

## A Stability Indicating RP- HPLC Method for Determination of Anticancer Agents Cytarabine in Lyophilized Dosage Form

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### Abstract

An isocratic High performance liquid chromatographic method with UV detection at 280nm is described for the determination of cytarabine in finished dosage form. Cytarabine peak was obtained on a HC-C18(2) column with dimension 250mm × 4.6mm, 5 $\mu$ m using mobile phase contained Acetonitrile and purified water with previously adjusted pH 2.8 with orthophosphoric acid (2: 98 v/v), was delivered at flow rate of 0.7 mL per minute. The literature revealed number of reported method cytarabine alone or combination with other drug, but no stability indicating method was reported for cytarabine. The degradation study was conducted on finished dosage form of cytrabine .The cytrabine was exposed to thermal, photolytic, oxidative, acid-base hydrolysis stress condition. The degradation behaviour was observed that significant degradation under hydrogen peroxide but its value was found within specification. Under other the influences of UV light, thermal, Acidic and Alkaline conditions, there was not any significant difference. The developed Performance Liquid Chromatographic method offers stability indicating assay method as well as peak purity, symmetric peak shape, good resolution and reasonable retention time for the drug. Linearity, accuracy and precision were found to be acceptable over the concentration range of 25-150 ppm. The described LC method can be used for the quality control of formulated products containing Cytarabine.

### Keywords:

Cytarabine; stability indicating method; reversed-phase HPLC; degradation study

### 1. Introduction

Cytarabine (Cytosine Arabinoside-ARA-C) is one of the most effective agent in the treatment of nonlymphocytic leukemia in man [1]. Mainly there are two main mechanism involved in its anti tumor activity. Firstly, cytarabine is transported across the cell membrane and activated to the form of 5'triphosphate, ara-triphosphate, which directly inhibits DNA polymerase by competing with the binding of 2-deoxycytidine 5'triphosphate(dCTP) to this enzyme [2]. Secondly, incorporation of cytarabine into DNA, producing chain termination of polydeoxynucleotide elongation, leads to an inhibition of DNA synthesis. Cytarabine inhibit cytidylatedeoxycydidylated (Cyd-dCyd) deaminase and increase the activity of Cyd-dCyd kinase. Cytarabine is a S phase specific drug. Prolonged exposure of cells to cytotoxic concentration is critical to achieve maximum cytotoxic activity. Activity of cytarabine is decreased by its rapid deamination to the biologically inactive metabolite uracil arbinoside [3].

Literature survey shows there are methods available for the quantization of cytarabine, it includes, High performance liquid chromatography alone [4] and liquid chromatography in combination with tandem mass spectrometry [5, 6]. Porous graphitic carbon chromatography/ tandem mass spectrometric was also used for the determination of cytarabine in mice plasma [7]. An ion-pairing liquid chromatography/tandem mass spectrometric method is also reported for the analysis of cytarabine [8].

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The present communication describes isocratic high performance liquid chromatographic method for estimation of Cytarabine, which would be a better choice for the quality control laboratory of the pharmaceutical industry. This study achieved satisfactory results in terms of formulation stability as well as behavior of cytarabine degradation under the influence of different stress conditions. This confirms the suitability of methods for the effectively separate drug from its degradation products. As indication of stability indicating assay methods. The method confirms suitability of selectivity, linearity, precision and accuracy under simple chromatographic condition. Adding to its advantage, the method is simple and time saving.

## **2. Materials and method**

### **2.1. Reagents and Standard**

Cytarabine reference standard (RS) of United States Pharmacopoeia (USP) was bought from Sigma, United States. Acetonitrile was obtained from Merck, India. All other chemicals were of analytical reagent grade unless specified.

### **2.2. Apparatus**

Agilent 1200 series liquid chromatographic system equipped with G1311A quaternary pump was used for the chromatographic separation. Agilent variable DAD detector and a G1329A Auto Injector. EZ Chrome Elite software was employed for data collecting and processing.

