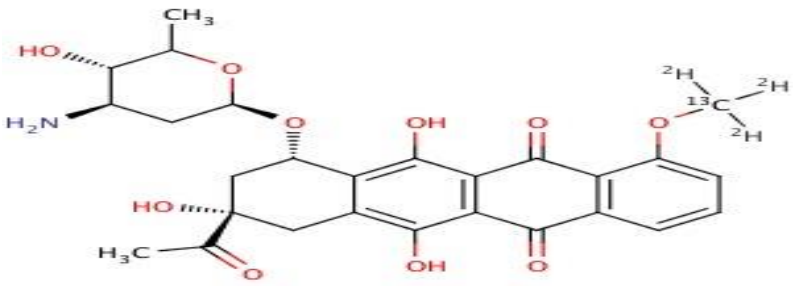
A simple, Accurate, precise method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine in Tablet dosage form. Chromatogram was run through Standard Kromosil (250 x 4.6 mm, 5µ). Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 65:35v/v was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 240.0 nm. Retention time of Daunorubicin and Cytarabine were found to 2.729 min and 2.211. %RSD of the Daunorubicin and Cytarabine were and found to be 0.7 and 0.8 respectively. %Recovery was obtained as 99.99% and 99.54% for Daunorubicin and Cytarabine respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.98, 2.96 and 1.03, 3.12 respectively. Regression equation of Daunorubicin is y = 73448.x+38469, y = 69369x+25775 of Cytarabine. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

**KEYWORDS:** Daunorubicin, Cytarabine, RP-HPLC.

## INTRODUCTION

**Daunorubicin** is a very toxic anthracycline aminoglycoside antineoplastic isolated from Streptomyces peucetius and others, used in treatment of leukemia and other neoplasms. Daunorubicin is an antineoplastic in the anthracycline class. General properties of drugs class include: interaction with DNA in a variety of different ways including intercalation (squeezing between the base pairs), DNA strand breakage and inhibition with the enzyme topoisomerase II. Most of these compounds have been isolated from natural sources and antibiotics. However, they lack the specificity of the antimicrobial antibiotics and thus produce significant toxicity. Anthracyclines are among the most important antitumor drugs available.

Doxorubicin is widely used for the treatment of several solid tumors while daunorubicin and idarubicin are used exclusively for the treatment of leukemia. Daunorubicin may also inhibit polymerase.




**Chemical Structure Of Daunorubicin**

activity, affect regulation of gene expression, and produce free radical damage to DNA. Daunorubicin possesses an antitumor effect against a wide spectrum of tumors, either grafted or spontaneous. anthracyclines are cell cycle the -nonspecific.

**Mechanism of action**

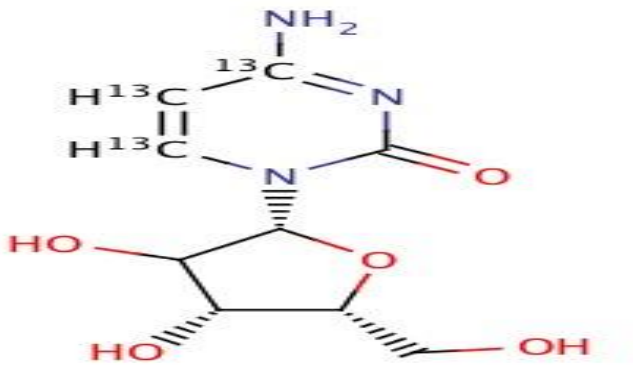
Daunorubicin has antimitotic and cytotoxic activity through a number of proposed mechanisms of action: Daunorubicin forms complexes with DNA by intercalation between base pairs, and it inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the relegation portion of the ligation-religation reaction that

topoisomerase II catalyzes.

