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Research Article

A VALIDATED ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF CYTARABINE AND DAUNORUBICIN IN BULK AND INFUSION FORMULATION BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The novel liposomal infusion formulation of cytarabine and daunorubicin liposomal infusion is considered as new hope in acute myeloid leukemia treatment. The objective of the present study is to develop and validate a simple, rapid, accurate, precise and sensitive reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of cytarabine and daunorubicin in bulk and infusion formulation.

Methods: The chromatographic separation of the drugs was achieved on Denali C18 (250 mm×4.6 mm, 5 µm) in isocratic mode with mobile phase consisting of water (pH was adjusted to 3):acetonitrile in the ratio of 55:45 with a flow rate of 1 ml/min at a detection wavelength of 240 nm using photodiode array (PDA) detector. The column temperature was set at 30°C with 10 µl injection volume. The proposed method was validated as per the International council for Harmonisation (ICH) guidelines.

Results: The retention times for cytarabine and daunorubicin were found to be 2.323±0.12 min and 3.140±0.16 min, respectively. Linearity (r^2=0.999) was observed over a concentration range of 16.2–97.5 µg/ml for cytarabine and 7.2–43.5 µg/ml for daunorubicin. The percentage relative standard deviation (RSD) for precision studies was found to be 0.2 for both the drugs.

Conclusion: A simple, rapid, economic, accurate, and precise RP-HPLC method was developed for simultaneous quantitative estimation of cytarabine and daunorubicin, and the method was validated as per the ICH guidelines. Hence, the method can be employed for the routine analysis of cytarabine and daunorubicin in bulk and infusion formulation.

Keywords: Cytarabine, Daunorubicin, Reverse-phase high-performance liquid chromatographic, Method development, Method validation.

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INTRODUCTION

Cytarabine (Fig. 1) is a pyrimidine nucleoside analog used mainly in the treatment of leukemia, especially acute non-lymphoblastic leukemia, acute myelogenous leukemia, and meningeal leukemia. It is chemically 4-amino-1-[(2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyrimidin-2-one. It has moderate emetogenicity which has been managed with antiemetic drugs [1].

Daunorubicin (Fig. 2) is an anthracycline antibiotic that has antineoplastic activity and is used for the treatment of acute leukemia and acquired immune deficiency syndrome-related Kaposi sarcoma. It is chemically (1S,3S)-3-Acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydro-1-tetracenyl 3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoside. It is one among six Topo II inhibitors which is prescribed as highly antineoplastic drug in clinical use [2].

A detailed literature survey revealed that there were liquid chromatographic methods for the estimation of daunorubicin in bulk and pharmaceutical formulation [3-6]. An ultraviolet spectrophotometric method was reported for the determination of cytarabine [7]. Few reverse-phase high-performance liquid chromatography (RP-HPLC) methods were published for the estimation of cytarabine in bulk and pharmaceutical formulation [8-10]. In the above reported methods developed for the individual estimation of daunorubicin and cytarabine, they used buffers and a combination of three solvents as mobile phases. Usage of buffers in separation chromatography decreases the life of the column and complex mobile phase usage will increase the time of analysis. Hence, a method which uses simple mobile phase is needed for the estimation of drugs. In addition, this no analytical method has been reported for the simultaneous estimation of cytarabine and daunorubicin. This work accomplishes the criteria as a simple and rapid RP-HPLC method was developed using a simple mobile phase with shorter run time. The developed method was validated as per the validation of analytical procedures, i.e. ICH guidelines: Q2(R1) Validation of Analytical Procedures: Text and Methodology [11].

METHODS

Chemicals and reagents

Pharmaceutical grade cytarabine and daunorubicin were provided as gift samples by Spectrum Pharma Research Solutions, Hyderabad, and the marketed formulation is available as Vyxeos liposome for injection (cytarabine-65 mg and daunorubicin-29 mg per vial), for intravenous use. The formulation is not available in India. Hence, the injection was prepared with the available excipients in the laboratory using evaporation method and was extracted with the mobile phase during the study. Acetonitrile, orthophosphoric acid, and HPLC grade water were purchased from Merck.

Instrument

Chromatography was performed on Waters HPLC 2695 equipped with PDA detector. The chromatographic separation was performed using Denali C18 (250 mm×4.6 mm, 5 µm). The data acquisition and integration were performed using Empower 2 software.

