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Research Article

Method Development of an Analytical Procedure for the Determination of Clofarabine in Pharmaceutical Formulations

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Abstract

A novel, simple and economic high performance liquid chromatography (HPLC) method has been developed for the estimation of Clofarabine in bulk and tablet dosage form with greater precision and accuracy.The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed HPLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Clofarabine in bulk and tablet dosage forms. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

Key words: clofarabine, RP-HPLC, method development, validation, ICH guidelines

Introduction

For many years, the Southern Research Institute has had a programme, supported by the US National Cancer Institute, searching for new nucleoside anticancer drugs. In the early 1980s, two adenine-containing nucleosides, now known as fludarabine (Fludara; Berlex Oncology) and cladribine (Leustatin; Ortho Biotech) were in clinical trials. At the time, it was not clear whether either drug would gain approval by the FDA because some concerns were raised during preclinical and clinical development of these agents. Both drugs were susceptible to glycosidic bond cleavage with fludarabine subject to some phosphorylase cleavage and cladribine subject to both hydrolytic and enzymatic cleavage [1].

Clofarabine administered intraperitoneally had significant activity against a wide variety of human tumourxenografts implanted subcutaneously in athymic nude or severe combined immune deficiency mice [2]. Moderate to excellent sensitivity to tumour growth delays were seen in all eight human colon tumours, three out of four human renal tumours, all four non-small-cell lung tumours, and all three prostate tumours. This spectrum of widespread anticancer activity has been confirmed by other investigators in human tumourxenograft models in mice [3]. The anticancer activity of clofarabine was dose- and schedule-dependent, and greater antitumour activity was associated with more frequent administration [4].Clofarabine is a second generation purine nucleoside analog with antineoplastic activity. Clofarabine is phosphorylated intracellularly to the cytotoxic active 5'-triphosphate metabolite, which inhibits the enzymatic activities of ribonucleotide reductase and DNA polymerase, resulting in inhibition of DNA repair and synthesis of DNA and RNA [5-7].

Acute leukaemia is the most common paediatric cancer, with acute lymphoblastic leukaemia (ALL) and acute myelogenous leukemia (AML) being the two most common types. In the United States alone, ~2,000 children are diagnosed each year with ALL and 500 with AML [8]. Successful treatment of paediatric ALL and AML involves intensive, multi-cyclic therapy with multiple drugs that have various mechanisms of action and dosing regimens [9-10]. Such intense, cyclic treatment regimens with many different agents have reported a projected 5-year disease-free survival of 70% for paediatric patients with ALL and a complete response (CR) rate of 90% in certain forms of childhood acute leukaemia [11].

In this regard and view of the need for a suitable analytical HPLC method for routineanalysis of Clofarabinein formulations.Attempts were made to developsimple, precise and accurate analytical methods for estimation of Clofarabineand extend it for their determination in formulation.

Aim and Objective

The aim of the method is to develop an analytical procedure for the determination of Clofarabine in Pharmaceutical Formulations. The analytical procedure for determination of Assay in finished product of Clofarabine Injection, 1mg/mL is an In-House procedure.

The method shall be validated for the following parameters:

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B) **Error! Reference source not found.**
   - Interference
   - **Error! Reference source not found.**
   - Linearity

C) **Error! Reference source not found.**

D) **Error! Reference source not found.**

E) **Error! Reference source not found.**

F) Stability of Analyte in solution

G) Filter compatibility.

System Suitability **Error! Reference source not found.**

Method of Preparation

Instrumentation,Chromatographic Conditions & Method:

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 85:15 (v/v) of buffer solution and acetonitrileoperated on isocratic mode. The flow rate is 1.0 ml/min. Chromatographic Estimation of Clofarabinewas performed onInertsil ODS-2 (150 x 4.6) mm, 5µm column. The wavelength of detection is 263 nm. The injection volume is 25µL.

