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Development and Validation of Stability Indicating RP - HPLC Method for the Estimation of Clofarabine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple reverse phase HPLC method was developed and validated for the estimation of Clofarabine in bulk and pharmaceutical dosage form. The analysis was carried out on Alliance waters(2487) HPLC model equipped with Develosil C-18 MG-5(250*4.6mm), 5µm column and variable wavelength programmable UV-Detector. Trifluoro acetic acid: Methanol: Acetonitrile in the ratio 75:10:15v/v at a flow rate of 1.0mL/min was employed for elution and the components were detected at 282 nm at 30^0C. The method was validated as per the ICH guidelines. The retention time for Clofarabine was found to be 3.905min. Linearity was observed in the concentration range of 10-50ppm with correlation coefficient 0.9995. The percent of relative standard deviation of six replicate measurements was found to be 0.151%which indicates that the proposed method was precise. Recovery studies were conducted at three different concentration levels and the average percent of recovery was 99.05%. The amount of Clofarabine present in parenteral dosage form was found to be 99.736%. Forced degradation studies were carried out at different stress conditions such as acid, base, oxidative, photolytic, thermal degradation. The degraded products showed no interference with analyte peak. Therefore, the developed method was simple, precise and accurate and can be successfully applied for the estimation of Clofarabine in bulk and pharmaceutical dosage form.

KEYWORDS: Clofarabine, Parenteral dosage form, RP-HPLC, Validation.

INTRODUCTION:

Clofarabine is a purine nucleoside antimetabolite that is being studied in the treatment of cancer. It is marketed in the U.S. and Canada as Clolar. In Europe and Australia/New Zealand the product is marketed under the name Evoltra.

Clofarabine is used in paediatrics to treat a type of leukaemia called relapsed or refractory acute lymphoblastic leukaemia (ALL), only after at least two other types of treatment have failed. It is not known if the drug extends life expectancy. Some investigations of effectiveness in cases of acute myeloid leukaemia (AML) and juvenile myelomonocytic leukaemia (JMML) have been carried out.

Clofarabine is a novel, next-generation, halogenated-adenosine analogue. Clofarabine has several distinguishing features compared to other drugs in its class including greater resistance to cellular degradation by adenosine deaminase, increased stability in gastric acid, decreased susceptibility to phosphorolytic cleavage, more efficiency as a substrate for deoxycytidine kinase (exceeding cladribine and the natural substrate deoxycytidine).

Mechanism of Action:-

Clofarabine is metabolized intracellularly to the active 5'-monophosphate metabolite by deoxycytidine kinase and 5'-triphosphate metabolite by mono- and di-phospho-kinases. This metabolite inhibits DNA synthesis through an inhibitory action on ribonucleotidereductase, and by terminating DNA chain elongation and inhibiting repair through competitive inhibition of DNA polymerases. This leads to the depletion of the intracellular deoxynucleotide triphosphate pool and the self-potentiation of clofarabine triphosphate incorporation into DNA, thereby intensifying the effectiveness of DNA synthesis inhibition. The affinity of clofarabine triphosphate for these enzymes is similar to or greater than that of deoxyadenosine triphosphate. In preclinical models, clofarabine has demonstrated the ability to inhibit DNA repair by incorporation into the DNA chain during the repair process. Clofarabine 5'-triphosphate also disrupts the integrity of mitochondrial membrane, leading to the release of the pro-apoptotic mitochondrial proteins, cytochrome C and apoptosis-inducing factor, leading to programmed cell death.

Materials & Methods:

Instrument:

The equipments used are double beam Uv-Visible spectrophotometer (Make: Schimadzu) equipped with Uv probe software, analytical balance (Make: Mettler Toledo), pH meter (Make: Mettler Toledo), Sonicator: Ultrasonic bath sonicator (Make: SV Scientific). The chromatographic separation was carried out using HPLC Alliance Waters (2487) equipped with gradient system, connected to Uv/visible detector. The data was acquired by Empower Pro.

Chemicals & Reagents:

Clofarabine working standard was used for analysis. Methanol, Acetonitrile used are of HPLC grade solvents. Chemicals Trifluoro acetic acid, Triethyl amine, Ammonium acetate, Acetic acid are of AR grade.

METHOD DEVELOPMENT

A new RP-HPLC method was developed for the determination of Clofarabine in parenteral dosage form.

STUDY OF SPECTRA AND SELECTION OF WAVELENGTH:

Preparation of clofarabine standard solution:

About 20 mg of clofarabine was taken in 100 ml volumetric flask and 60 ml of methanol was added and sonicated till it dissolved and made up the volume upto the mark with methanol. From the above solution 5ml was withdrawn and transferred into 50 ml volumetric flask and volume was made to mark with diluent.

