ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 5, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

# A STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DAUNORUBICIN AND CYTARABINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

**AKKALA SAI VENEELA[1], GALLA RAJITHA[2], ASHRITHA NARIKIMALLI[1]***

**[1]Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences and Technologies, JNTUK, Kakinada, Andhra Pradesh, India. [2]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India. Email: ashrithanarikimalli@yahoo.com**

***Received: 24 November 2022, Revised and Accepted: 04 March 2022***

**ABSTRACT**

**Objective:** A simple, accurate, and precise method was developed for the simultaneous estimation of daunorubicin and cytarabine dosage form using reverse-phase HPLC and validated with different parameters such as accuracy, precision, repeatability, linearity, limit of detection (LOD), and limit of quantitation (LOQ) as per ICH Q2R1 guidelines.

**Methods:** The chromatogram was run through Agilent C18 Column of dimensions 150×4.6 mm, 5 m. Mobile phase containing 0.01N $KH_2PO_4$: Methanol taken in the ratio 50:50 was pumped through the column at a flow rate of 1.0 ml/min. The temperature was maintained at 30°C. The optimized wavelength selected was 240 nm.

**Results:** Retention times for daunorubicin and cytarabine were found to be 2.433 min and 3.045 min. The %RSD of the daunorubicin and cytarabine was found to be 0.7 and 0.4, respectively. The %Recovery was obtained as 99.96% and 100.40% for daunorubicin and cytarabine, respectively. LOD and LOQ values obtained from regression equations of daunorubicin and cytarabine were 0.08 µg/ml, 0.24 µg/ml and 0.94 µg/ml, 2.86 µg/ml, respectively. The regression equation for daunorubicin is y=28587x+3141 and y=35995x+37534 for cytarabine.

**Conclusion:** The method developed was simple and economical that can be adopted in regular quality control tests in industries.

**Keywords:** Daunorubicin and cytarabine, RP-HPLC, Validation, Stability studies.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i5.43682. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

## INTRODUCTION

Daunorubicin, also known as daunomycin, is a chemotherapy medication used to treat cancer. Specifically, it is used for acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and Kaposi's sarcoma [1]. Cytarabine, also known as cytosine arabinoside (ARA-C), is a chemotherapy medication used to treat acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma [2].

HPLC is an accurate and sensitive method used for the quantitative analysis of several drugs [3,4]. The literature shows a few methods for simultaneous estimation of daunorubicin and cytarabine [5-10]. The present study aimed to develop and validate an economical and effective HPLC method for the simultaneous determination with good linearity and sensitivity for both drugs which could be used in quality control analysis (Fig. 1).

## EQUIPMENT AND CHEMICALS

The API daunorubicin and cytarabine were from MSN Pharma Limited, Hyderabad. The marketed formulation VYXEOUS (Cytarabine 100 mg and Daunorubicin 44 mg), MSN Pharma Limited, Hyderabad, India was used. Phosphate buffer, methanol, potassium dihydrogen orthophosphate, and orthophosphoric acid were from Rankem Chemicals, Haryana, India. Denver Electronic balance, BVK Enterprise pH Meter and Ultrasonicator, Thermo Scientific Hot air oven and Refrigerator, Millipore BM2EA9672R, T60 UV-Visible spectroscopy, Waters 2695 HPLC with Empower 2 software, Autoinjector, and PDA detector were used.

## METHODS

### Selection of detection wavelength

An ideal wavelength was used to get a good response for the analyte. Initially, daunorubicin and cytarabine were individually dissolved in diluent to scan the spectra on UV-visible spectrophotometer in the range of 200 nm–400 nm against diluent as blank. From the results, simultaneous estimation of three spectra's showed stable and maximum absorbance at 240 nm.

### Preparation of solutions

*Preparation of buffer: 0.01N Potassium dihydrogen orthophosphate ($KH_2PO_4$)*

1.36 g of Potassium dihydrogen orthophosphate was added to 900 ml milli-Q water in a 1000 ml volumetric flask and sonicated. The final volume is made up with water. 1 ml of triethylamine was added and pH was adjusted to 3.8 with dil. orthophosphoric acid solution.

*Preparation of diluent*

Preparation of diluent: Mixture of 0.01N $KH_2PO_4$ (Mobile phase A) and methanol (Mobile phase B) in the ratio of 50:50 v/v.