Chromatographic conditions

A High Performance liquid chromatography equipped with UV detector and an auto sampler or its equivalent

Column	:	Inertsil ODS-2 (150 x 4.6) mm, 5µm
Detection wavelength	:	263 nm
Flow rate	:	1.0 mL / min
Injection volume	:	25µL
Run time	:	10 min
Column temperature	:	40°C
Sample cooling rack	:	25°C

Standards

Clofarabine working standard.

Preparation of Buffer Solution

Dilute 1.0 mL of Glacial acetic acid in 1000 mL of water and mix.

Preparation of Mobile phase

Mix buffer solution and acetonitrile in 85:15 (v/v) portion, sonicate well.

Preparation of Diluents

Methanol 0.9% sodium chloride

Preparation of 0.9% Sodium Chloride Solution

Weigh and transfer accurately 0.9 g of sodium chloride into 100 mL volumetric flask dissolve and make up to the volume with water.

Preparation of Blank

Dilute 1.0 mL of methanol in 10 mL volumetric flask and make up to the mark with 0.9 % sodium chloride solution.

Preparation of Standard Solution

Weigh and transfer about 30 mg of Clofarabine standard in a 50 mL volumetric flask. Add about 10 mL of methanol and sonicate to dissolve. Make up to the volume with methanol and mix well. Transfer 5 mL of above solution in to 50 mL volumetric flask, dilute and make up to the volume with 0.9% sodium chloride solution.

Note: Standard solutions are stable up to 48 hrs at both room temperature and 2-8°C.

Preparation of Test Solution

Transfer 3.0 mL of sample solution into 50 mL volumetric flask. Add 5.0 mL methanol and 30 ml of 0.9% sodium chloride and mix well. Dilute up to the mark with 0.9% sodium chloride solution.

Note: Test solutions are stable up to 48 hrs at both room temperature and 2-8°C.

Procedure

Separately inject each solution into the chromatographic system in the following order.

Blank injection	-	Single
Standard solution injections	-	Five
Test solution	-	Two injections
Standard solution bracketing	-	Single injection

Results and Discussion

Experimental:

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Intermediate precision expresses within-laboratories variations such as different days, different analysts, different columns, different equipment's etc. Ruggedness incorporates the concept described under the terms "Intermediate Precision" as defined in USP <1225>.

Intermediate precision is established by doing same exercise as system and method precision by different analyst on different day using different column and different equipment. The same Lot/Batch of standard and sample were used within the laboratory. The results of Intermediate Precision are tabulated in below table. Comparison of Method Precision and Intermediate Precision result is summarized in the table below.

Results of Intermediate Precision (Sample Solution)

Sample #	% Assay
1	101.1
2	101.1
3	101.1
4	101.1
5	100.2
6	100.2
Mean	101.1
% RSD	0.05

Comparison of Method Precision and Intermediate Precision Results

Parameter	Method Precision	Intermediate Precision
Analyst	Analyst 1	Analyst 2
HPLC ID.	EP-QCI-012	EP-QCI-013
Column ID.	HPLCC-008	HPLCC-009
Comparison of Method Precision and Intermediate precision		
Sample #	**% Assay of** Clofarabine	
	Method Precision	**Intermediate Precision**
1	101.9	101.1
2	101.9	101.1
3	101.9	101.1
4	101.9	101.1
5	101.9	101.2
6	102.1	101.2
Mean	101.9	101.1
% RSD	0.02	0.05
Overall Mean (12 samples)	101.5	
Cumulative % RSD (12 samples)	0.42	

Acceptance Criteria:

1. The relative standard deviation of results obtained from six sample preparations should not be more than 2.0%
2. The cumulative relative standard deviation of method precision and intermediate precision results obtained from twelve sample (6 methods precision and 6 intermediate precision) preparations should not be more than 2.0%.

Conclusion:

The result meets the acceptance criteria and found comparable, indicates that the method is precise and rugged with respect to analyst to analyst, day to day, column to column and equipment to equipment for its intended use.