Chromatographic conditions

The developed method used a reverse-phase octadecyl column, Denali C18 (250 mm×4.6 mm, 5 µm), with a mobile phase consisting of water (pH adjusted to 3 with orthophosphoric acid):acetonitrile (55:45) which was pumped at a flow rate of 1.0 ml/min and a detection wavelength of 240 nm using PDA detector.

Preparation of diluent

The diluent used was water and acetonitrile in the ratio of 50:50.

Preparation of standard solutions

The standard stock solution of cytarabine and daunorubicin was prepared by accurately weighing 16.25 mg of cytarabine and 7.25 mg of daunorubicin pure drugs and was transferred into a 25-ml clean dry volumetric flask, and then, 10 ml diluent was added and sonicated for 10 min and made up to the final volume with diluent. From the above stock solution, 1 ml was taken into a 10-ml volumetric flask and made up to the volume with diluent so as to get the final concentration of 65 µg/ml of cytarabine and 29 µg/ml of daunorubicin.

Preparation of sample solutions

The sample stock solution of cytarabine and daunorubicin was prepared by taking volume of infusion equivalent to 65 mg of cytarabine and 29 mg of daunorubicin, transferred into a 100-ml volumetric flask, and then, 50 ml of diluent was added and vertexed for 25 min; further, the volume was made up with diluent and filtered by HPLC filters. 1 ml of filtered sample stock solution was transferred to 10-ml volumetric flask and made up with diluent.

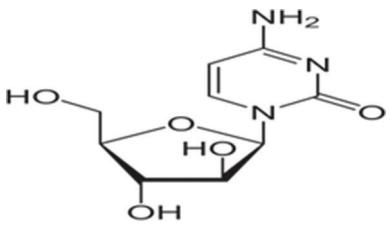

Fig. 1: Structure of cytarabine

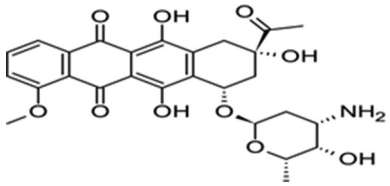

Fig. 2: Structure of daunorubicin

Validation of the developed method

The proposed analytical method was validated for accuracy, precision, linearity and range, limit of detection (LOD) and limit of quantitation (LOQ) in accordance with the ICH guidelines for analytical procedures Q2(R1).

Accuracy

Accuracy of the developed method has been carried out by recovery studies by applying the standard addition method. A known quantity of drug substance corresponding to 50%, 100%, and 150% of the label claim of drug was added to pre-determined sample solution. Each set of addition was repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay.

Precision

The precision of the developed analytical method was carried out at target assay concentration level. Six determinations were performed and the results were expressed in terms of percent relative standard deviation (RSD).

Linearity and range

The linearity expresses the proportional relationship between concentration and responses. This was evaluated at six concentration levels in the range between 16.2–97.5 µg/ml and 7.2–43.5 µg/ml for cytarabine and daunorubicin, respectively. A calibration curve was plotted by considering concentration against corresponding peak area, and using least square regression analysis, the correlation coefficient was determined.

LOD and LOQ

LOD and LOQ of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curve of cytarabine and daunorubicin using the following formula:

Table 1: Results of system suitability study with acceptance limits

Parameters	Acceptance limits	Cytarabine	Daunorubicin
Retention time* (min)	-	2.323±0.12	3.140±0.16
Resolution*	NLT 2	-	6.5±0.01
Theoretical plates*	NLT 2000	5287±2	7159±4
Tailing factor*	NMT 2	1.4±0.02	1.2±0.03

