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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHODS FOR THE QUANTIFICATION OF CLOFARABINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

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 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.

KEYWORDS: *Clofarabine, HPLC, Method Development, Validation, ICH Guidelines*

INTRODUCTION

Clofarabine is a purine nucleoside analog indicated for treatment of relapsed or refractory acute lymphoblastic leukaemia (ALL) in children [1].The drug is also increasingly used, outside of its Food and Drug Administration (FDA) approved indication, for treatment of relapsed or refractory acute myeloid leukemia (AML) in adults [2]. It acts by inhibiting DNA synthesis, the enzyme ribonucleotidereductase and repair and activation of mitochondrial repair processes [3]. We recently observed a case of acute kidney injury (AKI) associated with clofarabine treatment. We conducted a review of the literature and utilized the Food and Drug Administration Adverse Event Reporting System (FAERS)[4] to identify spontaneous reporting of renal adverse events with this drug.

Clofarabine administered intraperitoneally had significant activity against a wide variety of human tumour xenografts implanted subcutaneously in athymic nude or severe combined immune deficiency mice [5]. 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 tumour xenograft models in mice [6]. The anticancer activity of clofarabine was dose- and schedule-dependent, and greater antitumour activity was associated with more frequent administration [7].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 ribonucleotidereductase and DNA polymerase, resulting in inhibition of DNA repair and synthesis of DNA and RNA [8-10].

In this context, Assay procedures are intended to measure the analyte present in a given sample. The assay represents a quantitative measurement of the major component(s) in the drug product. Each drug has different physico-chemical properties like acidic or basic character, Pka value, solubility, polarity, UV absorption characteristics. Apart from these Chromatographic parameters like resolution, capacity factor, separation factor, column efficiency and peak asymmetry should also be the ideal for estimation.

Clofarabine is a next generation deoxyadenosine analogue which is used for the treatment of pediatricleukemia. The mechanism of its anti-cancer activity involves the combination of direct inhibition of DNA synthesis and ribonucleotidereductase and induction apoptosis. This drug is effective against various sub types of leukemia and solid tumors. The Chemical name of Clofarabineis(2R, 3R, 4S, 5R)-5-(6-amino-2-chloro-9H-purin-9yl)-4-fluoro-2-(hydroxymethyl) oxolan-3-ol and chemical formula is $C_{10}H_{11}ClFN_5O_3$ and molecular weight is 303.68 g/mol.

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.

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

PROCEDURE

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

Blank: Single injection

Standard Solution: Five injections

Test Solution: Two injections

Standard Solution Bracketing: Single injection

System Suitability Solution

- The tailing factor for Clofarabine peak in standard solution should be not more than 2.0.
- The relative standard deviation for Clofarabine peak from five replicate injection of standard solution should be not more than 2.0 %.

- The theoretical plates for Clofarabine peak in standard solution should be not less than 3000.
- The cumulative relative standard deviation for Clofarabine peak from five replicate injections of standard solution and bracketing standard should be not more than 2.0 %.

CALCULATION

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{V} \times \frac{P}{100} \times \frac{100}{LA}$$

Where,

AT: Average peak area of Clofarabine obtained from chromatogram of Sample preparation

AS: Average peak area of Clofarabine obtained from chromatogram of Standard preparation

WS: Weight of Clofarabine reference / working standard in mg

DS: Dilution of standard preparation

DT: Dilution of sample preparation

P: Potency of Clofarabine reference / working standard on as is basis

V: Volume of sample taken

LA: Label amount of Clofarabine in mg/mL.

Standard and Samples Used in Method Validation Study

The detail of standard and samples used in validation study is summarized in the below table

Table 1

Name	Lot / Batch No.
Clofarabine working standard	WS/001A/15
Alpha anomer	BP/2075/IP/14/008/32
2-Chloroadenine	BP/2075/IP/13/006/87
Clofarabine injection 1mg/mL	1-PD002
Placebo for Clofarabine injection	1-PD002P

RESULTS & DISCUSSIONS

Precision

System Precision

System precision is assessed from the five replicate injections of the standard preparation from the same vial. The results of system precision are summarized in Table. Typical chromatogram of diluent and standard preparation is exhibited below

Results of System Precision

Table 2

Injection #	Clofarabine	
	Retention Time	Area
1	3.679	4365531
2	3.681	4366135
3	3.678	4366853
4	3.669	4364875
5	3.667	4366851
Mean	3.675	4366049
% RSD	0.17	0.02

Chromatogram of Standard (System Precision)

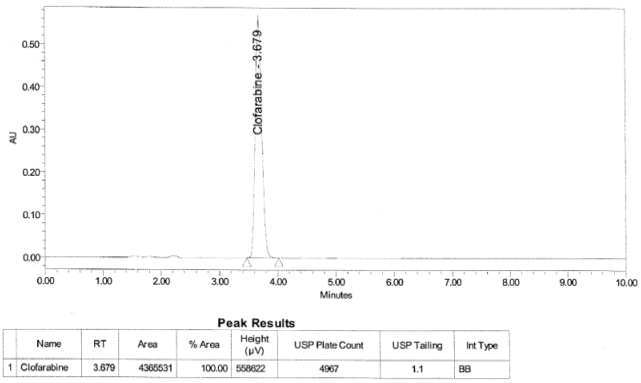

Figure 1

Acceptance Criteria

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

Conclusions

The result meets the acceptance criteria; hence the analytical procedure is precise with respect to the chromatographic system.