*Preparation of standard stock solutions*

11 mg of daunorubicin and 25 mg of cytarabine were transferred to 25 ml volumetric flasks individually and 3/4$^{th}$ of diluents were added to the flasks and sonicated for 10 min. Volumes were made up with diluent and labeled as standard stock solution. (440 µg/ml of daunorubicin and 1000 µg/ml cytarabine).

*Preparation of standard working solution*

1 ml from each stock solution was pipetted 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 solution*

1 vial equivalent to 44 mg daunorubicin and 100mg cytarabine was transferred into a 100 ml volumetric flask. 50 ml of diluent was added and sonicated for 25 min. Further, the volume was made up with diluent and filtered with HPLC filters (440 µg/ml of daunorubicin and 1000 µg/ml of cytarabine).

*Preparation of sample working solution*

1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (44 µg/ml of daunorubicin and 100 µg/ml of cytarabine).

**Optimization of chromatographic conditions**

HPLC method was developed and standardized for the analysis of daunorubicin and cytarabine. In the optimization process, various systems of stationary phase and mobile phase with several combinations were tested. Finally, acceptable retention times, good resolution, and theoretical plates were observed with Agilent $C_{18}$ (4.6×250 mm, 5 µm) column and 0.01N $KH_2PO_4$ and methanol (50:50) at pH 3.8 at a flow rate of 1.0 ml/min. Optimized chromatographic conditions are mentioned in Table 1. and the optimized chromatogram is shown in Fig. 2. Validation and stability studies of the optimized method were performed according to the ICH guidelines.

**Method validation**

Validation was performed as per the ICH Q2B (R2) guidelines [11-13]. The method was validated for the parameters such as system suitability, specificity, linearity, precision (system precision and repeatability), accuracy, limit of detection, limit of quantification, robustness, and assay. Stability studies such as acid degradation, base degradation, oxidative degradation, thermal degradation, photostability degradation, and aqueous degradation were carried out as per ICH guidelines [14,15].

**Table 1: Optimized chromatographic conditions**

| Serial number | Parameter | Condition |
|---|---|---|
| 1 | Mobile phase | 50% 0.01N $KH_2PO_4$: 50% methanol |
| 2 | Diluent | 50% 0.01N $KH_2PO_4$: 50% methanol |
| 3 | Column | Agilent $C_{18}$ (4.6 mm×250 mm, 5 µm) |
| 4 | Wavelength | 240 nm |
| 5 | Column temperature | 30°C |
| 6 | Injection volume | 10 µl |
| 7 | Flow rate | 1.0 ml/min |
| 8 | Run time | 5 min |
| 9 | Retention time | 2.433 min (Daunorubicin)<br>3.045 min (Cytarabine) |

*System suitability*

It is performed to verify that the analytical system is working properly and can give accurate and precise results. Standard solution of daunorubicin (44 ppm) and Cytarabine (100 ppm) was injected 6 times and the parameters such as resolution, peak tailing, and USP plate count were determined. The chromatogram was presented in Fig. 3 and results of system suitability were shown in Table 2. According to the ICH guidelines, plate count should be more than 2000, tailing factor should be <2 and resolution must be more than 2. All the system suitability parameters were passed and were within the limits.

*Specificity*

The specificity of the method is performed by separately injecting the blank, placebo, and sample solutions. The interference observed (if any) at the retention times of each analyte in all the chromatograms is evaluated. Chromatograms are shown in Figs. 4-6. Retention times of daunorubicin and cytarabine were 2.433 min and 3.045 min, respectively. The method is specific as no interfering peaks were observed in blank and placebo at retention times of the drugs.

*Linearity*

Standard solutions of 25%, 50%, 75%, 100%, 125%, and 150% concentrations were prepared by taking 0.25, 0.5, 0.75, 1.0, 1.25,

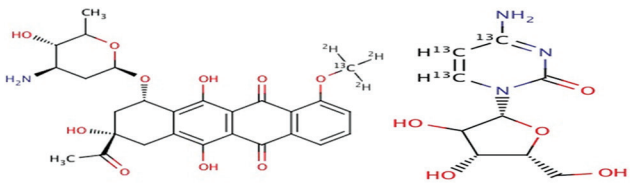

**Fig. 1: (a) Structure of daunorubicin [(8S,10S)-8-acetyl-10-{[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy}-6,8,11-trihydroxy-1-methoxy-5,7,8,9,10,12-hexahydrotetracene-5,12-dione] [11]; b) Structure of cytarabine [4-amino-1-[(2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-1,2-dihydropyrimidin-2-one] [12]**