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The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study is performed by analyzing the standard at different conditions. The results obtained with altered conditions are compared against results obtained under normal chromatographic conditions.

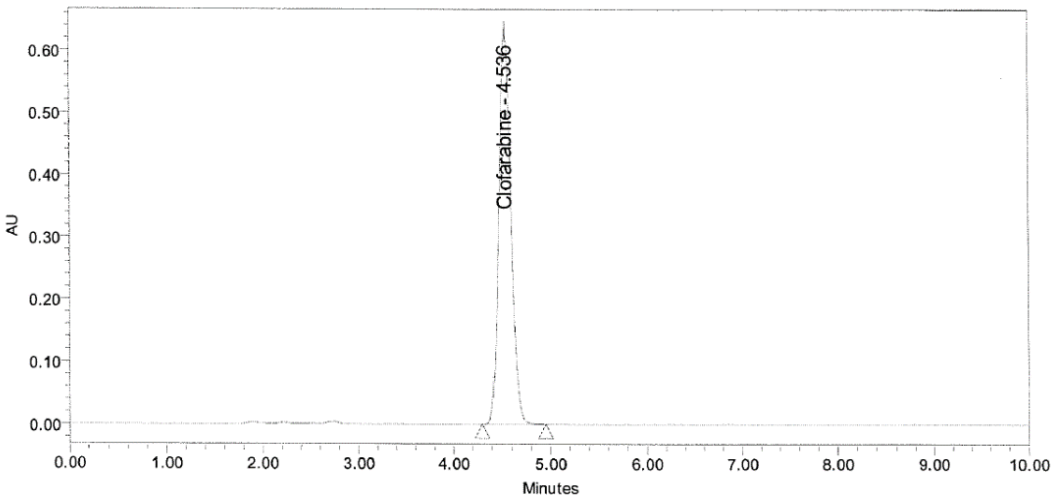
Variation in Flow Rate (± 0. 2 mL/min.)

The standard was carried out by varying the flow rate of mobile phase to 0.8 mL/min. and 1.2 mL/min. in place of actual flow rate 1.0 mL/min. The results are summarized in the below table. Typical chromatogram of Robustness for variation in flow rate (0.8 mL/min and 1.2 mL/ min) is exhibited below.

Results of robustness -Variation in flow rate for Clofarabine

Injection #	Flow Rate 0.8 mL/min.	Actual Flow Rate 1.0 mL/min.	Flow Rate 1.2 mL/min.
1	5449522	4365531	3613880
2	5448777	4366135	3612625
3	5451470	4366853	3613455
4	5444058	4364875	3613092
5	5449326	4366851	3614659
Mean	5448631	4366049	3613542
% RSD	0.05	0.02	0.02
Tailing factor	1.1	1.1	1.0
Theoretical plates	6243	4943	3940

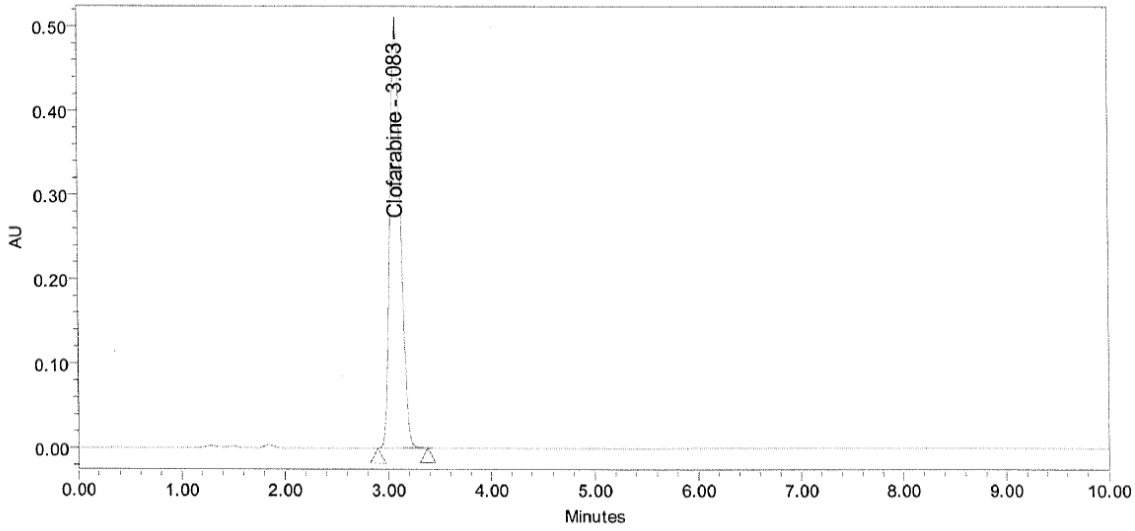
Chromatogram of Robustness for variation in flow rate (0.8 mL/min)