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The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample under the prescribed condition.

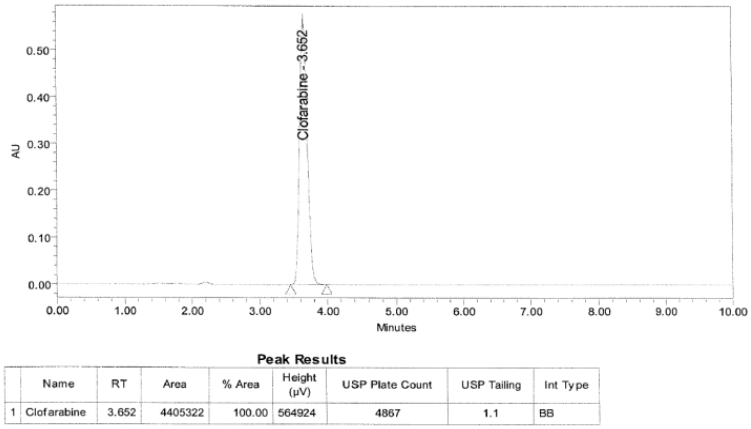
To evaluate the method precision, six individual samples were prepared for Assay and analyzed the samples as per the analytical procedure. The results of method precision for assay are tabulated in the below table. Chromatogram of sample solution of assay is exhibited below.

Results of Method Precision

Table 3

Sample #	% Assay
1	101.9
2	101.9
3	101.9
4	101.9
5	101.9
6	102.1
Mean	101.9
% RSD	0.08

Chromatogram of Sample Solution (Method precision)



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.652	4405322	100.00	564924	4867	1.1	BB

Figure 2

Acceptance Criteria

The relative standard deviation of assay results obtained from six sample preparations should not be more than 2.0%

Conclusions

The result meets the acceptance criteria; hence the analytical procedure is precise with respect to chromatographic method.

Specificity

Specificity is the ability of the analytical procedure to assess unequivocally the analyte in the presence of components which may be expected to be present.

Specificity by Diluent, Placebo and known impurities

Specificity has been evaluated by assuring no interference observed at the retention time of in the chromatogram obtained from the diluent, placebo and known impurities by injecting diluent, placebo solution, standard solution, sample solution and spike sample solution as per the analytical procedure. All peaks are well separated from blank, placebo and any known impurity peak. The specificity results are tabulated in the below Table.

Specificity Results

Table 4

Name	Retention Time (min.)	Results	Cross Reference to Chromatogram
Diluent	No Peak detected	No interference observed	1
Placebo	No Peak detected	No interference observed	2
Clofarabine in Standard solution	3.679	N/A	3
Clofarabine in Sample solution	3.674	N/A	4
Clofarabine in Spiked sample solution	3.676	N/A	5
2-Chloroadenine	2.508	N/A	6
Alpha anomer	4.327	N/A	7

Chromatogram of Diluent (Specificity)

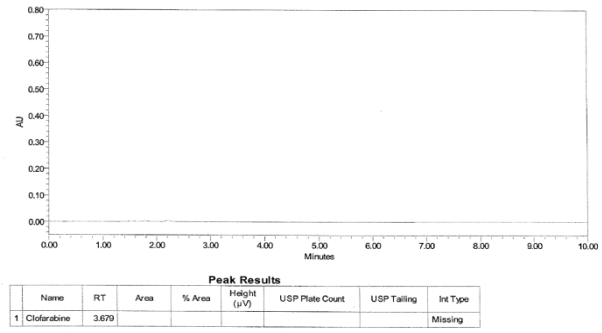


Figure 3

Chromatogram of Placebo (Specificity)

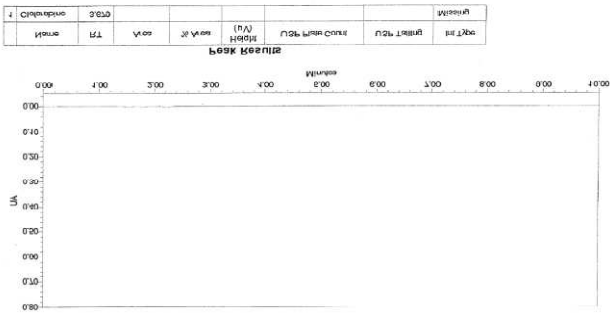


Figure 4

Chromatogram of Standard (Specificity)