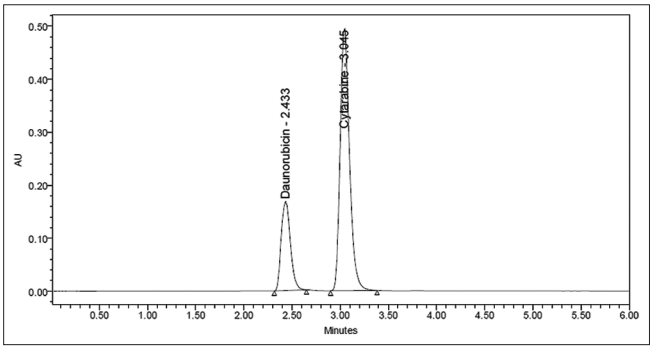


**Fig. 2: Optimized chromatogram**

**Table 2: System suitability parameters for daunorubicin and cytarabine**

| Serial number | Injection | | | | | | |
|---|---|---|---|---|---|---|---|
| | Daunorubicin | | | Cytarabine | | | |
| | Rt (min) | USP plate count | Tailing | Rt (min) | USP plate count | Tailing | Resolution |
| 1 | 2.410 | 3220 | 1.20 | 3.013 | 5269 | 1.25 | 3.6 |
| 2 | 2.423 | 3426 | 1.23 | 3.030 | 5456 | 1.25 | 3.6 |
| 3 | 2.424 | 3400 | 1.22 | 3.031 | 5393 | 1.25 | 3.6 |
| 4 | 2.425 | 3338 | 1.22 | 3.031 | 5408 | 1.25 | 3.6 |
| 5 | 2.425 | 3364 | 1.20 | 3.031 | 5432 | 1.25 | 3.6 |
| 6 | 2.427 | 3311 | 1.19 | 3.032 | 5443 | 1.25 | 3.6 |