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	4.536	5449522	100.00	633436	6296	1.1	BB

Chromatogram of Robustness for variation in flow rate (1.2 mL/min)



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.083	3613880	100.00	497328	3911	1.0	BB

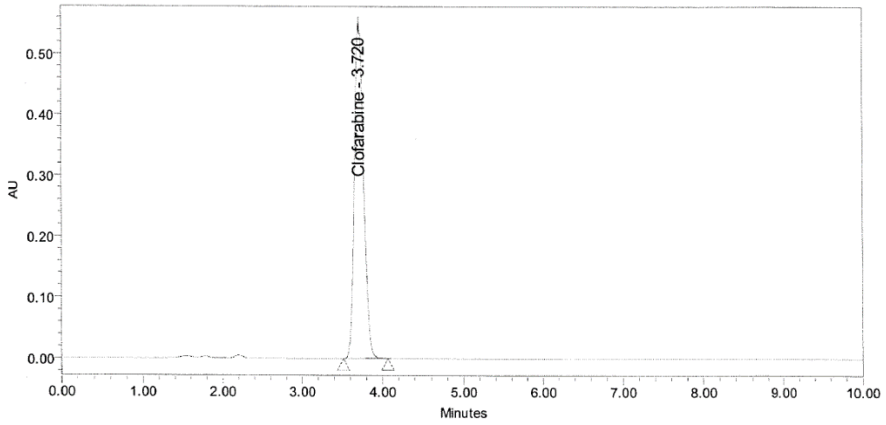
Variation in Column Oven Temperature (± 2°C)

The standard was carried out by varying the column oven temperature of 38°C and 42°C in place of actual column oven temperature 40°C. The results are summarized in the below table. Chromatogram of Robustness for variation in Column Oven Temperature (38°C and 42°C) is exhibited below.

Results of Robustness-Variation in Column oven Temperature for Clofarabine

Injection #	Column Oven Temperature 38°C	Actual Column Oven Temperature 40°C	Column Oven Temperature 42°C
1	4356727	4365531	4354231
2	4357687	4366135	4357138
3	4359938	4366853	4354543
4	4357320	4364875	4352076
5	4358888	4366851	4361549
Mean	4358112	4366049	4401322
% RSD	0.03	0.02	0.08
Tailing factor	1.1	1.1	1.1
Theoretical plates	4914	4943	4875

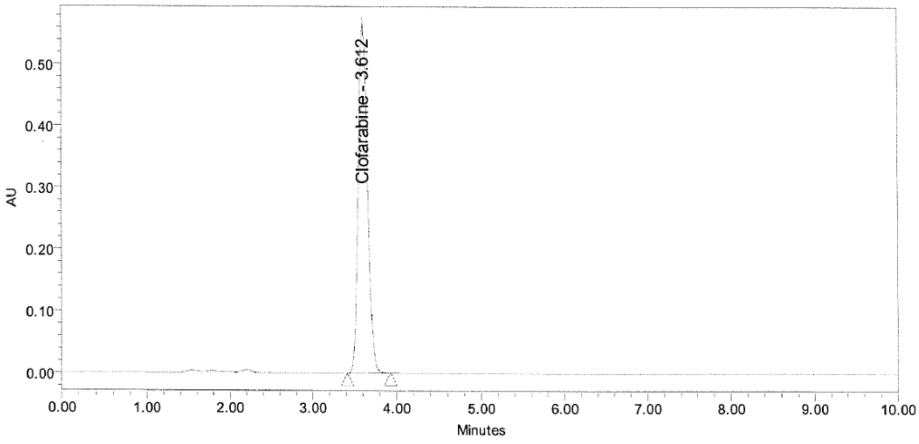
Chromatogram of Robustness for variation in Column Oven Temperature (38°C)



