

## PHARMACEUTICAL DEVELOPMENT AND COMPATIBILITY STUDIES ON CYTARABINE INJECTION

NOVEEN KONDA\*, PRASHANTH P, ARVIND G, SUMIT SHAH

Celon Laboratories Limited, Hyderabad, Andhra Pradesh, India. Email: noveen.konda@gmail.com

Received: 13 April 2013, Revised and Accepted: 7 May 2013

### ABSTRACT

The aim of present work was to develop a stable Cytarabine injectable formulation for the treatment of various cancers. Cytarabine is freely soluble and stable in water. Different compatibility studies of Cytarabine formulation was carried out. The fluororesin coated chlorobutyl rubber stopper was given least % impurities as compare to bromobutyl and teflon coated rubber stoppers. The platinum cured silicon tubing of Masterflex was observed the best suitable tubing in terms of % impurities. The filter made of the Polyethersulfone (PES) was compatible with Cytarabine formulation in terms of % impurities and loss of drug during filtration. Thermal cycling was performed at 40°C for 4 days and there was found that there was no significant decrease in assay and related substances. Filter validation was carried out with PES capsule filter and it was found to be compatible with Cytarabine formulation. Chemical stability of Cytarabine formulation with infusion solutions was performed and (0.9% NaCl, 5% Dextrose & Water) it was observed that the final solution for injection was stable after 5 days. Osmolality of the Cytarabine injection with 0.9% sodium chloride & 5% dextrose was performed and it was found to be optimum for injectable formulation.

**Keywords:** Cytarabine, Compatibility, Container closure, Filter validation.

### INTRODUCTION

Cytarabine or cytosine arabinoside is a chemotherapy agent used mainly in the treatment of cancers of white blood cells such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma. It kills cancer cells by interfering with DNA synthesis. It is called cytosine arabinoside because it combines a cytosine base with an arabinose sugar. Cytosine normally combines with a different sugar, deoxyribose, to form deoxycytidine, a component of DNA. Certain sponges, where it was originally found, use arabinoside sugars to form a different compound (not part of DNA). Cytosine arabinoside is similar enough to human cytosine deoxyribose (deoxycytidine) to be incorporated into human DNA, but different enough that it kills the cell. This mechanism is used to kill cancer cells. Cytarabine is the first of a series of cancer drugs that altered the sugar component of nucleosides. Other cancer drugs modify the base [1]. Cytarabine is rapidly delaminated in the body into the inactive uracil derivative and therefore is often given by continuous intravenous infusion [2, 3]. The marketed preparation of Cytarabine injection in the form of solution was manufactured by Fresenius Kabi USA. The marketed preparation of Cytarabine for injection in the form of lyophilized cake was available on the name of CYTOSAR-U manufactured by Teva paranteral [4, 5]. The objective of this study was to design, formulate, and manufacture a sterile parenteral formulation of Cytarabine injection suitable to reach Regulatory guidelines. For this purpose, analytical methods were developed and validated, forced degradation studies were conducted, and the degradation pathway was elucidated. A liquid formulation was developed and subjected to compatibility studies with rubber stoppers, tubings, capsule filter, thermal cycling, filter validation and chemical stability in infusion solutions.

### MATERIALS AND METHODS

#### Materials

Cytarabine was obtained from Celon Organics, India. The laboratory grade chemicals such as Sodium hydroxide, Hydrochloric acid, Sodium chloride, Chloroform, were purchased from Merck Chemicals Pvt., Ltd. Mumbai. The USP Type I glass vials and rubber stoppers were obtained from Kimble Glass Company (Vineland, NJ) and The West Company (Lionville, PA), respectively.

#### Assay

Carry out the method for liquid chromatography, using the following solutions. For solution (1) dissolve 20 mg of cytarabine in water and dilute to 100 ml with the same solvent. Dilute 10 ml to 100 ml with water. For solution (2) dilute a volume of the injection to produce a solution containing 0.002% w/v of Cytarabine. The chromatographic procedure may be carried out using (a) a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 μm) (Spherisorb ODS 2 is suitable) and maintained at 30°, (b) a mixture of 7.5 volumes of methanol and 100 volumes of 0.005M sodium pentane sulphonate adjusted to pH 2.8 using glacial acetic acid as the mobile phase with a flow rate of 1 ml per minute and (c) a detection wavelength of 280nm.