### **2.3. Chromatographic Conditions**

Chromatographic Separation was performed on HC-C18(2) column with dimension 250mm × 4.6mm, 5 $\mu$ m. The mobile phase contained acetonitrile and purified water with previously adjusted pH 2.8 with ortho phosphoric acid (OPA) (2:98), was delivered at rate of 0.7 mL per minute. The mobile phase was filtered through 0.45  $\mu$ m membrane filter (Milli pore) and degassed prior to use. Separation was performed at ambient condition and detection was made at 280nm. The injection volume was 20  $\mu$ L with a run time of 10 min.

### **2.4. Preparation of Standard and Sample solutions**

#### **2.4.1. Standard preparation**

Dissolve an accurately weighed quantity of Cytarabine RS in water and dilute quantitatively and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg mL<sup>-1</sup>.

#### **2.4.2. Sample solution**

Prepare the test sample in water such that the final concentration corresponds to the standard solution.

### **2.5. Degradation studies**

Degradation studies of cytarabine was carried out by treating the sample with Acid, base, oxidant, exposing the drug to the UV light and thermal.

#### **2.5.1. Solution stability**

The stability of the test solution was carried out by keeping the solution at room temperature as well as refrigerated (at 2°C-8°C) of Cytarabine at 0, 6, 12 and 24 h.

#### **2.5.2. Acid Degradation**

Accurately weighed quantity of Cytarabine equivalent to 10 mg was transferred to

25 mL beaker dissolved in minimum quantity of water and then 0.1N HCl was added to up to its mark and kept the solution for 12 h at room temperature. Neutralized with 0.1N NaOH, sample was diluted up to 100 mL with water. This solution was injected into the HPLC System.

### **2.5.3. Alkaline degradation**

Accurately weighed quantity of Cytarabine equivalent to 10 mg of Cytarabine was transferred to 25 mL beaker, dissolved in minimum quantity of water and then 0.1N NaOH was added to it up to the mark and kept the solution for 12 h at room temperature. Neutralized with 0.1N HCl, sample was diluted up to 100 mL with water. This solution was injected into the HPLC System.

### **2.5.4. Thermal degradation (dry heat)**

Accurately weighed quantity of Cytarabine equivalent to 10 mg of cytarabine was transferred to 100 mL beaker and was kept as such in oven at 60°C for 12 hours. Sample was cooled and diluted in 100 mL water. This solution was injected into the HPLC system.

### **2.5.5. Oxidative degradation**

Accurately weighed quantity of Cytarabine equivalent to 10 mg of cytarabine was transferred to 25 mL beaker, dissolved in minimum amount of water then 3% H<sub>2</sub>O<sub>2</sub> was added to it up to the mark and kept the solution for 12 h at room temperature. Sample was cooled and diluted in 100 mL water. This solution was injected into the HPLC system.

### **2.5.6. UV degradation**

Accurately weighed quantity of Cytarabine equivalent to 10 mg of cytarabine was transferred to 100 mL beaker and was kept as such under UV light for 12 hours. Then sample was diluted up to the mark with water. This solution was injected into the HPLC system.

## **2.6. Method validation**

The analytical method validation was carried out as ICH method validation guideline. The method was validated to meet requirements for a global the regulatory filing procedure and validation parameter included as specificity, linearity, accuracy, precision, LOD and LOQ.