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.720	4356727	100.00	549731	4911	1.1	BB

Chromatogram of Robustness for variation in Column Oven Temperature (42°C)



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.612	4354231	100.00	566163	4948	1.1	BB

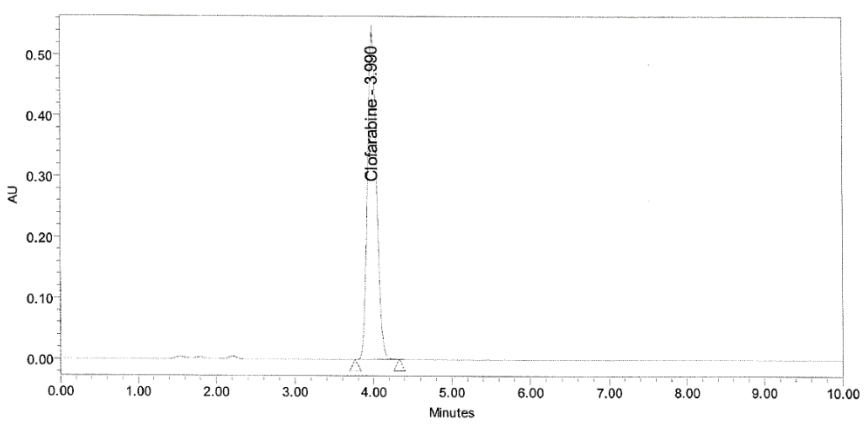
Variation in Organic composition (142.5 and 157.5)

The standard was carried out by varying the Organic composition 142.5 mL and 157.5 mL in place of actual the 150mL. The results are summarized in the below table. Chromatogram of Robustness for variation in the Organic composition (142.5 mL and 157.5 mL) is exhibited below.

Results of Robustness-Variation in pH of Buffer for Clofarabine

Injection #	Low Organic composition 142.5 mL	Actual Organic composition 150 mL	High Organic composition 157.5 mL
1	4360826	4365531	4363298
2	4359986	4366135	4360568
3	4362608	4366853	4359309
4	4360929	4364875	4360570
5	4361818	4366851	4358112
Mean	4361234	4366049	4360371
% RSD	0.02	0.02	0.04
Tailing factor	1.1	1.1	1.1
Theoretical plates	5489	4943	4545

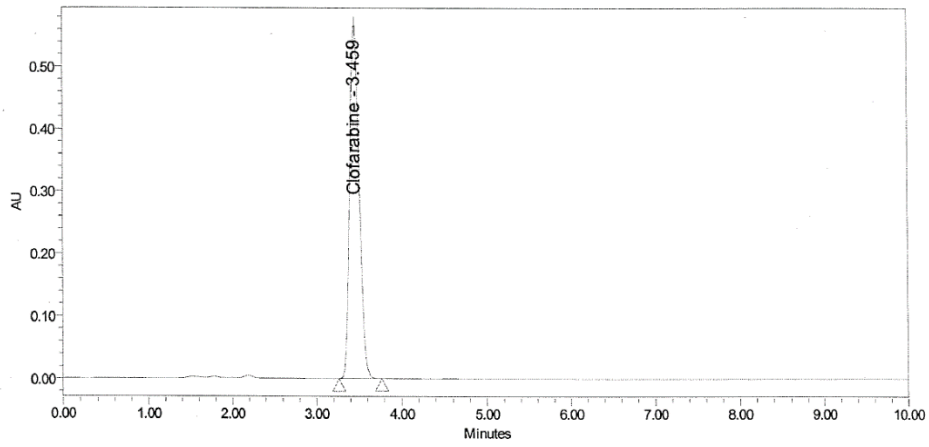
Chromatogram of Robustness for variation in organic composition of mobile phase (Low organic).



