IJPSR (2013), Vol. 4, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 July, 2013; received in revised form, 13 August, 2013; accepted, 17 November, 2013; published 01 December, 2013

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF CYTARABINE IN BULK AND PHARMACUTICAL DOSAGE FORMS

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Keywords:

Cytarabine, ICH Guidelines, RP-HPLC, Validation, Linearity

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ABSTRACT: A simple, rapid, accurate, specific and sensitive reverse phase liquid chromatography method (RP-HPLC) was developed and validated for the estimation of Cytarabine in Pharmaceutical dosage form. The method was standardized using a Nova pack C_{18} column (250 mm x 4.6 mm ID; Particle size 3 µm) and the mobile phase consisted of acetonitrile: buffer (Ammonium acetate) at 30:70% v/v. The eluents were monitored at 272 nm and at 1 ml/min flow rate. The retention time was found to be 2.734 min for Cytarabine. The developed method was validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantification in accordance with the ICH guidelines. Linearity was obtained with the correlation coefficient value r^2 =1 for Cytarabine. The proposed method can be used for the estimation of Cytarabine in bulk and Pharmaceutical dosage forms.

INTRODUCTION: Cytarabine (**Fig. 1**) (cytosine arabinoside, 1-b-D-arabinofuranosyl cytosine, ara-C) is a pyrimidine nucleoside analog which is predominantly used against acute myelogenous leukemia and non-Hodgkin's lymphoma [1]. It is also used in combination with other anticancer drugs for the treatment of leukemia and solid tumors. Cytarabine is a polar nucleoside and has a short plasma half-life. The low bioavailability of cytarabine is created by its low permeability across the membrane and rapid conversion into inactive 1-b-Darabinofuranosyl uracil [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(12).4573-76

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(12).4573-76

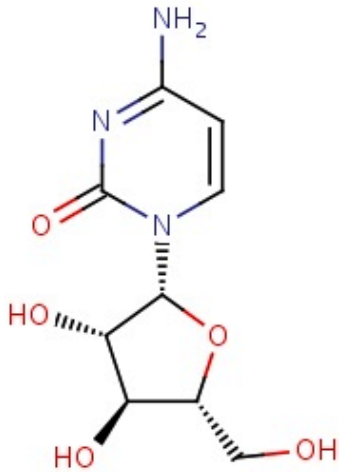

FIGURE 1: STRUCTURE OF CYTARABINE

Thus, continuous intravenous infusion of higher doses is required to maintain a constant plasma level of the drug in 8-24 hr. The higher doses of cytarabine lead to side effects and toxicity to normal organs [3-6].

Literature survey [7-9] reveals that, there is no single method was reported for the estimation of Cytarabine using RP-HPLC. Therefore, the present work describes the development of a validated RP-HPLC method for cytarabine in Bulk and Pharmaceutical dosage forms.

MATERIALS AND METHODS:

Materials: The bulk drug of Cytarabine was obtained from Naprod Life Sciences Pvt. Ltd., Mumbai. All analytical grade chemicals and solvents were purchased from Merck Specialties Pvt. Ltd., Mumbai, India. Acetonitrile and water of HPLC grade were used.

Instrumentation: The HPLC system used was Waters-2695 separation module, equipped with UV-Visible detector and auto-sampler controlled by Empower software, a Nova pack C_{18} column (250 mm x 4.6 mm ID; Particle size 3 µm).

Chromatographic conditions: The analysis was carried out on an HPLC system using a C_{18} column (250 mm x 4.6 mm ID; Particle size 3 µm) with UV detection at 272 nm. An injection volume of 20 µl was used, keeping the flow rate at 1.0 ml/min.

Preparation of Ammonium acetate Buffer: Accurately weighed about 1.927 g of ammonium acetate and transferred into 500 ml volumetric flask. To it added 50 ml of Milli-Q water, shaken well and made up the volume with same water, adjusted the pH to 3.

Preparation of Mobile Phase: Accurately weighed and transferred about 20 ml of Acetonitrile into a 100 ml flask. To it added about 70 ml of buffer and shaken well. Later made up the volume with Acetonitrile, degas it and filtered through 0.45µm nylon membrane filter.