**Cytarabine** is a pyrimidine nucleoside analog that is used mainly in the treatment of leukemia, especially acute non-lymphoblastic leukemia. Cytarabine is an antimetabolite antineoplastic agent that inhibits the synthesis of DNA. Its actions are specific for the S phase of the cell cycle. It also has antiviral and immunosuppressant properties. (From Martindale, The Extra Pharmacopoeia, 30th ed, p472)

**Mechanism of action:** Cytarabine acts through direct DNA damage and incorporation into DNA.

Cytarabine is cytotoxic to a wide variety of proliferating mammalian cells in culture. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Although the mechanism of action is not completely understood, it appears that cytarabine acts through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported.




**Chemical Structure Of Cytarabine**

Very few methods are reported in the literature and the proposed liquid chromatographic method was applied for the determination of Daunorubicin & Cytarabine in tablet formulations and the assay values for both the drugs were comparable with the corresponding labeled amount using HPLC for analyzing of Daunorubicin and Cytarabine in bulk and dosage forms. The present research work is to develop a rapid, sensitive, HPLC method for the simultaneous determination of Daunorubicin and Cytarabine in bulk and the proposed HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines.

## MATERIALS AND METHODS

**Table 1: List of equipments.**

| S. No. | Instrument | Model No. | Software | Manufacturer'sName |
|---|---|---|---|---|
| 1 | HPLC Alliance | Waters 2695 | Empower2 | Waters |
| 2 | UV Double Beam Spectrophotometer | | UV Win 6 | PG Instruments T60 |
| 3 | Electronic Balance | | - | Denver |
| 4 | pH meter | | - | BVK enterprises |
| 5 | Ultra Sonicator | | - | BVK enterprises |
| 6 | Suction Pump | | - | Lab India |

**Table 2: List of Chemicals.**

| S. No. | Chemical | Manufacturer | Grade |
|---|---|---|---|
| 1 | Water | Rankem | HPLC Grade |
| 2 | Methanol | Rankem | HPLC Grade |
| 3 | Acetonitrile | Rankem | HPLC Grade |
| 4 | Potassium dihydrogen orthophosphate | Rankem | A.R |
| 5 | Ortho Phosphoric Acid | Rankem | A.R |

**Wavelength detection:** The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Danorubicin and Cytarabine was obtained and the isobestic point of Danorubicin and Cytarabine showed absorbance's maxima at 240nm.

**Method development Preparations and Procedures**

**Preparation of Diluent:** Based up on the solubility of the drugs diluent was selected, Acetonitrile and HPLC water taken in the ratio of 50:50v/v.

**Preparation of Standard stock solutions:** 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 flask and sonicated for 10 minutes. Volume was adjusted upto the mark with diluent. (440µg/ml of Daunorubicin and 1000µg/ml Cytarabine)

**Preparation of Standard working solutions (100% solution):** 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and volume was adjusted upto the mark diluent. (44µg/ml of Daunorubicin and 100µg/ml of Cytarabine).

**Preparation of Sample stock solutions:** 20 tablets were weighed and the average weight of

each tablet was calculated. Weigh equivalent to 44 mg Daunorubicin & 100mg Cytarabine was transferred into a 100ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, further the volume was adjusted upto the mark with diluent and filtered by HPLC filters (440µg/ml of Daunorubicin and 1000µg/ml of Cytarabine).

**Preparation of Sample working solutions (100% solution):** 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and volume was adjusted upto the mark with diluent. (44µg/ml of Daunorubicin and 100µg/ml of Cytarabine).

**Preparation of buffer:** Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of HPLC water added and degassed by sonication and finally the volume was adjusted with HPLC water.

**Preparation of mobile phase:** 0.1% orthophosphoric acid and acetonitrile in the ratio of 50:50v/v was used for separation of these drugs after filtering through 0.45 micro-membrane filter and sonicated each solvent for 10mins.

**Validation**

**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 flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution.

440µg/ml of Daunorubicin and 1000µg/ml Cytarabine).