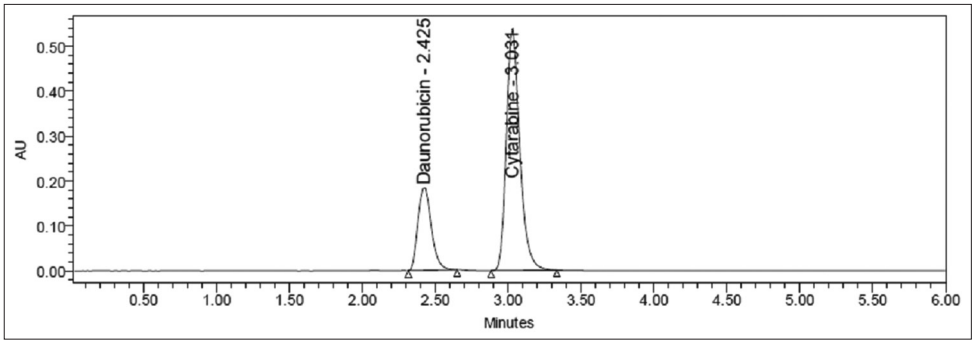


**Fig. 3: Chromatogram for system suitability**

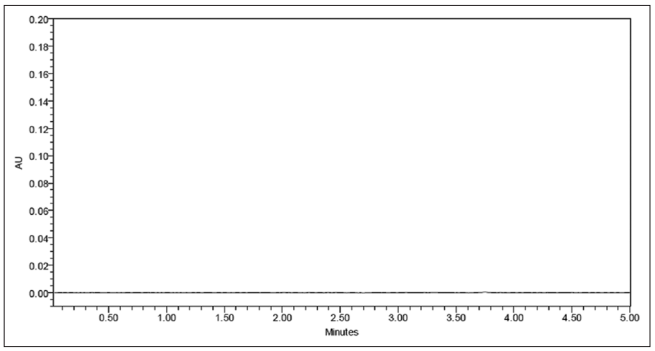


**Fig. 4: Chromatogram of blank**

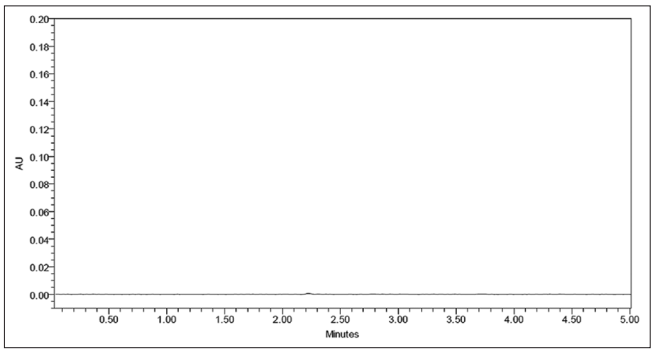


**Fig. 5: Chromatogram of placebo**

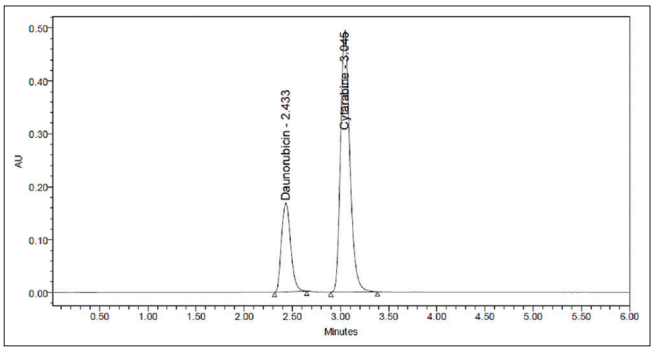


**Fig. 6: Typical chromatogram**

and 1.5 ml each from two standard stock solutions and made up to 10 ml. Six linear concentrations of daunorubicin (11–66 µg/ml) and cytarabine (25–150 µg/ml) were injected in a duplicate manner. Peak areas were recorded for each injected concentration and the calibration curves – concentration versus peak area were constructed Figs. 7 and 8. The results are given in Table 3. Linearity equations

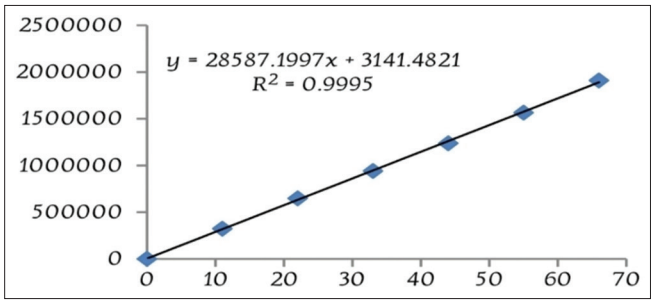


**Fig. 7: Calibration curve of daunorubicin**

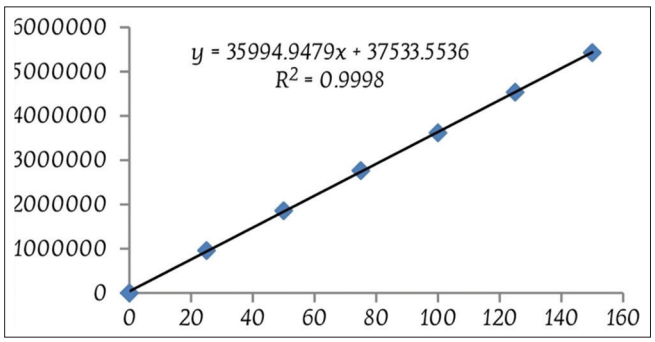


**Fig. 8: Calibration curve of cytarabine**

**Table 3: Results for linearity of daunorubicin and cytarabine**

| Daunorubicin | | Cytarabine | |
|---|---|---|---|
| **Concentration (µg/ml)** | **Peak area** | **Concentration (µg/ml)** | **Peak area** |
| 0 | 0 | 0 | 0 |
| 11 | 322962 | 25 | 958464 |
| 22 | 648201 | 50 | 1857576 |
| 33 | 941175 | 75 | 2763833 |
| 44 | 1237618 | 100 | 3616994 |
| 55 | 1565668 | 125 | 4535677 |
| 66 | 1910010 | 150 | 5427540 |

obtained for daunorubicin and cytarabine were y=28587x+3141 and y=35995x+37534. The correlation coefficient obtained was 0.999 for both drugs.