(Concentration of clofarabine was 20µg/ml)

The solutions were scanned in the UV range of 400 – 200 nm in 1 cm cell against blank (methanol). The absorbance maximum was found to be 282nm. The spectrum was shown in fig.No.1

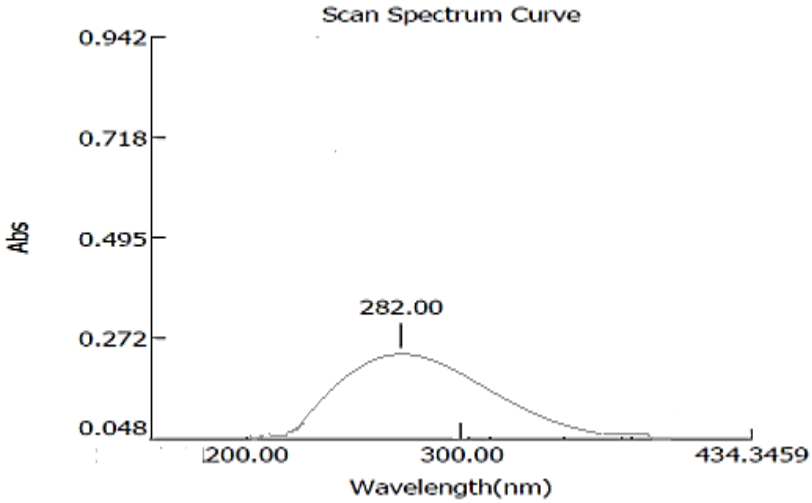

Fig.No.1. UV spectra of Clofarabine

METHOD VALIDATION:

PROPOSED METHOD:

Preparation of Buffer:

Accurately 1 ml of trifluoro acetic acid was transferred into 1000 ml of purified water and mixed. Adjusted the pH of the solution to 3.6 +/- 0.05 with triethyl amine.

Preparation of Mobile Phase:

Mixture of buffer, methanol and acetonitrile was taken in the ratio of 70:20:10 v/v then filtered and degassed.

Preparation of Diluent: methanol was used as diluent.

Preparation of Standard Solution:

Accurately weighed and transferred about 2 mg of clofarabine working standard into 100 ml volumetric flask, to it 60 ml of methanol was added and sonicated for 5 min, then the volume was made up to the mark with the methanol.

Preparation of Sample Solution:

Accurately 2 ml of clofarabine injection was transferred into 100 ml volumetric flask without any loss of solution. Diluted with methanol.

CHROMATOGRAPHIC CONDITIONS: -

PARAMETERS	CONDITIONS
Column	Develosil C-18 MG-5 (250 X 4.6 mm) , 5 µm particle size
Mobile phase	Trifluoro acetic acid : methanol : acetonitrile (75:10:15)
Detector	UV detector
Injection volume	10µl
Wavelength	263 nm
Column temperature	30^0c
Sample cooler temperature	5^0c
Run time	10 mins
Flow rate	1 ml/min
Mode	isocratic

Table no.1: Chromatographic condition of Optimized method

Estimation of Clofarabine from Injection:

Chromatogram of blank:

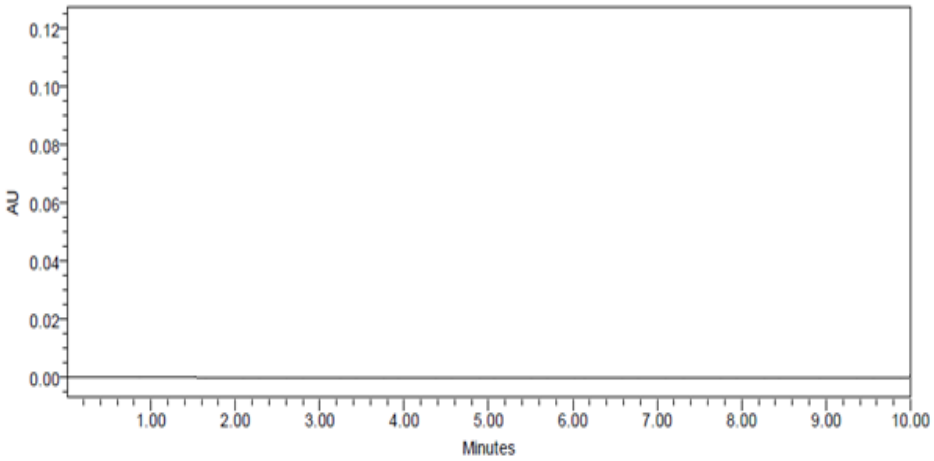

Fig.No.2: Chromatogram of Blank

Chromatogram of standard:

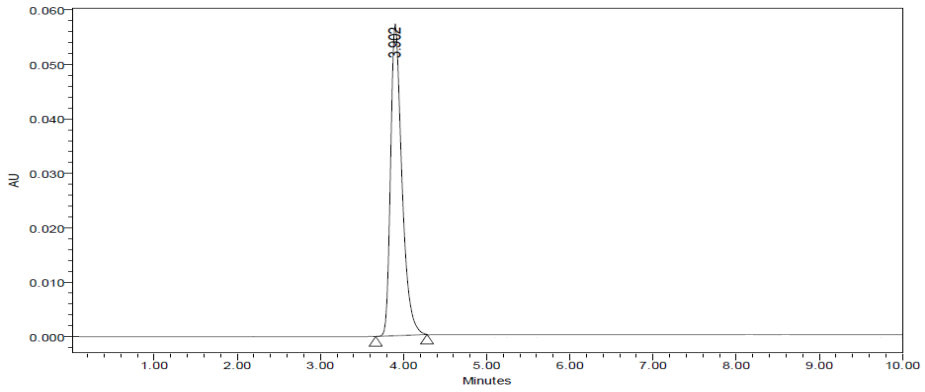

Fig. No.3: Chromatogram of Standard

Chromatogram of sample:

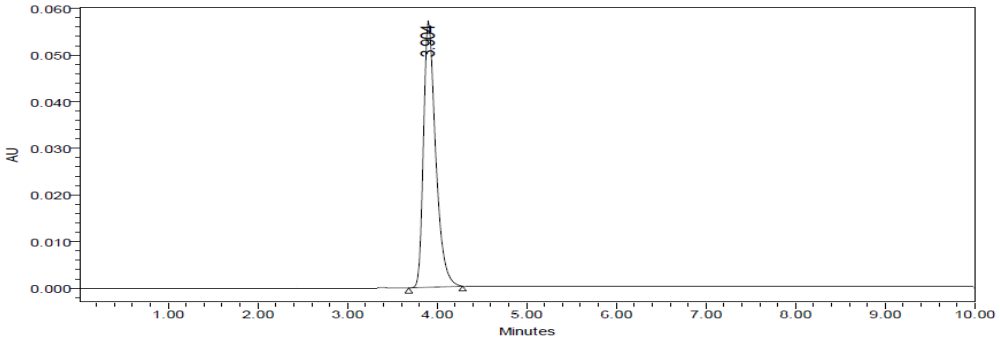

Fig.No.4: Chromatogram of Sample

	Name	**Vial**	**RT(min)**	**Peak area**	**USP plate count**	**Usp tailing**
1	Clofarabine Standard	2	3.902	588407	9216	1.11
2	Clofarabine Sample	3	3.904	588624	9420	1.0

Table no. 2: Results for Assay

The % content of Clofarabine was calculated.

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

Where:
AT = Peak Area of Clofarabine obtained with test preparation
AS = Peak Area of Clofarabine obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

$$\text{Assay \%} = \frac{588624}{588407} \times \frac{10}{10} \times \frac{0.3}{10} \times \frac{10}{10} \times \frac{10}{0.3} \times \frac{99.7}{100} \times 100 = 99.736$$

VALIDATION PARAMETERS

System Suitability:-

Standard solution was prepared as per the proposed method and injected into the HPLC system in five replicates and chromatograms were recorded. Measure the peak area response for the analyte peak and evaluate the system suitability parameters as directed. Results are tabulated in Table 3.

Name	vial	RT (min)	Peak Area	USP Plate Count	USP Tailing
Clofarabine	11	3.905	538420	5512	1.1
Clofarabine	12	3.902	541804	5884	1.0
Clofarabine	13	3.901	541854	5684	1.0
Clofarabine	14	3.908	541869	5956	1.1
Clofarabine	15	3.904	540524	5617	1.0
Mean			540894.2	5780.6	1.04
% RSD			0.226	1.97	1.86

Table no. 3: System suitability results for clofarabine

Data Interpretation

It was observed from the data tabulated above that all the system suitability parameters meet the predetermined acceptance criteria as per the test method and indicates the suitability of the selected system.

Linearity:

A series of standard concentrations of clofarabine were prepared from 10µg/mL to 50µg/mL. 3replicate injections of 10,20,30,40,50µg/mL were injected.

A linearity graph of concentration (µg/mL) versus average peak area response was plotted for clofarabine and the correlation coefficient was calculated.

The correlation coefficient should be NLT0.99.

Preparation of the stock solution:

About 10 mg of clofarabine working standard was weighed and transferred into a 100 ml volumetric flask and 60 ml of methanol was added and sonicated till it dissolved. Then diluted with methanol upto the mark. Results are tabulated in Table:4.

Preparation of Linearity Dilutions for clofarabine

Linearity level (ppm)	Volume of stock taken (mL)	Diluted to (mL)	Final conc.(µg/ml)
10	1	10	10.02
20	2	10	21.32
30	3	10	29.265
40	4	10	40.562
50	5	10	50.231