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

**Linearity**

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

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

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

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

**125% Standard solution:** 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (55µg/ml of Daunorubicin and 8125µg/ml of Cytarabine)

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

**Accuracy**

**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 flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 440µg/ml of Daunorubicin and 1000µg/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:** 1.0ml 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.

**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 change in the result and are within range as per ICH Guide lines.

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 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 solutions 0.1ml each of Daunorubicin, Cytarabine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

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

## RESULTS AND DISCUSSION

**Method development**

**Optimized chromatographic method**

**Chromatographic conditions**

**Mobile phase** **:** 0.1% OPA: Acetonitrile (65:35v/v)
**Flow rate** **:** 1 ml/min
**Column** **:** Kromosil C18 (4.6 x 250mm, 5µm)
**Detector wave length :** 240nm
**Column temperature :** 30°C
**Injection volume** **:** 10µL
**Run time** **:** 5 min
**Diluent** **:** Water and Acetonitrile in the ratio 50:50 v/v

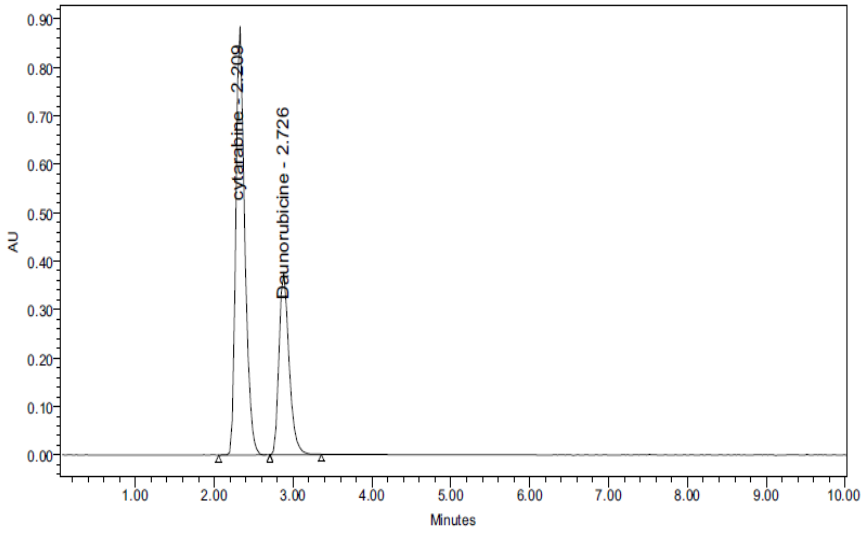



**Fig. 1: Chromatogram of optimized condition.**

**Results:** Both peaks have good Fronting, tailing Factor, theoretical plate count and resolution but retention time of Daunorubicin is in void time. So, further trial is Carried out.

**Observation:** Daunorubicin and Cytarabine were eluted at 2.729 min and 2.211 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

**Method validation**

**Table 3: System suitability parameters for Daunorubicin and Cytarabine.**

| S. no | Daunorubicin | | | Cytarabine | | |
|---|---|---|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing | RT(min) | USP Plate Count | Tailing |
| 111 | 2.727 | 8751 | 1.33 | 2.210 | 6132 | 1.34 |
| 22 | 2.729 | 8835 | 1.27 | 2.211 | 6608 | 1.32 |
| 33 | 2.729 | 9438 | 1.26 | 2.212 | 6529 | 1.28 |
| 44 | 2.731 | 9291 | 1.26 | 2.213 | 6752 | 1.29 |
| 55 | 2.731 | 9692 | 1.27 | 2.213 | 6158 | 1.27 |
| 66 | 2.733 | 9653 | 1.25 | 2.214 | 6449 | 1.28 |