*Precision*

System precision

System precision was determined by injecting 15 µl standard working solution 6 times and the chromatograms were recorded. Average area, standard deviation, and %RSD were calculated for the two drugs and

**Table 4: Results for system precision and repeatability of daunorubicin and cytarabine**

| Serial number | System precision | | Repeatability | |
|---|---|---|---|---|
| | Area of daunorubicin | Area of cytarabine | Area of daunorubicin | Area of cytarabine |
| 1 | 1276435 | 3551797 | 1268366 | 3609348 |
| 2 | 1269906 | 3539751 | 1281255 | 3581141 |
| 3 | 1264947 | 3524435 | 1271755 | 3599632 |
| 4 | 1276927 | 3561195 | 1266393 | 3531947 |
| 5 | 1271333 | 3529081 | 1270972 | 3578407 |
| 6 | 1252996 | 3530147 | 1271099 | 3548987 |
| Mean | 1268757 | 3539401 | 1271640 | 3574910 |
| SD | 8906.0 | 14456.0 | 5127.4 | 29550.1 |
| RSD (%) | 0.7 | 0.4 | 0.4 | 0.8 |

SD: Standard deviation

results are shown in Table 4. The %RSD obtained was 0.7% and 0.4%, respectively, for daunorubicin and cytarabine. As the limit of precision was <2, the method was precise.

*Repeatability*

Repeatability (method precision) was determined by multiple sampling from a sample stock solution and six working sample solutions of the same concentrations were prepared. 15 µl injection from each working sample solution was given and their peak areas are mentioned in Table 4. Average area, standard deviation, and % RSD were calculated for the two drugs and obtained as 0.4% and 0.8%, respectively, for daunorubicin and cytarabine. As the limit of precision was <2, the method was repeatable.

*Accuracy*

Three levels (50%, 100%, and 150%) of accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy. The results are shown in Table 5 and 6. The mean % recovery was obtained as 99.96% and 100.04% for daunorubicin and cytarabine, respectively.

*Limit of detection and limit of quantification*

LOD and LOQ were calculated based on signal to noise ratio. The results are shown in Table 7.

*Robustness*

The method was performed in duplicate under varied conditions of flow rate (±0.1 ml/min), mobile phase compositions (±5.0 ml of both organic components), and column temperature (±5°C) and results are given in Table 8. System suitability parameters were not affected and %RSD was within the limit. Hence, the method was considered to be robust.

*Assay*

Assay was performed with Vyxeous, bearing the label claim cytarabine 100mg and daunorubicin 44 mg. 20 µl of the standard and sample working solutions were injected into chromatographic system and areas for daunorubicin and cytarabine were measured and results are shown in Table 9. The average % assay for daunorubicin and cytarabine was found to be 99.83% and 100.60%, respectively.

*Stability studies*

Acid degradation studies

1 ml 2N hydrochloric acid was added to 1 ml stock solution of daunorubicin and cytarabine, refluxed for 30 min at 60°C. Resultant solution was diluted to obtain 44 µg/ml and 100 µg/ml solution and 10 µl solution was injected into the system to assess the stability of the sample. The results are given in Table 10.

**Table 5: Accuracy results for daunorubicin**

| Level (%) | Amount spiked (µg/ml) | Amount recovered (µg/ml) | Recovery (%) | Mean recovery (%) |
|---|---|---|---|---|
| 50 | 22 | 22.40 | 101.82 | 99.96 |
| | 22 | 22.16 | 100.73 | |
| | 22 | 21.80 | 99.09 | |
| 100 | 44 | 43.66 | 99.23 | |
| | 44 | 43.85 | 99.66 | |
| | 44 | 43.81 | 99.57 | |
| 150 | 66 | 66.27 | 100.41 | |
| | 66 | 65.80 | 99.70 | |
| | 66 | 65.63 | 99.44 | |

**Table 6: Accuracy results for cytarabine**

| Level (%) | Amount spiked (µg/ml) | Amount recovered (µg/ml) | Recovery (%) | Mean recovery (%) |
|---|---|---|---|---|
| 50 | 50 | 49.76 | 99.52 | 100.04 |
| | 50 | 49.71 | 99.42 | |
| | 50 | 50.06 | 100.12 | |
| 100 | 100 | 99.80 | 99.80 | |
| | 100 | 100.51 | 100.51 | |
| | 100 | 99.96 | 99.96 | |
| 150 | 150 | 150.02 | 100.01 | |
| | 150 | 150.48 | 100.32 | |
| | 150 | 151.05 | 100.70 | |

**Table 7: Results for limit of detection and limit of quantitation of daunorubicin and cytarabine**

| Molecule | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| Daunorubicin | 0.08 | 0.24 |
| Cytarabine | 0.94 | 2.86 |