Preparation of standard stock solution: Accurately weighed quantity of 10 mg of cytarabine was transferred to a 100 ml volumetric flask, dissolved in 25 ml of diluent, sonicated for 15 min and the volume was made up to 100 ml. From this standard stock solution 0.25 ml, 0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml and 1.5 ml of the solution was transferred into a 10 ml volumetric flask and diluted with the diluent to give the following concentrations i.e., 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 1 µg/ml, 1.25 µg/ml, 1.5 µg/ml of Cytarabine.

Calibration: Dilutions containing 2.5 - 15 µg/ml of cytarabine were prepared by proper dilutions of primary stock solution with mobile phase to obtain working standards. A 20 µl of the sample was injected into the chromatographic system and the chromatograms were for 8 min acquisition. The flow rate was maintained at 1 ml/min and the eluents were monitored at 272 nm. The separation was done with C_{18} column using developed mobile phase which contains acetonitrile: buffer (30:70%v/v) (**Fig. 2**)

Analysis of formulation: Twenty tablets were accurately weighed and average weight was calculated. All twenty tablets were triturated and a tablet powder weight equivalent to 10 mg cytarabine was transferred to a separate 100 ml volumetric flask containing 25 ml of diluent. Prepared solution was sonicated for 10 minutes for proper solubilisation of the drug and the final volume was made up to 50 ml with the mobile phase. Further pipetted out 1.0 ml of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with mobile phase. Filtered aliquots were analysed using the proposed method.

RESULTS AND DISCUSSION: Using the above chromatographic conditions, the method developed was validated in terms of linearity, accuracy, precision and specificity [8, 9].

Linearity: The linear regression data for the calibration curves indicate that the response is linear over the concentration range (2 - 15 µg/ml of cytarabine) with correlation coefficient values r^2 is 1 (**Fig. 3**).

Precision

Method precision (Repeatability) was performed by assaying the tablet solution at a concentration having 10 µg/ml of cytarabine, under the same experimental conditions. System precision was also performed by six replicate injections of the freshly prepared mixed standard solution at the same concentration (10 µg/ml of cytarabine). The %RSD values were found to be 0.16 for method precision and 0.13 for system precision.

Accuracy: The accuracy of the method was determined by use of standard additions at three different levels, i.e., at multiple level recovery studies. The sample stock solution was prepared at a concentration of 10 µg/ml of cytarabine. This solution was spiked with 50%, 100% and 150% of the mixed standard solution at the same concentration. The mean % recoveries were found to be 100 ± 0.15 for cytarabine and are shown in **Table 1**.

Specificity: The specificity was determined by comparing the test results from the analysis solution containing active substances. The method allows active substances to be separated and the common excipients present in the formulation did not interfere with the elution or quantification of the method. Thus the established method is suitable or specific for desired separation.

System suitability: The system suitability test was performed to check the various parameters such as column efficiency, resolution, peak tailing and retention time. The number of theoretical plates for cytarabine was 3572. All these parameters were evaluated with the background of regulatory requirements, which also suggest the good chromatographic condition. The results were shown in **Table 2**.

Robustness: Robustness was tested by introducing small deliberate variations in liquid chromatography conditions that are

a) Mobile phase composition was changed by ±5 %

b) The flow rate was changed by ±0.1 units. The %RSD values thus obtained showed that the method is robust.