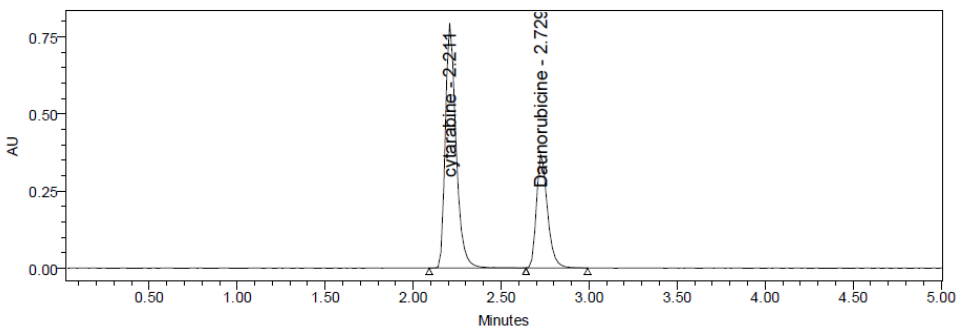



**Fig. 2: System suitability Chromatogram.**

**Discussion:** According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Specificity**

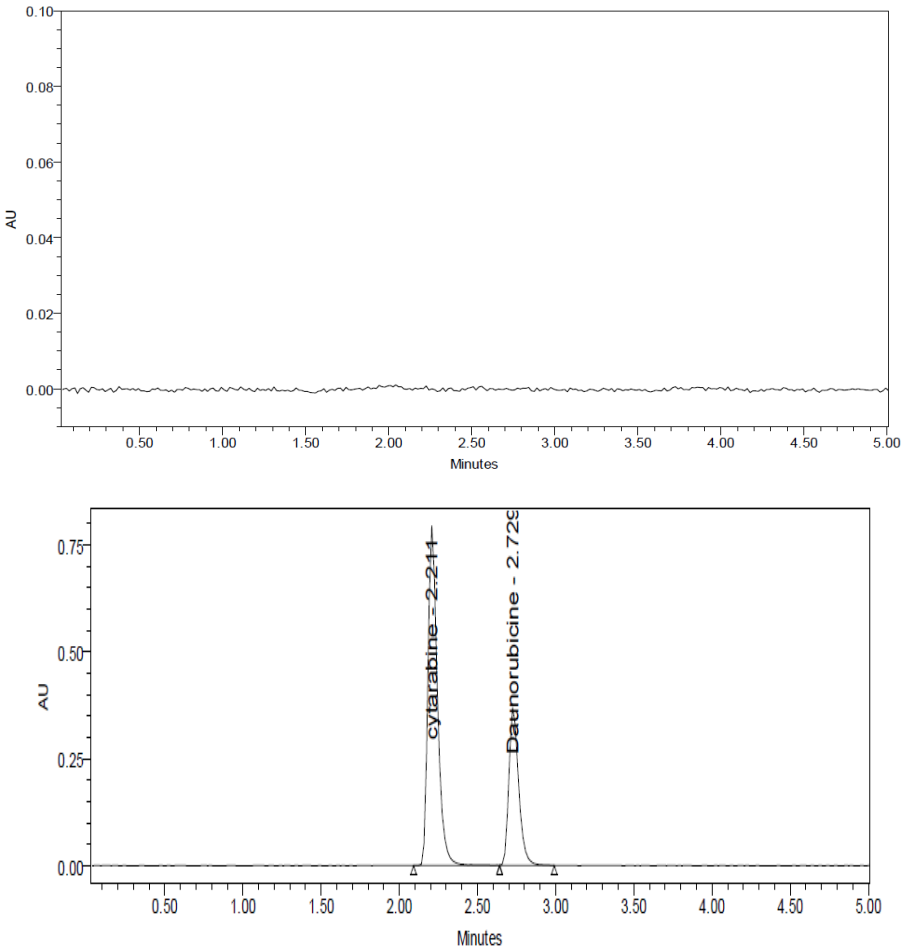



**Fig. 4: Chromatogram of blank & placebo.**

**DISCUSSION**

Retention times of Daunorubicin and Cytarabine were 2.729 min and 2.211 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Linearity:** Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line.