Table no. 4: Linearity Dilutions of clofarabine

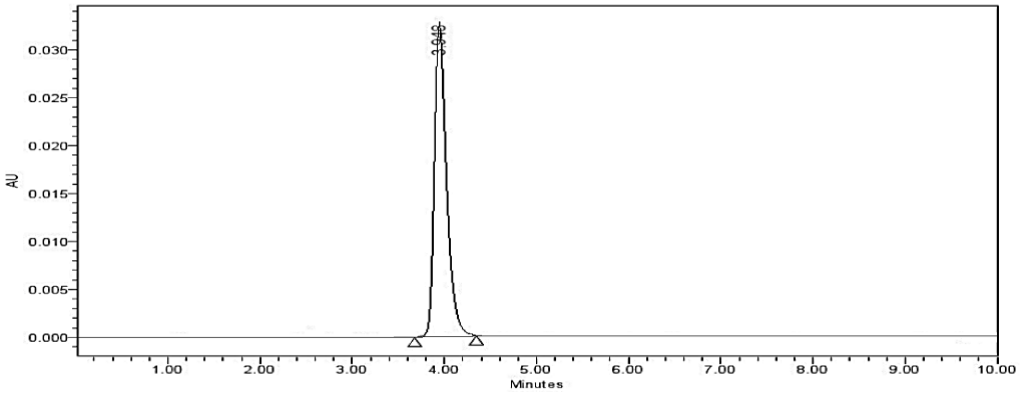

Fig.no.5: Chromatograms for Linearity at 10 PPM

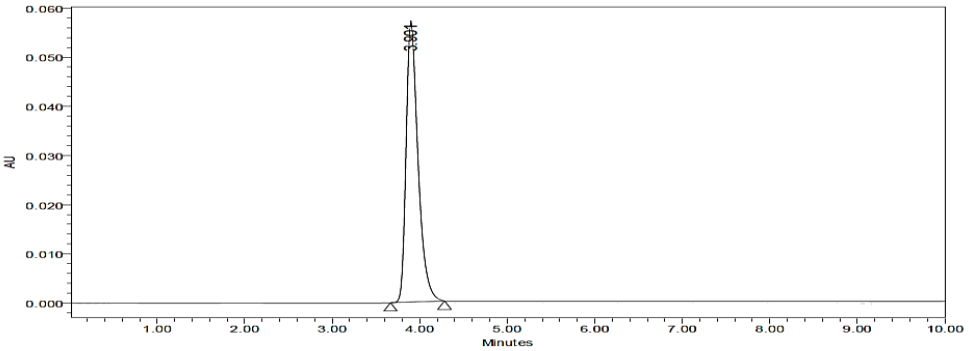

Fig.no.6: Chromatograms for Linearity at 20 PPM

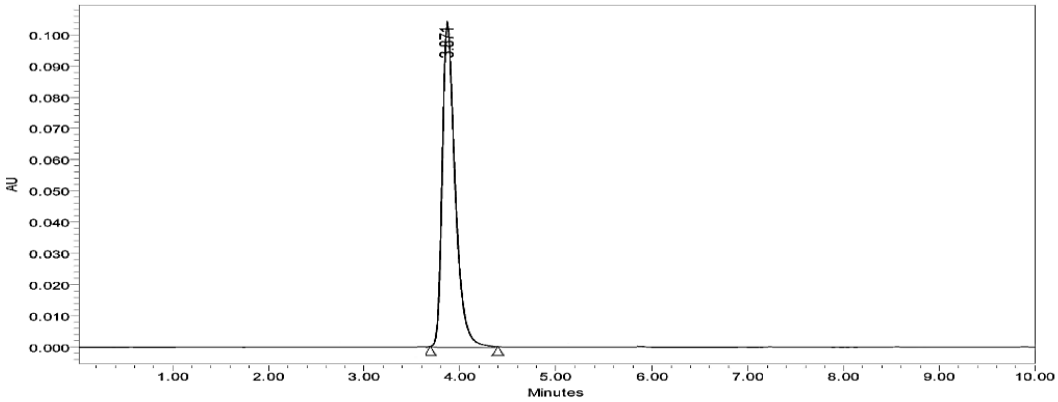

Fig.no.7: Chromatograms for Linearity at 30 PPM

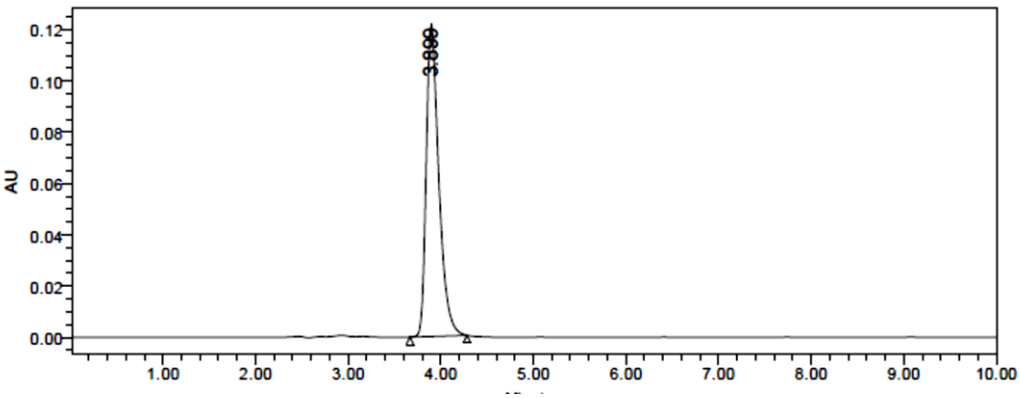

Fig.no.8: Chromatograms for Linearity at 40 PPM

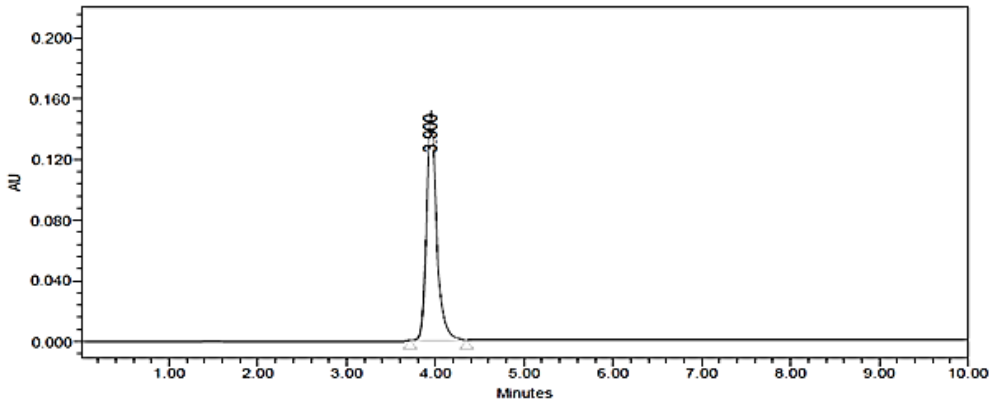

Fig.no.9: Chromatograms for Linearity at 50 PPM

Table no.5: Calculations for Linearity of clofarabine

Linearity Level	Final conc.	Inj 1	-Inj 2	Inj-3	Avg Response
10	10.02	284562	289652	286957	286576
20	21.32	541526	549586	544125	545079
30	29.265	851236	854562	853512	853104
40	40.562	1184844	1132546	1126564	1147985
50	50.231	1426598	1426456	1496415	1455683
Correlation coefficient			**0.9995**		
Intercept (y)			**28739**		

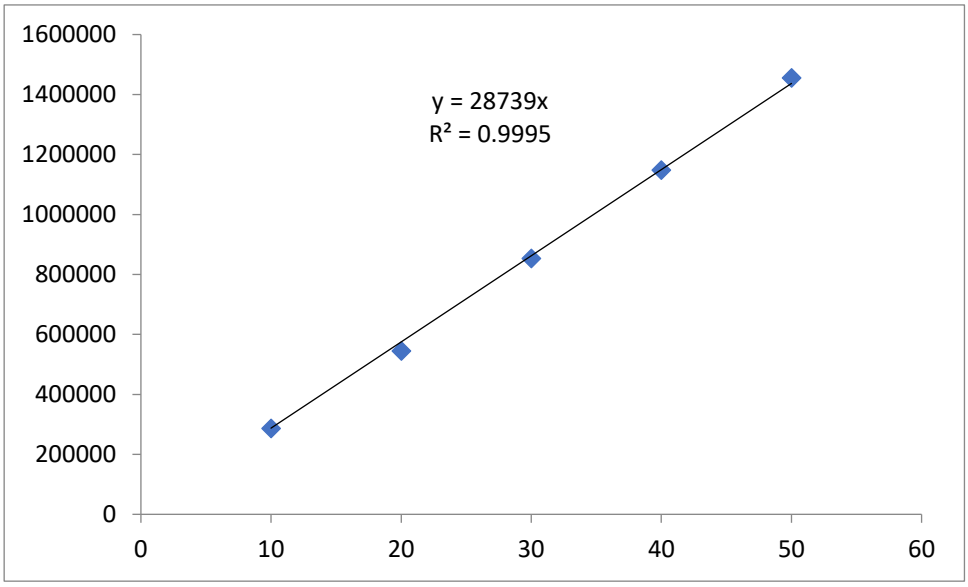

Fig.no.10: Linearity Plot of clofarabine

Data Interpretation

The method for clofarabine was found to be linear in the concentration range of 10 µg/ml to 50 µg/ml. Correlation coefficient was found to be 0.9995

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement.

Method Precision

Preparation of stock solution:10mg of Clofarabine Working standard was accurately weighed and transferred into 10mL volumetric flask and 7mL of diluent was added and sonicated to dissolve it completely and the volume was made upto the mark with the same diluent.

Preparation of working standard solution: Further 0.3mL of the above stock solution was pipetted into 10mL volumetric flask and diluted to the mark with diluent. The solution was mixed well and filtered through 0.45µm filter.