#### Related Substances

The method was carry out on thin-layer chromatography, using a silica gel 60 F254 precoated plate (Merck plates are suitable) and a mixture of 15 volumes of water, 20 volumes of acetone and 65 volumes of butan-2-one as the mobile phase. Apply separately to the plate 10 μl of each of the following solutions in water. For solution (1) dilute, if necessary, a volume of the injection to produce a solution containing 2% w/v of Cytarabine. For solution (2) dilute 1 volume of solution (1) to 200 volumes. Solution (3) contains 0.040% w/v of uracil arabinoside EPCRS. Solution (4) contains 0.020% w/v each of uridine and uracil arabinoside EPCRS. After removal of the plate, allow it to dry in air and examine under ultraviolet light (254 nm). In the chromatogram obtained with solution (1) any spot corresponding to uracil arabinoside is not more intense than the spot in the chromatogram obtained with solution (3) (2%). Any other secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2) (0.5%). The test was not valid unless the chromatogram obtained with solution (4) shows two clearly separated spots.

#### Forced Degradation studies

The stability of Cytarabine was assessed by subjecting it to thermal, acidic, basic, and oxidative stress. A 1 mg/mL solution was prepared in water and heated to 100°C for 30 minutes and then assayed.

Acidic stress was performed by mixing 0.1 mL of a 100 mg/ml Cytarabine in water with 0.1 ml of 1N hydrochloric acid and allowed to react for 24 hours. For the basic stress, Cytarabine in water was treated with 1N Sodium hydroxide. Samples were removed at 0, 25, 60, and 90 minutes for assay. Oxidative stress was induced by mixing an equal volume of drug solution with 30% hydrogen peroxide solution and allowed to react for 24 hours. The final concentration of all the samples prepared for this study was 100 mg/ml. The samples were analyzed for Cytarabine by HPLC.

#### Formulation of Cytarabine Injection

Weighed quantity of Cytarabine was added in water under continuous stirring; ensure complete dissolution through "visible clarity with no lumps". Adjust the pH of the solution between 7.5 to 9.0 using 1N Hydrochloric acid or 1N Sodium hydroxide solution (Note: maintain the nitrogen purging throughout the compounding process.). Following studies are conducted with Cytarabine formulation (Table 1)

#### Compatibility with Rubber stoppers

The container-closure system is an essential part of the final presentation of a pharmaceutical product. It defines the closure, protection, and functionality of a container while it ensures the safety and quality of the drug product over the product shelf life. The unfiltered formulation was filled in 5-ml glass vials and stoppered with different types of rubber closures such as Fluorotec coated rubber stoppers, Butyl coated rubber stoppers, Teflon coated rubber stoppers and Chlorobutyl coated rubber stoppers. The study was carried out for 96 hrs and analyzed for the drug content by HPLC to determine the degradation of drug and related substance along with the control sample. The vials were placed upright and inverted in the stability chambers maintained at 25°C with 60% RH. The vials were inverted to obtain maximum exposure of the rubber closure to the formulation. The contents of the vials were periodically analyzed for Cytarabine injection [6].

#### Compatibility study with tubing

The tubing study is required for in-process control check point of any injectable formulation. Unfiltered formulations with different types of tubing's were taken in a ratio of 10.0 cm of the tubing per 50 ml of the formulation. Compatibility study with different types of tubing's (peroxide cured and platinum cured tubing's) from different manufacturers (Sanitech and Masterflex) were carried out by keeping Cytarabine solution in those tubing for 96 hrs and analyzed for the drug content by HPLC along with the control sample [7].

#### Compatibility study with filters

The compatibility study of filter is the most important test for sterility of final formulation. Unfiltered formulations with different types of Capsule filter (PES, PVDF and nylon) were filtered through all these filters and analyzed for the drug content by along with the control sample [8].

#### Osmolality

As the product is meant intravenous administration after dilution with either 0.9% sodium chloride injection or 5% dextrose injection, the osmolality of the product should match with the osmolality of blood. The measured osmolality of Cytarabine injection was diluted to 0.5mg/ml and 0.05mg/ml with either 0.9% sodium chloride injection or 5% dextrose injection [9].

#### Thermal cycling Study

The thermal cycling study was carried out to see the effect of accidental change in temperature on formulation. Thermal cycling study was performed based on the storage condition of the formulation, as the storage condition of the Cytarabine injection is at 25°C, thermal cycling study was performed by freezing at -20°C for 2 days followed by thawing at 40°C±2°C /75%±5% RH for 2 days. Three thermal cycles was carried out [10, 11].

#### Bacterial challenging test for filter validation

Bacterial challenge test was carried out to check the sterility and integrity of filter. The bacterial challenge test serves two major

functions. The filter manufacturer uses it to classify filters as sterilizing grade if the filter provides a sterile effluent with a minimum of  $10^7$  cells of *B. diminuta* /cm<sup>2</sup> of effective filter surface area. Bacterial challenge tests also required to validate the sterilizing filtration process of a specific product. The filter challenge test must be performed with actual product or, where justified, suitable surrogate fluid. The viable bacteria were mixed with Cytarabine solution, minimum of  $10^7$  cells of *B. diminuta* /cm<sup>2</sup> of effective filter surface area. Filtration was carried out with 0.22µm PES capsule filter and sterility was performed for 14 days.