**Table 4: Linearity table for Daunorubicin and Cytarabine.**

| Daunorubicin | | Cytarabine | |
|---|---|---|---|
| Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area |
| 0 | 0 | 0 | 0 |
| 11 | 857023 | 25 | 1654701 |
| 22 | 1692298 | 50 | 3429236 |
| 33 | 2502298 | 75 | 4944236 |
| 44 | 3242163 | 100 | 6706250 |
| 55 | 4039863 | 125 | 8378842 |
| 66 | 4902163 | 150 | 9911094 |

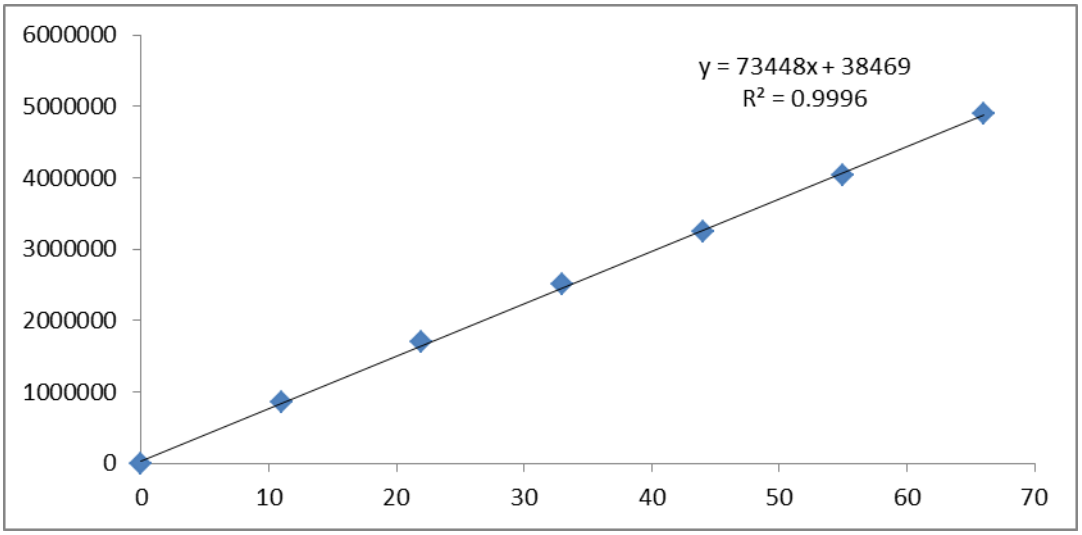



**Fig. 5: Calibration curve of Daunorubicin.**

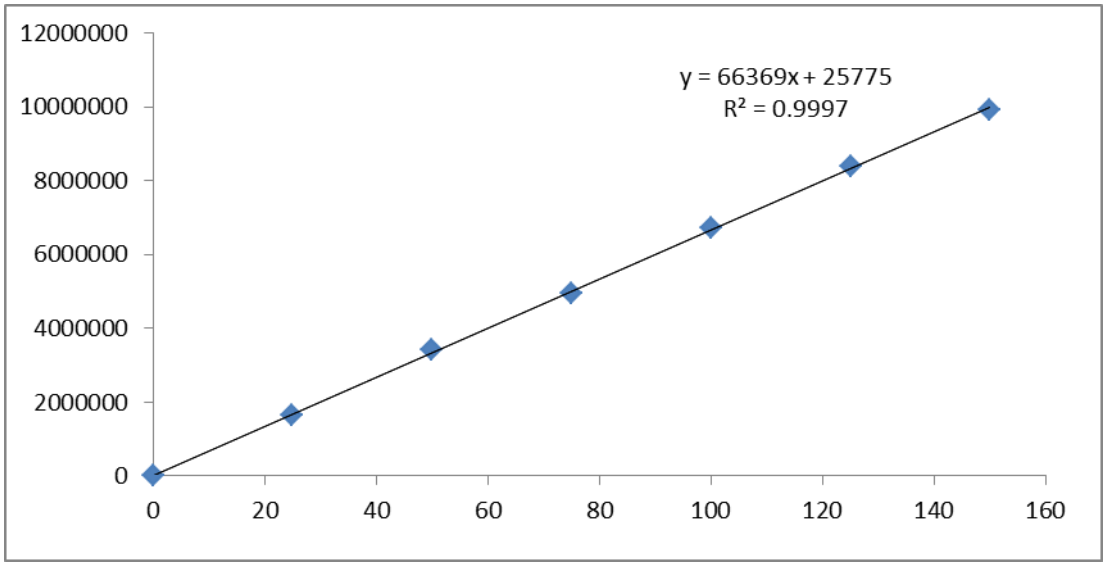



**Fig. 6: Calibration curve of Cytarabine.**

**Discussion:** Six linear concentrations of Daunorubicin (11-66µg/ml) and Cytarabine (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and

linearity equations obtained for Daunorubicin was y = 73448x+38469 and of Cytarabine was y = 66369.x + 25775 Correlation coefficient obtained was 0.999 for the two drugs.

**Precision**

**System Precision**

**Table 5: System precision table of Daunorubicin and Cytarabine.**

| S. No | Area of Daunorubicin | Area of Cytarabine |
|---|---|---|
| 1. | 3291043 | 6640146 |
| 2. | 3221371 | 6695430 |
| 3. | 3274112 | 6677227 |
| 4. | 3267569 | 6730206 |
| 5. | 3255899 | 6662736 |
| 6. | 3260500 | 6788005 |
| Mean | 3261749 | 6698958 |
| S.D | 23285.3 | 53219.1 |
| %RSD | 0.7 | 0.8 |

**DISCUSSION**

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.7% and 0.8% respectively for Daunorubicin and Cytarabine. As the limit of Precision was less than "2" the system precision was passed in this method.