## **2.7. Data Analysis**

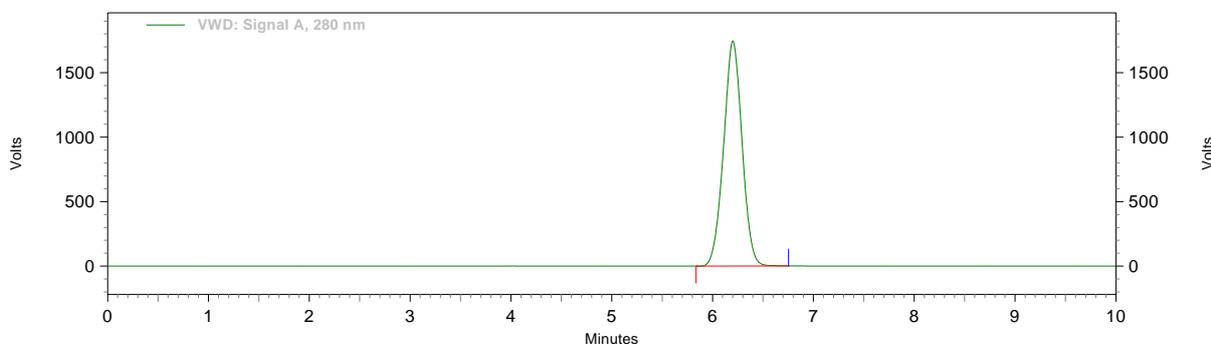
For determination of Cytarabine inject equal volumes of the standard preparation and the assay preparation, record the chromatograms and measure the responses for the major peak.

## **3. Results and discussion**

### **3.1. Method Development and optimization of stability-indicating HPLC**

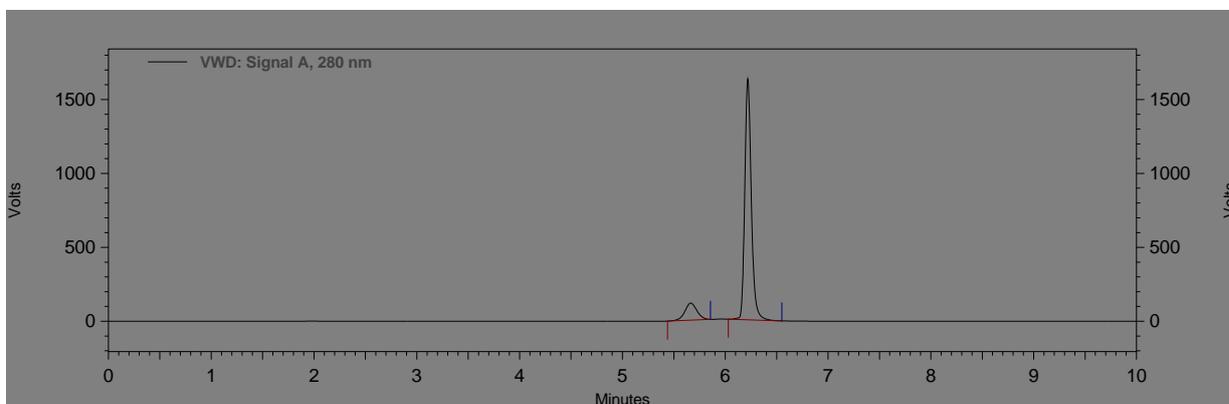
For the method development of cytarabine different mobile phases were investigated for the analysis of cytarabine in bulk and finished commercial dosage form. This include methanol - phosphate buffer (pH 3-7), Acetonitrile – phosphate buffer (pH 3-7), Tetrabutyl ammonium hydroxide–acetonitrile (pH 3-7) on variety of columns. Lastly, when Acetonitrile and purified water, which has previously adjusted pH 2.8 with OPA. An isocratic method is suitable for separation of major degradation products formed under stress conditions. The resolution was not as good intially but when after a number of trials

by changing mobile phase ratio. The best resolution was observed with finally selected mobile phase ratio Acetonitrile and Purified water with previously adjusted pH 2.8 with OPA (2:98). The retention time was obtained 6.2 with symmetric peak shape, high theoretical plate value and asymmetry less than 1.2. The mobile phase was delivered by the flow rate  $0.7 \text{ mL min}^{-1}$ . The retention time of Cytarabine for six repetition was  $6.221 \pm 0.086$ . A typical chromatogram of a sample solution is shown in Fig. 1.



**Fig. 1.** A typical Chromatogram of Cytarabine,  $0.1 \text{ mg mL}^{-1}$ .

Stress samples were analyzed by this method for suitability of method that peak purity results suggest that no other co-eluting, interfering peak from degradants, impurities and excipients from products due to variable stress conditions. The estimation of cytarabine by this method is specific in presence of degradants and impurities. The method was optimized to separate major degradation products formed under different stress conditions. The main target of chromatographic method to get separation for closely eluted degradation product. A chromatogram obtained under stressed condition is shown in Fig. 2.