*Results of six determinations; NLT: Not less than, NMT: Not more than

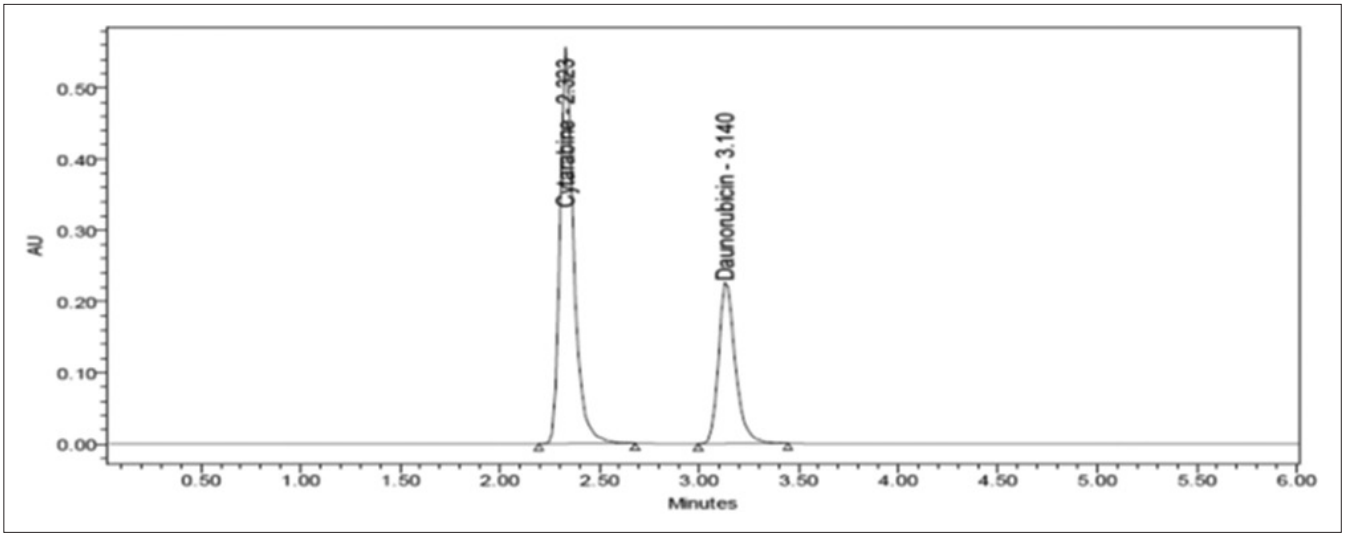

Fig. 3: Chromatogram of cytarabine and daunorubicin standards

Table 2: Results of accuracy

Level of standard added (%)	Cytarabine		Daunorubicin	
	% Recovery	% Mean recovery*	% Recovery	% Mean recovery*
50	99.45	99.05	99.50	99.45
50	98.36		99.25	
50	99.36		99.60	
100	100.22	99.81	99.38	99.32
100	99.68		99.51	
100	99.55		99.07	
150	98.98	99.30	99.28	99.44
150	99.53		99.11	
150	99.39		99.95	

*Average of three determinations; Acceptance criteria: % recovery must be 98%–102%

Table 3: Results of repeatability of precision studies

Number of injections	Cytarabine	Daunorubicin
	Peak area	Peak area
Injection 1	2,682,197	1,334,905
Injection 2	2,678,407	1,339,524
Injection 3	2,678,537	1,335,420
Injection 4	2,679,682	1,336,952
Injection 5	2,666,181	1,339,277
Injection 6	2,676,157	1,335,590
Mean*±SD	2,676,157±5948.9	1,338,111±2557.0
% RSD#	0.2	0.2

*Each value is represented as a mean±SD of six observations (n=6), SD: Standard deviation, RSD: Relative standard deviation, #Acceptance criteria: RSD <2

LOD = 3.3 σ/s

LOQ = 10 σ/s

Where, σ is the standard deviation of the y-intercepts and s is the slope of the calibration curve.

RESULTS AND DISCUSSION

Method development

Various chromatographic conditions have been tried for best resolution and separation. After number of trials being performed, highly resolved symmetrical peaks were obtained by using Denali C18 (250 mm×4.6 mm, 5 µm) with a mobile phase consisting of water (pH adjusted to 3 with orthophosphoric acid):acetonitrile (55:45) pumped at a flow rate of 1.0 ml/min. The detection wavelength of 240 nm was fixed using PDA detector. A typical RP-HPLC chromatogram for simultaneous determination of cytarabine and daunorubicin from standard preparation is shown in Fig. 3. All the system suitability parameters such as theoretical plates, resolution, and tailing factor were found to be within the limits and are summarized in Table 1.

Method validation

Accuracy

The percentage recoveries of cytarabine and daunorubicin at three different levels (50%, 100%, and 150%) were evaluated and are shown in Table 2. The results of accuracy specify that the recovery values were within the acceptance range of 98–102%. Hence, the developed method was accurate for the determination of specified drugs.

Precision

The precision of the method was verified by repeatability. The standard solution of cytarabine and daunorubicin was prepared at working concentration and was injected six times into the chromatographic system. The results of precision are tabulated in Table 3. The RSD was calculated and reported. The RSD values were found to be <2%. This clearly assured that the developed method was found to be precise, repeatable, and reproducible.