**Repeatability**

**Table 6: Repeatability table of Daunorubicin and Cytarabine.**

| S. No | Area of Daunorubicin | Area of Cytarabine |
|---|---|---|
| 1. | 3238050 | 6643486 |
| 2. | 3262737 | 6598165 |
| 3. | 3228045 | 6642660 |
| 4. | 3239218 | 6716185 |
| 5. | 3291916 | 6690012 |
| 6. | 3288822 | 6757301 |
| Mean | 3258131 | 6674635 |
| S.D | 27461.6 | 57683.3 |
| %RSD | 0.8 | 0.9 |

**DISCUSSION**

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation

and % RSD were calculated for two drugs and obtained as 0.8% and 0.9% respectively for Daunorubicin and Cytarabine. As the limit of Precision was less than "2" the system precision was passed in this method.

**Intermediate precision (Day_ Day Precision)**

**Table 7: Intermediate precision table of Daunorubicin and Cytarabine.**

| S. No | Area of Daunorubicin | Area of Cytarabine |
|---|---|---|
| 1. | 3181063 | 6542246 |
| 2. | 3165261 | 6492430 |
| 3. | 3223112 | 6477627 |
| 4. | 3227269 | 6536206 |
| 5. | 3245899 | 6662736 |
| 6. | 3221500 | 6488205 |
| Mean | 3210684 | 6533242 |
| S.D | 30748.2 | 68768.5 |
| %RSD | 1.0 | 1.1 |

**DISCUSSION**

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 1.0% and 1.1% respectively for Daunorubicin and Cytarabine. As the limit of Precision was less than "2" the system precision was passed in this method.