**Fig. 2.** A Chromatogram of Cytarabine under stressed conditions

### 3.2. Validation of Analytical Method

#### 3.2.1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix. The response of analyte in test mixtures under stressed conditions and all potential degradants and impurities of components in compound with response to its degradation behaviour. The analyte peak is evaluated for peak purity from the nearest eluting peak. For this purpose a solution containing 100 ppm of Cytarabine was injected and peak purity was checked along with stress sample treated with oxidative, acid-base, photolytic, thermal and

solution stability. The acceptance criteria for peak purity is that the purity angle should be less than purity threshold. Result of peak purity analysis was found to be satisfactory, purity angle and purity threshold (peak purity) for Cytarabine was found to be 0.99.

### 3.2.2. Accuracy/ recovery

Accuracy was determined by applying the described method to synthetic mixtures of excipients to which known amount of each drug corresponding to 75, 100 and 125% of label of claim had been added. The accuracy was then calculated as the percentage of analyte recovered by the assay. Recoveries of cytarabine in drug formulation was found in the range of 100.45% -100.80% indicating good accuracy of the method for determination of the drug. The accuracy was determined and results are shown in Table 1.

**Table 1.** Recovery/Accuracy study data of Cytarabine

Sample No.	Concentration spiked (ppm)	Concentration Found (ppm)	Recovery (%)
1	75	75.6	100.80%
2	100	100.45	100.45%
3	125	125.76	100.61%

System precision is a measure of method variability that can be expected for a given analyst performing the analysis. Precision of the method was determined with the product. An amount of the product powder equivalent to 75, 100 and 125 % of label claim was weighed accurately and assayed in five replicate determinations for each of the three weighing amounts. The results for precision indicate that acceptable precision was achieved, as revealed by relative standard deviation data (RSD<2.0%) in all of the levels of cytarabine. The results of Inter and Intra precision are shown in Table 2.

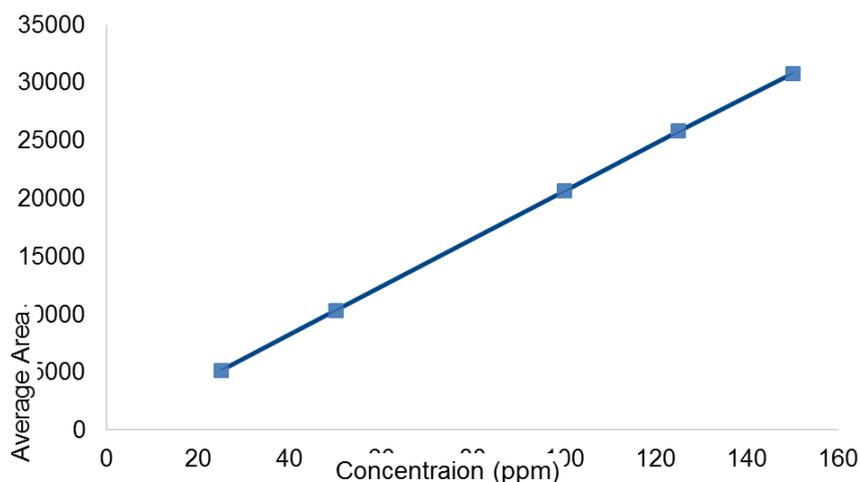
**Table 2.** Inter and Intra day precision

Parameters	Intra Precision	Inter Precision
Mean $\pm$ SD	100.53 $\pm$ 0.196	100.89+ 0.199
RSD (% , n = 6)	0.20%	0.10%

### 3.2.4. Linearity

Under the experimental conditions described above, linear calibration curves for cytarabine was obtained with five concentration level each from Fig. 3.