LOD: Limit of detection, LOQ: Limit of quantitation

**Table 8: Results for robustness of daunorubicin and cytarabine**

| Serial number | Condition | RSD of Daunorubicin (%) | RSD of Cytarabine (%) |
|---|---|---|---|
| 1 | Flow rate (−) 0.90ml/min | 1.4 | 1.2 |
| 2 | Flow rate (+) 1.1ml/min | 0.4 | 0.3 |
| 3 | Mobile phase (−) 55B: 45A | 1.1 | 0.6 |
| 4 | Mobile phase (+) 45B: 55A | 0.9 | 0.6 |
| 5 | Temperature (−) 25°C | 0.9 | 0.3 |
| 6 | Temperature (+) 35°C | 0.2 | 1.1 |

**Table 9: Results for assay of daunorubicin and cytarabine**

| Serial number | Daunorubicin | | | Cytarabine | | |
|---|---|---|---|---|---|---|
| | Standard area | Sample area | Assay (%) | Standard area | Sample area | Assay (%) |
| 1 | 1276435 | 1268366 | 99.4 | 3551797 | 3609348 | 101.6 |
| 2 | 1269906 | 1281255 | 100.9 | 3539751 | 3581141 | 101.2 |
| 3 | 1264947 | 1271755 | 100.5 | 3524435 | 3599632 | 102.1 |
| 4 | 1276927 | 1266393 | 99.2 | 3561195 | 3531947 | 99.2 |
| 5 | 1271333 | 1270972 | 100.0 | 3529081 | 3578407 | 101.4 |
| 6 | 1252996 | 1271099 | 101.4 | 3530147 | 3548987 | 100.5 |
| Average | 1268757 | 1271640 | 100.2 | 3539401 | 3574910 | 101 |
| SD | 8906.0 | 5127.4 | 0.90 | 14456.0 | 29550.1 | 1.0 |
| RSD (%) | 0.7 | 0.4 | 0.9 | 0.4 | 0.8 | 1.0 |

SD: Standard deviation

**Table 10: Degradation data of daunorubicin and cytarabine**

| S.NO | Degradation condition | Daunorubicin | | | Cytarabine | | |
|---|---|---|---|---|---|---|---|
| | | Area | % Recover | % Drug degraded | Area | % Recover | %Drug degraded |
| 1 | Acid | 1188333 | 93.29 | 6.71 | 3201132 | 90.08 | 9.92 |
| 2 | Alkali | 1206724 | 94.73 | 5.27 | 3373199 | 94.92 | 5.08 |
| 3 | Oxidation | 1230423 | 96.59 | 3.41 | 3249490 | 91.44 | 8.56 |
| 4 | Thermal | 1246892 | 97.88 | 2.12 | 3388688 | 95.36 | 4.64 |
| 5 | UV | 1253103 | 98.37 | 1.63 | 3443155 | 96.89 | 3.11 |
| 6 | Water | 1262922 | 98.37 | 1.63 | 3535415 | 99.49 | 0.51 |

Alkali degradation studies

1 ml 2N NaOH was added to 1 ml stock solution of daunorubicin and cytarabine, and refluxed for 30 min at 60°C. The resultant solution was diluted to obtain 44 µg/ml and 100 µg/ml solution and 10 µl solution was injected into the system to assess the stability of the sample. The results are given in Table 9.

Oxidative degradation studies

1 ml 20% $H_2O_2$ was added to 1 ml of stock solution of daunorubicin and cytarabine. The solutions were kept for 30 min at 60°C. The resultant solution was diluted to obtain 44 µg/ml and 100 µg/ml solution and 10 µl was injected into the system to assess the stability of sample. The results are given in Table 9.

Thermal degradation studies

The standard drug solution was placed in an oven at 105°C for 1 h to study dry heat degradation. The resultant solution was diluted to 44 µg/ml and 100 µg/ml solution and 10 µl was injected into the system to assess the stability of the sample. The results are given in Table 9.

Photostability studies

The photochemical stability of the drug was studied by exposing 440 µg/ml daunorubicin and 1000 µg/ml cytarabine solution to UV light by keeping the beaker in UV Chamber for 1 day. The resultant solution was diluted to obtain 44 µg/ml and 100 µg/ml solution and 10 µl was injected into the system to assess the stability of the sample. The results are given in Table 9.