**Accuracy**

**Table 8: Accuracy table of Daunorubicin.**

| % Level | Amount taken (µg/mL) | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| **50%** | 44 | 66 | 65.76 | 99.64 | 99.73% |
| | | 66 | 66.69 | 101.05 | |
| | | 66 | 65.01 | 98.50 | |
| **100%** | 44 | 88 | 88.74 | 100.83 | 100.43% |
| | | 88 | 87.16 | 99.06 | |
| | | 88 | 89.24 | 101.41 | |
| **150%** | 44 | 110 | 110.16 | 100.1 | 99.8% |
| | | 110 | 110.83 | 101.2 | |
| | | 110 | 107.83 | 98.1 | |

**Table 9: Accuracy table of Cytarabine.**

| % Level | Amount taken (µg/mL | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|---|
| **50%** | 100 | 150 | 149.13 | 99.42 | 99.3% |
| | | 150 | 147.6 | 98.40 | |
| | | 150 | 150.12 | 100.09 | |
| **100%** | 100 | 200 | 198.82 | 99.41 | 99.7% |
| | | 200 | 200.92 | 100.46 | |
| | | 200 | 198.8 | 99.40 | |
| **150%** | 100 | 250 | 246.86 | 98.75 | 99.5% |
| | | 250 | 250.91 | 100.37 | |
| | | 250 | 248.95 | 99.58 | |

## DISCUSSION

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.99% and 99.54% for Daunorubicin and Cytarabine respectively.

### Sensitivity

**Table 10: Sensitivity table of Daunorubicin and Cytarabine.**

| Molecule | LOD | LOQ |
|---|---|---|
| **Daunorubicin** | 0.98 | 2.96 |
| **Cytarabine** | 1.03 | 3.12 |

### Robustness

**Table 11: Robustness data for Daunorubicin and Cytarabine.**

| S.no | Condition | %RSD of Daunorubicin | %RSD of Cytarabine |
|---|---|---|---|
| 1 | Flow rate (-) 0.9ml/min | 0.7 | 1.0 |
| 2 | Flow rate (+) 1.1ml/min | 0.3 | 1.3 |
| 3 | Mobile phase (-) 60B:40A | 0.2 | 0.5 |
| 4 | Mobile phase (+) 70B:30A | 0.1 | 0.3 |
| 5 | Temperature (-) 25°C | 0.5 | 1.2 |
| 6 | Temperature (+) 35°C | 1.0 | 1.7 |

**Discussion:** Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60B:40A), mobile phase plus (70B:30A), 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.

**Assay:** Bearing the label claims Daunorubicin 44mg, Cytarabine 100mg. Assay was

performed with the above formulation. Average 20 tablets were weighed & % Assay for Daunorubicin and Cytarabine obtained was 99.49% and 99.44% respectively.

**Table 12: Assay Data of Daunorubicin.**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 3291043 | 3238050 | 98.88 |
| 2 | 3221371 | 3262737 | 99.63 |
| 3 | 3274112 | 3228045 | 98.57 |
| 4 | 3267569 | 3239218 | 98.91 |
| 5 | 3255899 | 3291916 | 100.52 |
| 6 | 3260500 | 3288822 | 100.43 |
| Avg | 3261749 | 3258131 | 99.49 |
| Stdev | 23285.3 | 27461.6 | 0.84 |
| %RSD | 0.7 | 0.8 | 0.8 |

**Table 13: Assay Data of Cytarabine.**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 6640146 | 6643486 | 98.97 |
| 2 | 6695430 | 6598165 | 98.30 |
| 3 | 6677227 | 6642660 | 98.96 |
| 4 | 6730206 | 6716185 | 100.06 |
| 5 | 6662736 | 6690012 | 99.67 |
| 6 | 6788005 | 6757301 | 100.67 |
| Avg | 6698958 | 6674635 | 99.44 |
| Stdev | 53219.1 | 57683.3 | 0.9 |
| %RSD | 0.8 | 0.9 | 0.9 |