#### Leachable and Extractable test

Extractable are chemical compounds that migrate from any product-contact material when exposed to an appropriate solvent under exaggerated conditions of time and temperature. Leachables are Chemical compounds, typically a subset of extractables that migrate into a drug formulation from any product contact material as a result of direct contact under normal process conditions or accelerated storage conditions. These are likely to be found in the final drug product. Current USP Leachable and extraction conditions require that the test article be extracted in purified water at a test article surface area of 6,000 cm<sup>2</sup> per liter of purified water at 70 °C for 24 hours [12].

#### Bubble point test

Bubble point test was carried out to check the integrity of the filter. Filter membrane was wetted with the product. Pressure was applied on the upstream side of the filter. The pressure at which a stream of air bubbles was detected downstream of the filter was known as the Minimum Bubble Point of the filter [13]. Pre and post bubble point test was carried out with Cytarabine formulation.

#### Chemical Stability in Infusion Solutions

Chemical stability of Cytarabine injection in different infusion solutions (0.9% sodium chloride solution, 5% dextrose solution and WFI) was studied for 5 days [14].

### RESULTS AND DISCUSSION

#### Forced Degradation studies

Thermal stress caused about 10% degradation of Cytarabine formulation. Acidic stress with strong acid, such as hydrochloric acid, did not degrade Cytarabine injection. However, basic stress caused a time dependent loss of drug. No degradation was seen under oxidative stress.

#### Compatibility with Rubber stoppers

Cytarabine injection has shown good stability with all type of rubber stoppers. The assay of Cytarabine injection and related substances were compared with each other after 96 hrs of study. The Fluorescein coated chlorobutyl rubber stoppers was selected as a primary packaging material based on the assay and impurity profiles (Table 2).

#### Compatibility study with tubing

After 96 hrs of study, the related substances were found to be higher for peroxide cured silicone tubing as compared to platinum cured silicone tubing. Hence platinum cured silicone tubing (Masterflex) was selected as a manufacturing process component during filtration and filling for the product (Table 3).

#### Compatibility study with filter

The assay of Cytarabine solution before filtration and after filtration, in three capsule filters was comparable with that of initial assay. The PES Capsule filter showed lower impurity as compared to PVDF and Nylon capsule filter. Hence PES capsule filter was selected as a manufacturing process component during filtration and filling for the product (Table 4).

#### Osmolality

The osmolality of Cytarabine solution with 0.9% sodium chloride injection and 5% dextrose injection was obtained 282 and 298

mOsm/l respectively. The normal plasma osmolality is in the range of 280- 300 mOsm/l; hence 5% dextrose and 0.9% NaCl can be used as infusion diluents (Table 5).

#### Thermal cycling Study

After 3 cycles of freeze-thaw, no significant (\*\*P < 0.01) difference in impurities and assay of Cytarabine injection was observed as compare to initial. This showed that the formulation was stable at varied temperatures (Table 6).

#### Bacterial challenging test

The sterility of Cytarabine solution was passed after filtered through PES capsule filter. So it was concluded that the challenge test was also passed.

#### Leachable and Extractable test

No leachable or extractable were found after analyzing the solution with LCMS and GCMS.

#### Bubble point test

The bubble point of PES capsule filter was detected at 52.0 psi and the limit of filter was 50 psi as per vendor COA.

#### Chemical stability in infusion solutions

Chemical stability of cytarabine injection in 0.9% sodium chloride solution, 5% dextrose solution and WFI was studied and there was no significant (\*\*P < 0.01) difference in assay and related substances after 5 days.

**Table 1: Qualitative and quantitative composition**

S.NO	Ingredients	Quantity
1.	Cytarabine	100 mg
2.	Sodium hydroxide	q.s. to pH
3.	Hydrochloric acid	q.s. to pH
4.	Water	q.s to 1.00 ml

**Table 2: Rubber stopper compatibility data**

S.No	Sample description	Duration	Assay	Related substances	Description
1.	Initial	0hrs	101.2%	0.2%	A clear colorless solution.
2.	Glass control	96hrs	101.1%	0.2%	A clear colorless solution.
3.	Fluororesin coated Bromobutyl rubber stoppers	96hrs	101.1%	0.3%	A clear colorless solution.
4.	Teflon coated rubber stoppers	96hrs	100.0%	0.25%	A clear colorless solution.
5.	Fluororesin coated Chlorobutyl rubber stoppers	96hrs	100.8%	0.20%	A clear colorless solution.