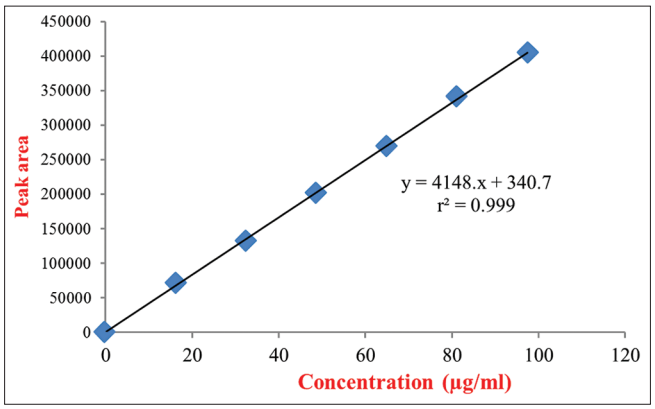

Fig. 4: Calibration plot of cytarabine

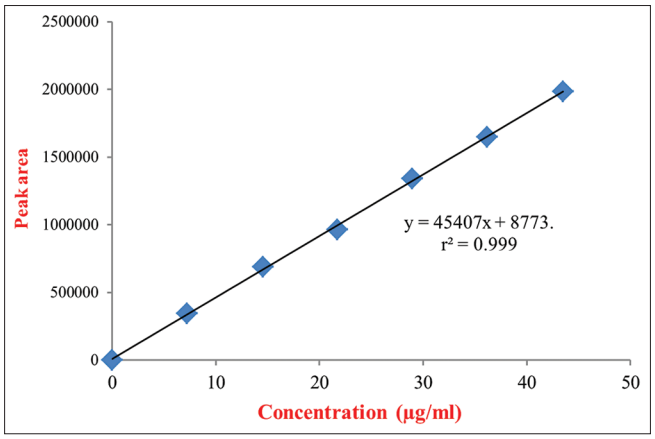

Fig. 5: Calibration plot of daunorubicin

Linearity and range

The linearity of the assay method was checked by preparing test solutions from the standard stock solution at five concentration levels from 25% to 150% of assay concentration. The concentration range at which there is a proportional relationship with peak area is considered as range. The peak area versus concentration data was treated by least square linear regression analysis. The calibration plots are shown in Figs. 4 and 5. The results were obtained. The results showed an excellent correlation between peak areas and concentration within the concentration range of 16.2–97.5 µg/ml for cytarabine and 7.2–43.5 µg/ml for daunorubicin. The correlation coefficients were found to be 0.999 for both the drugs. The results indicate linear relationship between concentration of specified range and response which meet the method validation acceptance criteria, and hence, the method was said to be linear for the specified concentration range.

Table 4: Results of system suitability during robustness studies

Parameter	Cytarabine		Daunorubicin	
	Plate count#	Tailing*	Plate count#	Tailing*
Less flow rate (0.8 ml/min)	5834	1.23	7268	1.26
More flow rate (1.2 ml/min)	5119	1.30	6506	1.28
Less column temperature (28°C)	5610	1.19	7141	1.22
More column temperature (32°C)	5262	1.28	6649	1.28
Less organic phase (60:40)	4962	1.27	6531	1.26
More organic phase (50:50)	5014	1.19	7012	1.20

Acceptance criteria: #Plate count: >2000, *Tailing: <2

LOD and LOQ

LOD and LOQ were estimated from the formula. The LOD and LOQ were found to be 0.12 µg/ml and 0.28 µg/ml for cytarabine and 0.35 µg/ml and 0.85 µg/ml for daunorubicin. The results of LOD and LOQ clearly indicate the sensitivity of the developed method.

Robustness

Robustness of the developed method was determined by deliberately changing the method parameters such as flow rate (±0.1 ml/min), column temperature (±2°C), and mobile phase (±5%). The solutions were prepared as per the test method and were injected at different variable conditions; system suitability parameters were assessed. The results are tabulated in Table 4. From the results, it was concluded that even small changes that have made in the conditions did not affect significantly on system suitability parameters and were found to be within the limits. Hence, the developed method was found to be robust.

CONCLUSION

In the present study, a simple, rapid, and economic RP-HPLC method was developed for the simultaneous analysis of cytarabine and daunorubicin in bulk and infusion formulation. The developed method was validated as per the ICH guidelines and was found to be applicable for routine quality control of drugs in bulk and pharmaceutical formulation either individually or simultaneously.

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AUTHORS' CONTRIBUTIONS

All authors have contributed equally for this research article.

CONFLICT OF INTERESTS

Authors declare that no conflicts of interest exist in this research work.

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