In method precision, a homogenous sample of a single batch should be analysed six times. This indicates whether a method is giving consistent results for a single batch. Results are tabulated in Table 6.

Sample name	**RT (min)**	**Peak area (injection 1)**	**Peak area (injection 2)**	**Average area**
Samp 1	3.899	1145165	1148456	1146811
Samp 2	3.889	1141265	1145121	1143193
Samp 3	3.899	1147562	1142523	1145043
Samp 4	3.889	1145165	1148512	1146839
Samp 5	3.899	1148652	1146523	1147588
Samp6	3.899	1149632	1148562	1149097
Average				**1146429**
Standard deviation				**1738.97**
%RSD				**0.151**

Table no. 6: Method Precision Results for clofarabine

Intermediate precision:

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Six replicate injections of target concentration of clofarabine sample was injected and the area was measured for all six injections in HPLC.

The % RSD of 12 results (six of method precision and six of intermediate precision) should be less than 2%. Results are tabulated in Table 7&8.

Sample name	**RT (min)**	**Peak area (injection 1)**	**Peak area (injection 2)**	**Average area**
Samp11	3.914	1138310	1137954	1138132
Samp12	3.907	1137692	1137895	1137794
Samp13	3.905	1137099	1136745	1136922
Samp14	3.905	1139256	1140265	1139761
Samp15	3.907	1134563	1135627	1135095
Samp16	3.901	1139499	1136521	1138010
Average				**1137619**
Standard deviation				**1303.36**
%RSD				**0.114**

Table No. 7: Intermediate Precision Results For Clofarabine

S. No	Sample ID	Peak Area
1	Samp 1	1146811
2	Samp 2	1143193
3	Samp 3	1145043
4	Samp 4	1146839
5	Samp 5	1147588
6	Samp6	1149097
7	Samp11	1138132
8	Samp12	1137794
9	Samp13	1136922
10	Samp14	1139761
11	Samp15	1135095
12	Samp16	1138010
	AVG	**1142024**
	SD	**4522.43**
	%RSD	**0.396**

Table no.8: Intermediate Precision Results

Data Interpretation

From the above results, it was concluded that the method is precise.