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.990	4360826	100.00	539588	5489	1.1	BB

Chromatogram of Robustness for variation in organic composition of mobile phase (High organic).



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.459	4363298	100.00	569667	4611	1.1	BB

Acceptance Criteria:

The System suitability defined in test procedure should meet in each condition.

- The Tailing factor for Clofarabine should be NMT 2.0.
- The relative standard deviation for Clofarabine peak from five replicate injections of standard solution should be NMT 2.0 %.
- The theoretical plates for Clofarabine peak in standard solution should be not less than 3000.

System suitability of overall validation study

The System suitability is an integral part of analytical procedure. The tests are based on the concept that the equipment, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. The system suitability results are tabulated in the below Table.

System Suitability of Overall Validation Study

Parameter		Tailing Factor	Theoretical plates
System Suitability/ System Precision	0.02	1.1	4943
Specificity by diluent, placebo and known impurities	0.02	1.1	4943
Specificity by Forced degradation	0.02	1.1	4983
Specificity by Forced degradation (Alkali)	0.03	1.1	5047
Specificity by Forced degradation (UV)	0.02	1.1	4983
Linearity	0.02	1.1	4943
Method Precision	0.02	1.1	4943
Intermediate Precision	0.04	1.1	6303
Accuracy (Recovery)	0.02	1.1	4943
Robustness-Flow rate: 0.8mL/minute	0.05	1.1	6243
Robustness-Flow rate: 1.2mL/minute	0.02	1.0	3940
Robustness-Column oven temperature: 38°C	0.03	1.1	4914
Robustness-Column oven temperature: 42°C	0.08	1.1	4875
Robustness-Low organic composition(142.5 mL)	0.02	1.1	5489
Robustness-High organic composition(157.5 mL)	0.04	1.1	4545
Stability of Analyte in Solution (Initial)	0.02	1.1	4943
Stability of Analyte in Solution (24 Hours)	0.02	1.1	4943
Stability of Analyte in Solution (48 Hours)	0.02	1.1	5489
Minimum	0.02	1.0	3940
Maximum	0.08	1.1	6243
Average	0.03	1.1	5078

Conclusion

The analytical procedure for Assay is validated and found suitable for its intended use and it meets the acceptance criteria for:

Specificity:

No Interference should be observed at the retention time of peak in the chromatograms obtained from the diluent and the placebo solution.

Forced Degradation:

The method is specific and stability indicating for its intended use.

Linearity:

The analytical procedure is linear within the concentration range from 50 % to 150 % (30.24µg/mL to 90.72µg/mL) for Clofarabine peak.

Intermediate Precision:

The method is precise and rugged with respect to analyst to analyst, day to day, column to column and equipment to equipment for its intended use.

Accuracy:

The analytical test procedure is accurate for its intended use.

Robustness:

The test method is robust enough as demonstrated by altering the Flow rate, Column oven temperature and Organic composition.

Stability of analyte in solution:

The Standard solution is stable up to 48 hours and sample solution is stable up to 48 hours at room temperature and 2-8°C for Clofarabine peak.

The data for each validation characteristic described in this report meets the acceptance criteria with respect to Specificity, Forced degradation, Stability of analyte in solution, Linearity, Precision, Intermediate Precision, Accuracy and Robustness.

The validation results reveal that the analytical procedure is suitable for determination of Assay in ClofarabineInjection, 1mg/mL. The method is stability indicating for determination of Assay of Clofarabine in ClofarabineInjection, 1mg/mL.

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