**Table 3: Tubings compatibility data**

S.No	Sample description	Duration	Assay	Related substances	Description
1.	Initial	96hrs	101.5%	0.2%	A clear colorless solution.
2.	Peroxide cured silicon tubing (Sanitech)	96hrs	99.3 %	0.4%	A clear colorless solution.
3.	Peroxide cured silicon tubing (Masterflex)	96hrs	98.9%	0.45%	A clear colorless solution.
4.	Platinum cured silicon tubing (Sanitech)	96hrs	101.0%	0.25%	A clear colorless solution.
5.	Platinum cured silicon tubing (Masterflex)	96hrs	100.8%	0.22%	A clear colorless solution.

**Table 4: Filter compatibility data**

S.No	Sample description	Duration	Assay	Related substances
1.	Initial	96hrs	101.3%	0.2%
2.	PVDF Capsule filter	96hrs	99.3 %	0.3%
3.	Nylon Capsule filter	96hrs	99.8%	0.3%
4.	PES Capsule filter	96hrs	100.0%	0.2%

**Table 5: Osmolality data of Cytarabine injection with different infusion**

S.No	Concentration	Osmolarity (mOsm/l)
1.	Cytarabin injection with 0.9% NaCl	282
2.	Cytarabin injection with 5% Dextrose	285

**Table 6: Thermal cycling data**

S. No	Parameters	Specifications	Conditions and schedule	
			Initial	Thermal cycling study
1.	Description	A clear colorless solution.	A clear colorless solution.	A clear colorless solution.
2.	Assay	90-105%	101.5%	100.1%
3.	Related substances	Not more than 0.5%	0.2%	0.4%

**CONCLUSIONS**

Different studies of Cytarabine injection was carried out such as forced degradation, stopper compatibility study, tubing compatibility study, filter compatibility study, thermo cycling study, Bacterial challenging study, leachable and extractable study and chemical stability in infusion solutions. It was concluded that the Cytarabine formulation was suitable for parenteral administration in the treatment of cancer therapy.

**ACKNOWLEDGEMENT**

The authors are grateful to Managing Director, Celon laboratories Ltd., Hyderabad, for providing facilities.

**REFERENCES**

1. Wang WS, Tzeng CH, Chiou TJ, et al. High-dose cytarabine and mitoxantrone as salvage therapy for refractory non-Hodgkin's lymphoma. *Jpn. J. Clin. Oncol.* 1997; 27 Suppl 3: 154-57.
2. Perry, Michael J. *The Chemotherapy source book*, Philadelphia; 2008. p. 80 - 90.
3. Lemke, Thomas L, Williams, David H, Foye, William O. *Foye's principles of medicinal chemistry*. Hagerstwon; 2002. p. 963 - 75.
4. The prescribing information provided by Pfizer Labs, <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=1315f89d-f530-4dff-98e5-27d51617d4a1>, Accessed April 2012.
5. The prescribing information provided by Bedford Laboratories, <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=8bfe6bb4-b240-440e-83b4-64b03d62e073>, Accessed August 2009.
6. Kostecka D, Duncan MR and Wagenknecht D. Formulation of a stable parenteral product; Clonidine Hydrochloride Injection, *PDA J Pharm Sci Technol.* 1998; 52 Suppl 6 : 320-25.
7. Selina Hines and Steve Pleasance. Compatibility of injectable hydromorphone formulations with typical diluents, components of giving sets and drugs for potential co-administration, *EJHP Practicel.* 2011; 17 Suppl 3: 47-53.
8. Xiaolin (Charlie) Tang and Michael J.Pikal. Design of freeze-drying processes for pharmaceuticals, practical advice, *Pharmaceutical Research.*2004, (21, Suppl 2):191-200.
9. Sunil kumar panda, Subas Chandra Dinda. Formulation and in-vitro evaluation of Clopidogrel bisulphate in parenteral dosage form using Lyophilization technique. *IJPRBS* 2012; 1 Suppl 5: 436-55.
10. Gopal Krishna, William F. Hodnick, Wengsheng Lang. Pharmaceutical Development and Manufacturing of a Parenteral Formulation of a Novel Antitumor agent, VNP4010M. *AAPS Pharm sci Tech*, 2001; 14 (2, Suppl 3): 1-9.
11. Sunitha S, Amareshwar P, Santhosh kumar M. a study on the effect of different cellulose polymers on release rate from tramadol loaded microspheres prepared by emulsion solvent evaporation method, *Asian Journal of Pharmaceutical and clinical research*; 2010 3 Suppl 4: 35-39.
12. John Bennan, Frank Bing, Heather Boone, Jim Fernandez. Evaluation of Extractables from Product-Contact Surfaces, *Bio Pharm International*; 2002. p. 22-34.
13. <http://wine.appstate.edu/sites/wine.appstate.edu/files/Bubble%20Point%20Test.pdf>, Accessed 2010.
14. Meor Mohd Affandi, T Julianto, ABA Majeed. Development and stability evaluation of Astaxanthin Nanoemulsion, *Asian Journal of Pharmaceutical and clinical research*; 2011 4 Suppl 1: 142-48.