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy study was conducted by spiking the known amount of active ingredients into the placebo at three different levels (50%, 100% and 150% of target concentration). The samples were analysed as per the proposed procedure and the % recovery for each spiked level was calculated.

The % recovery at each spike level should be NLT 98.0% and NMT 102.0% of the added amount.

Preparation of standard solution:

Preparation of stock solution: 10mg of Clofarabine Working standard was accurately weighed and transferred into a 10mL volumetric flask and 7mL of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent.

Preparation of working standard solution:

Further 3 mL of the above stock solution was pipetted into 10mL volumetric flask and diluted up to the mark with diluent. The solution was mixed well and filtered through 0.45µm filter.

Procedure for Accuracy

Placebo: 0.9 % w/v of NaCL (saline solution).

Accuracy at 50%:1ml of placebo was taken into a 10 ml of volumetric flask and to this 0.5 ml of working standard solution was spiked and diluted up to the mark with diluent. The solution was mixed well and filtered through 0.45µm filter.

Accuracy at 100%:1 ml of placebo was taken into a 10 ml of volumetric flask and to this 1 ml of working standard solution was spiked and diluted up to the mark with diluent. The solution was mixed well and filtered through 0.45µm filter.

Accuracy at 150%:1 ml of placebo was taken into a 10 ml of volumetric flask and to this 1.5 ml of working standard solution was spiked and diluted up to the mark with diluent. The solution was mixed well and filtered through 0.45µm filter.

Table 9: Accuracy Results for Clofarabine

S. No	Sample ID	Peak area	Std peak area	Std conc. (µg/mL)	Spiked conc. (µg/mL)	Obtained conc. (µg/mL)	% Recovery
1	Acc – 50% 1	268270	588407	29.45	14.89	14.79	99.1
2	Acc – 50% 2	268897	588407	29.45	14.92	14.82	99.6
3	Acc – 50% 3	260025	588407	29.45	14.43	14.33	96.0
4	Acc – 100% 1	581858	588407	29.45	29.79	29.88	100.1
5	Acc – 100% 2	581804	588407	29.45	29.95	29.87	100.4
6	Acc – 100% 3	581693	588407	29.45	29.84	29.84	100.0
7	Acc – 150% 1	800457	588407	29.45	44.69	44.15	98.6
8	Acc – 150% 2	800235	588407	29.45	44.80	44.25	99.1
9	Acc – 150% 3	801378	588407	29.45	44.74	44.20	98.6

Data Interpretation

The results were found within acceptance criteria. Hence the method is accurate throughout the selected range.

Specificity:

Blank & Placebo Interference: Placebo was injected in triplicate by weighing the equivalent amount present in the finished drug product and analysed for interference due to placebo.