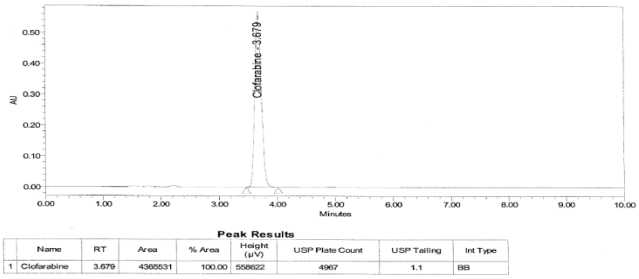
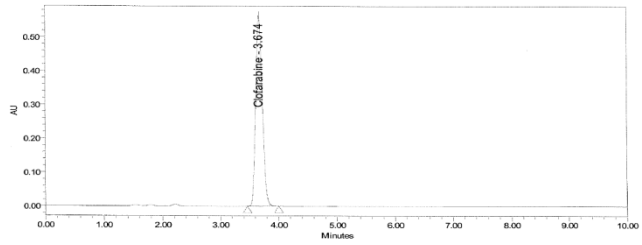


Figure 5

Chromatogram of Sample Solution (Specificity)

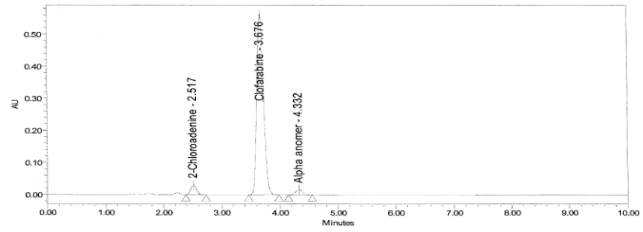


Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	Clofarabine	3.674	4418853	100.00	566317	5002	1.1	BB

Figure 6

Chromatogram of Spike Sample Solution (Specificity)

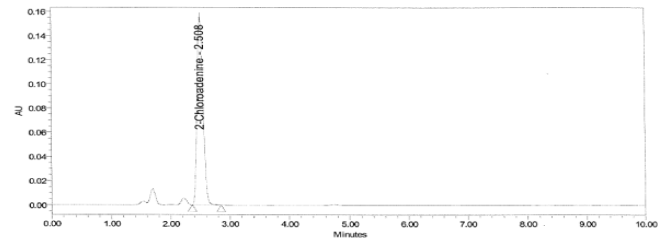


Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Plate Count	USP Tailing	Int Type
1	2-Chloroadeni	2.517	215496	4.47	31430	2950	1.1	BB
2	Clofarabine	3.676	4463595	92.64	565863	4917	1.1	BB
3	Alpha anomer	4.332	139081	2.89	16666	6037	1.1	BB

Figure 7

Chromatogram of 2-Chloroadenine (Specificity)

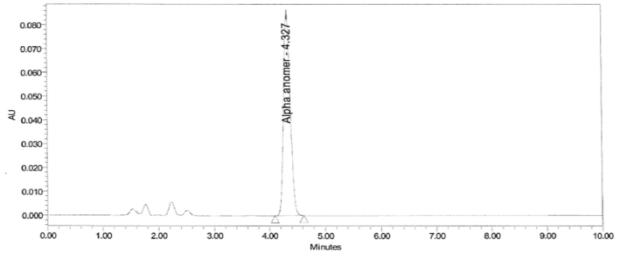


Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	s/n	USP Tailing	RT Ratio	Purity1 Flag	Int Type
1	2-Chloroadenine	2.508	1079380	100.00	0.174	0.216		1.1		No	BB

Figure 8

Chromatogram of Alpha Anomer (Specificity)



Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	s/n	USP Tailing	RT Ratio	Purity1 Flag	Int Type
1	Alpha anomer	4.327	714936	100.00	0.067	0.237		1.1		No	BB

Figure 9

ACCEPTANCE CRITERIA

No Interference should be observed at the retention time of Clofarabine peak in the chromatograms obtained from the diluent, placebo and known impurities.

CONCLUSIONS

No Interference is observed at the retention time of Clofarabine peak in the chromatograms obtained from the diluent placebo and known impurities. The results meet the acceptance criteria; hence the method is specific for its intended use.

Overall Summary of Validation Results

Table 5

Validation Parameters	Acceptance Criteria	Results	
Precision	**System Precision** The RSD of results obtained from six standard NMT 2.0%	**Component Name**	**% RSD**
		Clofarabine	0.02 %
	Method Precision The relative standard deviation results obtained from six sample preparations should not be more than 2.0%	**Component Name**	**% RSD**
		Clofarabine	0.08 %

Table 6

Validation Parameters	Acceptance Criteria	Results	
Specificity	**No Interference From Diluent, Placebo And Known Impurities** No Interference should be observed at the retention time of Clofarabine peak in chromatograms obtained from the diluent, placebo and the impurities	There is no interference is observed at the retention time of Clofarabine peak in the chromatogram obtained from the diluent, placebo and known impurities.	
	Forced Degradation Study • Calculate the % degradation against as such test preparation for each condition in any one of condition degradation should be achieved between 5.0% to 20.0%. • For each degradation sample, purity angle should less than the purity threshold for Clofarabine peak.	**Drug Product (FP)**	
		As Such (Unstressed)	0.0
		Acid degradation	-1.2
		Alkali degradation	8.0
		Peroxide degradation	-1.5
		UV degradation	-0.3
		Thermal degradation	-0.5

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