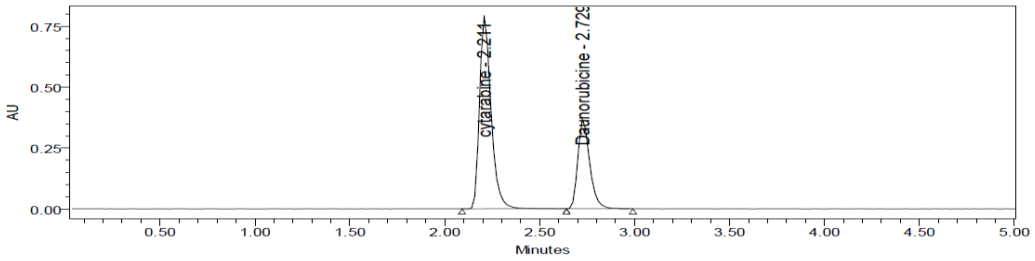



**Fig. 7: Chromatogram of working standard solution.**

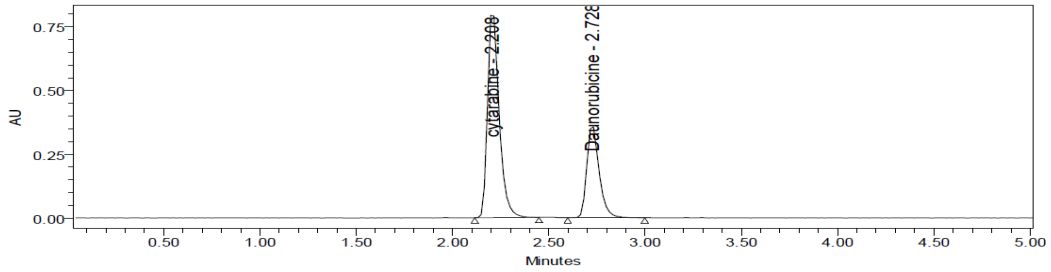



**Fig. 8: Chromatogram of working sample solution.**

## SUMMARY AND CONCLUSION

- A new RP-HPLC method was developed for the simultaneous estimation of Daunorubicin & Cytarabine by trial and error method i.e., by changing column and mobile phase.
- UV overlain spectra of Daunorubicin & Cytarabine shows that both the drugs absorbs appreciably at 240nm was selected as the detection wavelength in liquid chromatography.
- Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained.
- Different mobile phases were tried but, satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase 0.1% OPA: Acetonitrile (65:35).
- The retention time of daunorubicin was found to be 2.729 min and that of cytarabine was found to be 2.211 min respectively.
- Resolution between Daunorubicin & Cytarabine was found to be 0.7% which indicate good separation of both the compounds.
- The asymmetric factor for Daunorubicin & Cytarabine was found to be 1.428 and 1.423.
- The calibration curves for Daunorubicin & Cytarabine was obtained by plotting the respective peak areas versus their concentration over the range of 11-66µg/ml and 25-150µg/ml with correlation coefficient($r^2$)= 0.999 which indicates good correlation exist between conc. and response.
- Detection limit for Daunorubicin & Cytarabine was 0.98 and 1.03µg/ml and quantitation limit was 2.96µg/ml and 3.12µg/ml, which suggest that a nano gram quantity of both the compounds can be estimated accurately, the low values indicates that the method is sensitive.
- The results Daunorubicin & Cytarabine for stability studies revealed that the retention time and peak area of remained almost unchanged and no significant degradation was observed within the indicated period.
- The %Recovery was obtained as 99.99% and 99.54% Daunorubicin & Cytarabine for respectively. The % RSD was found to be less than 2%, which shows that the method is precise.
- The proposed liquid chromatographic method was applied for the determination of Daunorubicin & Cytarabine in tablet formulations and the assay values for both the drugs were comparable with the corresponding labeled amount.

## CONCLUSION

The proposed RP-HPLC developed method is found to be simple, precise accurate, economical & cost effective compare to the existing method. It can be used in any laboratory for the estimation of the simple, accurate, precise of the formulation of the dosage drugs, dissolution studies and quality control.

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