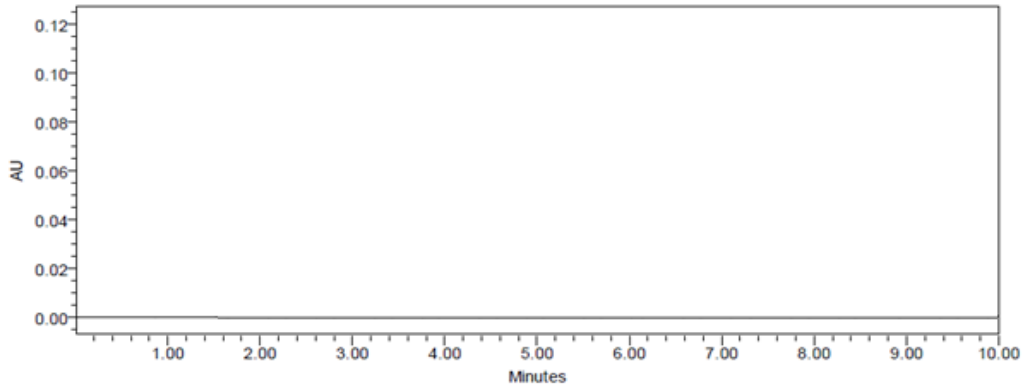

***Fig. No. 11*:** Chromatogram of Blank (Diluent)

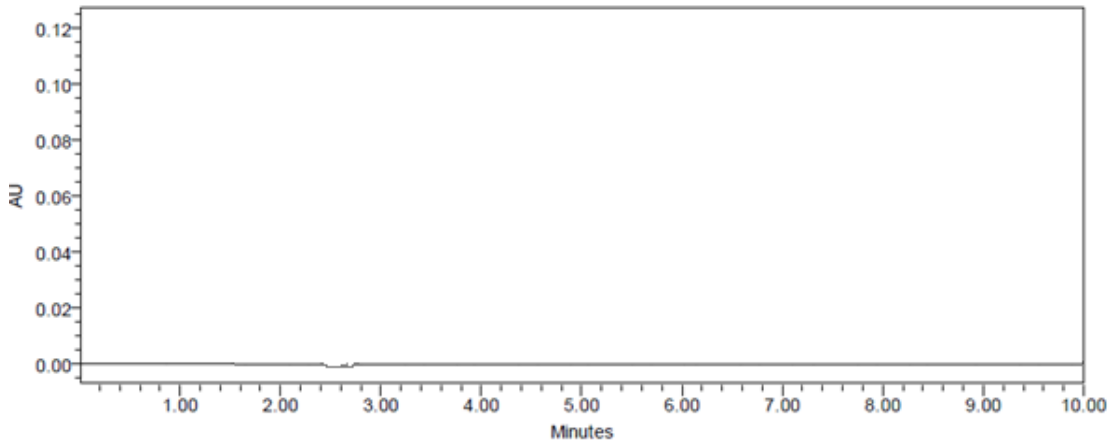

***Fig. No. 12*:** Chromatogram of Placebo

Data Interpretation

On the basis of these chromatograms, we can say that there is no interference of blank and placebo at the retention time of clofarabine. Hence the method is specific.

Robustness:

The robustness of an analytical method 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 of the method was investigated under a variety of conditions including changes in the composition of the mobile phase, column temperature and flow rate.% RSD of assay was calculated for each condition.

In order to demonstrate the robustness of the method, the following optimized conditions were slightly varied.

- 5% in mobile phase composition,
- 0.1 ml/min flow rate,
- 5^0C in column temperature.

Influence on variation in Mobile phase composition:

The robustness of the method was demonstrated by changing the organic solvent ratio by +/-5%. By injecting the replicate injections of standard solution with 5% change in organic phase ratio, it was found that the system suitability parameters were passed. The %RSD of peak area, tailing factor and theoretical plates were found to be within the limits.

Results for robustness

5% less organic solvent:

Sample name	**RT (min)**	**Peak area**	**USP plate count**
Samp32	4.336	1156688	4532
Samp33	4.352	1158451	4656
Samp34	4.341	1152364	4684
Average		1155834	4624
Standard deviation		2214.662	57.2014
% RSD		0.19	1.23

Table no. 10: Peak Results for Robustness at 5% less organic solvent

5% more organic solvent

Sample name	**RT (min)**	**Peak area**	**USP platecount**
Samp35	3.608	1149064	4076
Samp36	3.599	1148569	4265
Samp37	3.612	1149631	4032
Average		1149088	4124.3
Standard deviation		375.761	87.534
% RSD		0.032	1.45

Table no. 11: Peak Results for Robustness 5% more organic solvent

Influence on variation of Column Temperature:

The robustness of the method was demonstrated by changing the column temperature to +/-5°C of specified column temperature (30°C). By injecting the replicate injections of standard solution at the column temperature of 25°C and 35°C,it was found that the system suitability parameters were passed. The %RSD of peak area, tailing factor and theoretical plates were found to be within the limits.

Column temperature at 25^0c

Sample name	**RT(min)**	**Peak Area**	**USP plate count**
Samp38	3.914	1138310	4296
Samp39	3.912	1138624	4325
Samp40	3.914	1139245	4265
Average		1138726	4295.33
Satandard deviation		336.46	21.217

% RSD	0.0295	0.49

Table no. 12: Peak Results for Robustness at Column temperature 25^0c

Column temperature at 35^0c

Sample name	**RT(min)**	**Peak Area**	**USP plate count**
Samp41	3.901	1156688	4303
Samp42	3.900	1155621	4263
Samp43	3.901	1159651	4365
Average		1157320	4310.33
Satandard deviation		1477	36.340
% RSD		0.12	0.84

Table no. 13: Peak Results for Robustness at Column temperature 35^0c

Influence of Flow Variation

The robustness of the method was demonstratedby changing the flow rate to +/- 0.1mL/min of specified flow rate (1mL/min). By injecting the replicate injections of standard solution at a flow rate of 0.9mL/min and 1.1mL/min,it was found that the system suitability parameters were passed. The %RSD of peak area, tailing factor and theoretical plates were found to be within the limits.

Flow rate at 09.ml/min

Sample name	**RT(min)**	**Peak Area**	**USP plate count**
Samp46	4.318	1275158	4384.1
Samp47	4.326	1275126	4489
Samp48	4.319	1273954	4456
Average		1274746	4443
Satandard deviation		485.1309	37.96
% RSD		0.038	0.854

Table no. 14: Peak Results for Robustness at 0.9 ml/min flow rate

Flow rate at 1.1.ml/min

Sample name	**RT(min)**	**Peak Area**	**USP plate count**
Samp49	3.552	6044009	3895
Samp50	3.562	6058414	3866
Samp51	3.552	6054158	3927
Average		6052194	3896
Satandard deviation		5233.066	21.57545
% RSD		0.086	0.553

Table no. 15: Peak Results for Robustness at 1.1 ml/min flow rate

Data Interpretation

All the system suitability parameters were well within the acceptance criteria and % RSD for nine determinations were less than 2%.Hence, it was concluded that the method is robust**.**

Table 16: Data of robustness

Parameter change	% RSD	Inference
5% less organic solvent	0.19	Robust*
5% more organic solvent	0.032	Robust*
Column temperature 25^0c	0.029	Robust
Column temperature 35^0c	0.12	Robust
0.9 ml/min flow rate	0.038	Robust*
1.1 ml/min flow rate	0.086	Robust*

Degradation Studies*:*

Preparation of sample stock solution

10mL of Clofarabine sample solution was transferred from the vial (20mg/20mL) into100mL volumetric flask and the volume was made upto the mark with the diluent. The concentration of Clofarabine sample solution was 100µg/mL.