*Aqueous degradation studies*

Stress testing under neutral conditions was studied by refluxing the drug in water for 1 h at 60°C [16-19]. The resultant solution was diluted to 44 µg/ml and 100 µg/ml and 10 µl was injected into the system to assess the stability of the sample. The results are given in Table 9.

## CONCLUSION

A simple, accurate, and precise method was developed for the simultaneous estimation of the daunorubicin and cytarabine in tablet dosage form. Retention times for daunorubicin and cytarabine were found to be 2.433 min and 3.045 min. %RSD of the daunorubicin and cytarabine were found to be 0.7 and 0.4, respectively. %Recovery was obtained as 99.96% and 100.60% for daunorubicin and cytarabine, respectively. LOD and LOQ values obtained from regression equations of daunorubicin and cytarabine were 0.08 µg/ml, 0.24 µg/ml and 0.94 µg/ml, 2.86 µg/ml, respectively. The regression equation for daunorubicin is y=28587x+3141 and y=35995x+37534 for cytarabine. Compared to other methods, the retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control tests in industries.

## ACKNOWLEDGMENT

The author wish to thank MSN Pharma Limited, Hyderabad, for providing the facility to complete the work.

## AUTHORS CONTRIBUTION

Sai Veneela performed the work and Ashritha helped in drafting and editing the journal. Rajitha is the mentor.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR FUNDING

None.

## REFERENCES

1. Chemocare Place Unknown. Available from: https://www.chemocare.com/chemotherapy/drug-info/daunorubicin.aspx
2. Place Unknown. Available from: https://www.chemocare.com/chemotherapy/drug-info/cytarabine.aspx
3. Shabir GA. HPLC method development and validation for pharmaceutical analysis. Pharm Technol Eur 2004;16:1-5.
4. Vidushi Y, Meenakshi B. A review in HPLC method development and validation. Res J Life Sci Bioinform Pharm Chem Sci 2017;2:166-78.
5. Varanasi SN, Murthy AR, Pravallika KE, Rani AP, Rahaman SA. Development and validation of RP-HPLC method for estimation of cytarabine in bulk and pharmaceutical dosage forms. IJPSR 2013;4:4573-76.
6. Available from: https://www.go.drugbank.com/drugs/DB00694
7. Available from: https://www.go.drugbank.com/drugs/DB00987
8. Validation of Analytical Procedures: Text and Methodology. Vol. Q2. 2005; Geneva, Switzerland: ICH Guidelines. p. R1.
9. Rawat T, Pandey IP. Forced degradation studies for drug substances and

drug products-scientific and regulatory considerations. J Pharm Sci Res 2015;7:238-41.
10. Charde MS, Kumar J, Welankiwar AS, Chakole RD. Development of forced degradation studies of drugs. Int J Adv Pharm 2013;2:34-9.
11. Skoog AD, Holler FJ, Crouch RS. An Introduction to Chromatographic Separations. Instrumental Analysis. India: Cengage Learning: 2012. p. 836-9.
12. Khopkar SM. Principles of Analytical Chemistry. Basic Concepts of Analytical Chemistry. 3rd ed. New Delhi: New Age International Publishers; 2012. p. 111-25.
13. Skoog AD, Holler FJ, Crouch RS. Liquid Chromatography. Instrumental Analysis. India: Cengage Learning; 2012. p. 893-904.
14. Khopkar SM. High Performance Liquid Chromatography. Basic Concepts of Analytical Chemistry. 3rd ed. New Delhi: New Age International Publishers; 2012. p. 197-204.
15. Gupta V, Jain AD, Gill NS, Kapil. Development and validation of HPLC method. Int Res J Pharm Appl Sci 2012;2:1-5.
16. Hokanson GC. A life cycle approach to the validation of analytical methods during pharmaceutical product development. Part 1: The initial validation process. Pharm Technol 1994;21:92-100.
17. Green JM. A practical guide to analytical method validation. Anal Chem. 1996;68:305-9.
18. Aswathy SR, Muhas C, Sruthy AS, Devi Swapna PV, Gopinath U. Validation and application of RP-HPLC method for quantification of Enrofloxacin in pure and veterinary dosage forms. IJPPS 2021;14:42-47.
19. Pradhan N, Rajkhowa H, Giri H, Shrestha B. Simultaneous spectrophotometric estimation of moxifloxacin hydrochloride and doxorubicin hydrochloride. IJPPS 2015;7:21-6.