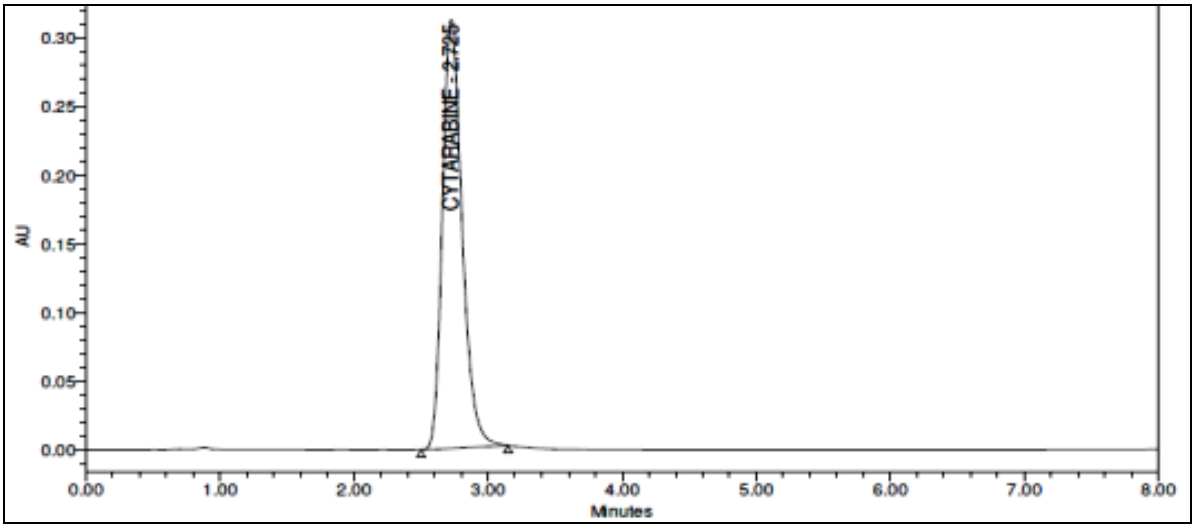

FIGURE 2: CHROMATOGRAM OF CYTARABINE

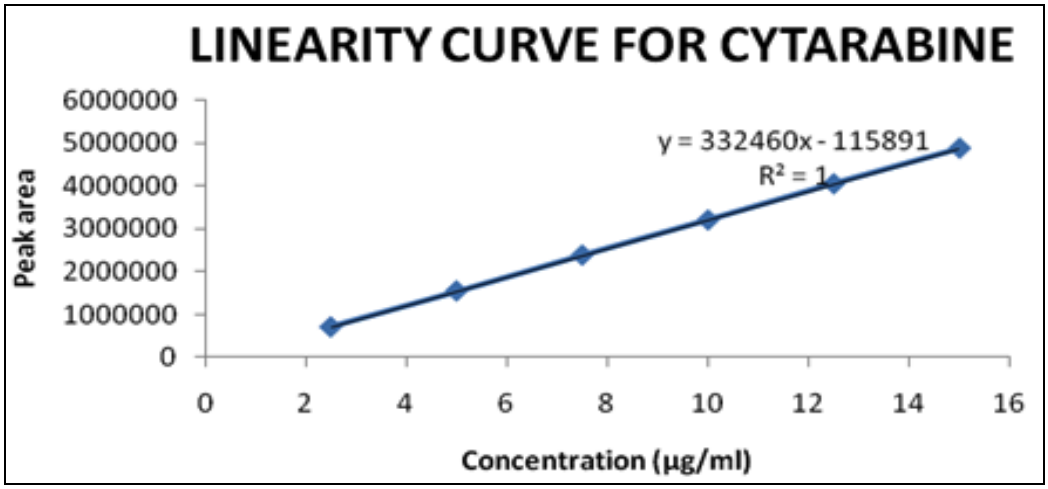

FIGURE 3: CALIBRATION CURVES OF CYTARABINE AT 272 NM

TABLE 1: ACCURACY OF CYTARABINE AT 272 nm

Cytarabine			
% Spiked	**Amount added**	**%Amount recovered**	**% Recovery of pure drug**
50	5	4.86	98.16
100	10	10.02	100.0
150	15	14.68	98.72

TABLE 2: VALIDATION AND SYSTEM SUITABILITY PARAMETERS

S. No.	Parameter	Cytarabine
1	Linearity	2-15 µg/ml
2	Regression equation Y= mx+c	y= 332460x – 115891
3	Correlation coefficient	1
4	Precision (%RSD) 1. Method precision	0.16
	2. System precision	0.13
5	% Assay	99.74%
6	Theoretical plates	3572
7	Tailing factor	1.26

CONCLUSION: The proposed RP-HPLC method for the estimation of Cytarabine was validated in accordance with the ICH guidelines and the method was found to be accurate, precise, linear, robust, simple and rapid. Hence the present RP-HPLC method is suitable for routine analysis of Cytarabine in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT: The authors express their gratitude to Naprod Life Sciences Pvt. Ltd., Mumbai for providing Cytarabine sample, and Management of Nirmala College of Pharmacy, Atmakur, Mangalagiri (M) for their constant support and encouragement.

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How to cite this article:
Murthy VSN, Rohini A, Pravallika KE, Rani AP and Rahaman SA: Development and validation of RP-HPLC method for estimation of Cytarabine in bulk and pharmaceutical dosage forms. *Int J Pharm Sci Res* 2013; 4(12): 4573-76. doi: 10.13040/IJPSR. 0975-8232.4(12).4573-76