Preparation of zero hour sample stock solution:- 1 ml of Clofarabine sample solution was transferred from the vial into100mL volumetric flask and the volume was made upto the mark with the diluent

Preparation of samples for force degradation

Procedure ID	Drug substance	Reagent1	Condition	Reagent2
Drug sample control	1mL of sample stock solution	NA	Reflux at 80^0c for 3 hrs	NA
Alkali solution	1mL of sample stock solution	Add 2mL of 1N NaOH	Reflux at 80^0c for 3 hrs	Add 2mL of1N HCL
Acidic solution	1mL of sample stock solution	Add 2mL of 1N HCL	Reflux at 80^0c for 3 hrs	Add 2mL of 1N NaOH
Acid/alkali control	NA	Add 2.0mL 1N NaOH	Reflux at 80^0c for 3 hrs	Add 2mL of 1N HCL
Oxidative solution	1mL of sample stock solution	Add 2mL of 30%H_2O_2	Reflux at 80^0c for 3 hrs	NA
Oxidative control	NA	Add 2mL of 30%H_2O_2	Reflux at 80^0c for 3 hrs	NA
Photolytic solution	1mL of sample stock solution	NA	UV lamp in UV cabinet at 254 nmfor 2 hrs	NA
Thermal solution	1mL of sample stock solution	NA	2 hrs at 100˚c	NA
Zero hour sample	1mL of sample stock solution	NA	NA	NA

Table no.17: Sample preparation for forced degradation

Samples were prepared as per the test method by adding reagent-1 specified in the above table-10 and neutralized before dilution with reagent-2 and then made to the mark with the diluent and injected into the HPLC system and % assay was calculated.

Results for force degradation studies

Table no. 18:- Results for Force Degradation Studies of Clofarabine

S.no	Sample Id	Retention Time	Peak Areas	% Assay	% Degradation
1	Zero hour sample	3.884	589411	99.87	NA
2	Sample Control	3.887	587987	99.62	0.25
3	Alkali Degradation	3.856	326835	55.32	44.60
4	Acid Degradation	3.884	526654	90.14	9.74

5	Peroxide Degradation	3.856	528541	89.55	10.33
6	Photolytic Degradation	3.869	584894	99.33	0.54
7	Thermal Degradation	3.856	573687	97.20	2.67

Table.No.19. Forced Degradation Summary of Clofarabine

Mechanism of degradation	**Observation**
Acid (1N Hcl refluxed for 3 hrs)	No interference at analyte peak
Base (1N NaOH refluxed for 3 hrs)	No interference at analyte peak
Peroxide (30%H_2O_2 refluxed for 3 hrs)	No interference at analyte peak
Heat at 100^0C for 2 hrs	No interference at analyte peak

CONCLUSION:

A new method has been developed for the estimation of Clofarabine by RP-HPLC method.

Chromatographic conditions were successfully developed for the separation of Clofarabine using:

- The instrument used was WATERS HPLC auto sampler.
- Develosil C-18 MG-5(250*4.6mm), 5µm column
- Detection wave length -282nm.
- Temperature -30^0C
- The mobile Phase optimized was Trifluoro acetic acid (adjusted to pH 3.1 +/- 0.05 with Triethyl amine), Methanol and Acetonitrile in the ratio of 75:10:15 % v/v.
- flow rate-1.0mL/min
- The retention timewas found to be 3.905mins.
- The % assay value was found to be 99.736%

The developed method was validated in accordance with the ICH guidelines. The results obtained were within the limits.

- Linearity was obeyed in the concentration range of 10-50µg/mL. Correlation coefficient value for calibration curve of Clofarabine was 0.9995 and the regression equation was found to be Y = 38542x.
- The %RSD for precision on replicate injection and intermediate precision was 0.151 and 0.396 respectively which indicates that the method was precise, robust and repeatable.
- The % Recovery was within limits(98%-102%) indicating that the proposed method was highly accurate.
- No interfering peaks were found in the chromatogram indicating that the excipients did not interfere with the estimation of the drug and thus the method was specific.
- For the robustness study, the flow rate and the detection wavelength were deliberately altered and in all the conditions system suitability parameters were found to be within acceptable limits, which indicate that the method is robust.
- Forced degradation studies were carried out at different stress conditions. The HPLC chromatograms of degraded products showed no interference at the analyte peaks; hence the method was stability indicating. It can be also concluded that Clofarabine is more unstable at alkaline pH.

Thus, a simple, sensitive, accurate, precise, robust, economical and stability indicating RP-HPLC method was developed for the estimation of Clofarabine which can be used for the routine analysis of Clofarabine in API as well as pharmaceutical dosage form.

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