Peak area (A) and concentration (C) of each drug substance was subjected to regression analysis to calculate the regression equation and the correlation coefficients. The regression equation obtained were  $f(x) = 205.30931(\pm 2.813)x + 58.62837(\pm 1.62)$  ( $r = 0.999$ ,  $n = 5$ ) The drug was found to be linear at a concentration of 25-150 ppm. The results showed that within the tested concentration range was excellent correlation between the peak area and the concentration of cytarabine. The linearity of cytarabine is shown in Fig. 3.



**Fig. 3.** Linearity graph for Cytarabine

### 3.2.5. Limit of detection and limit of quantitation

Limit of detection (LOD) were established at a signal to noise ratio (S/N) of 3.3. Limit of quantification (LOQ) was established at a signal to noise ratio (S/N) of 10. LOD and LOQ were experimentally verified by six injection of cytarabine at the LOD and LOQ concentration. The LOD was calculated to be 0.15 ppm and LOQ was found out to be 0.50 ppm are shown in Table 3.

**Table 3.** Summary of Validation Parameters

Parameters	Results obtained
Wavelength	280 nm
Range	25-150 ppm
Linearity	0.999
Intercept	58.62837
Slope	205.30931
Inter precision	%RSD < 2
Intra precision	%RSD < 2
LOD	0.15 ppm
LOQ	0.5 ppm

### 3.3. Degradation behavior of cytarabine

#### 3.3.1. Solution Stability

The stability of the test was determined by monitoring the peak area of the a sample solution of Cytarabine at 0, 6, 12 and 24 hours at room temperature as well as refrigerated condition (at 2°C-8°C). The results show that there is no significant difference in the area for 24 hour. The results of solution stability at room temperature and refrigerator are shown in Table 4.

#### 3.3.2. Degradation under stress conditions

Stress degradation study was carried out under acidic, alkaline, oxidative and thermal conditions and exposing to UV light and results indicated that Cytarabine was susceptible to oxidative stress under experimental condition than others stress condition,

but its value was found within specification. That there was not any significant difference in assay under the influence of UV light, thermal, Acidic and Alkali condition.

**Table 4.** Stability of analytical solution at Room Temperature

Time of injection	% relative difference from initial (Room Temperature)	% relative difference initial (Refrigerator)
Initial	100.53	100.53
6 hours	100.45	99.78
12 hours	99.69	99.63
24 hours	99.67	99.54
% RSD	0.448%	0.120%

Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drug under non degradation condition. Summary of degradation studies was given in Table 5. Cytarabine was well resolved from its degradation products. Peak purity was also investigated during degradation studies. The peak purity was found satisfactory that no interference was detected.

**Table 5.** Result of Forced degradation study

Degradation condition	% of drug obtained	% drug degradation
Acidic	99.86	0.67
Alkaline	98.08	2.45
Oxidative	85.43	15.1
Dry heat	98.49	2.04
UV light	99.57	0.96

### 3.4. Comparison of Proposed method with existing methods.

The Proposed method is comparable with other existing methods as it used simple isocratic method with good accuracy and recovery. The analytical methods shows that stability indicating assay method was offers isolation of impurities from main peak of cytarabine. There is no report on stability indicating assay of cytarabine in literature. This also gives information about degradation behaviour under influence of stress condition. It is cost effective method. The method was found to be suitable for analysis of Cytarabine in bulk and pharmaceutical dosage forms as well as the stability-indicating studies. The summary of results of Validation Parameters are shown in Table 3.

### 3.5. Method application

The validated LC method was applied to the determination of Cytarabine for injection. The three batches of the sample were analyzed and the assay results was found within 0.11% RSD, expressed as percentage of the label claim.

## 4. Conclusion

The developed Liquid chromatographic method with DAD detection offers, a simple and validated stability-indicating HPLC method for estimation of cytarabine in presence of degradation products. The developed method is specificity, precise accurate

and robust. The statistical parameter of this method showed good results. It produces symmetric peak shape and reasonable retention time for cytarabine. Moreover there is no buffers preparation which makes the method simple and easy to perform. It can be used for the simultaneous determination of cytarabine in the pharmaceutical companies for routine analysis in quality control and research laboratories.

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