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Research Paper

STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATIONOF CLOFARABINE IN PARENTERAL FORMULATION

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A Simple, accurate and precise Stability Indicating RP-HPLC method was developed for estimation of Clofarabine in Parenteral Formulation. Inertsil C_{18} (150mm×4.6mm) 5µ (particle size) was used as stationary phase. The mobile phase used was Buffer: Acetonitrile 90:10 v/v. The mobile phase was delivered at flow rate 1.0 ml/min. UV detection was set at 263nm. The retention time of Clofarabine was found to be 3.07 minutes. Linearity was observed over the concentration range of 5-25µg/ml for Clofarabine. Force degradation study was perform and maximum degradation of Standard and Test of Clofarabine was found to be 18.8% and 17.5% in Acidic condition. The LOD was found to be 0.071 µg/ml for Clofarabine. Whereas LOQ was found to be 0.21 µg/ml. Moreover, the % RSD for repeatability, inter and intraday precision was found to be less than 2%, which reveals that the method is precise. However, the change in flow rate and mobile phase ratio also did not show any significant variance. Assay of the dosage form finalized the applicability of this method for estimation of Clofarabine in Parenteral Formulation.

Key Words: Stability Indicating RP-HPLC method, Clofarabine, Parenteral Formulation, Force degradation study, % RSD.

INTRODUCTION

Pharmaceutical products formulated with more than one drug, typically referred to as combination products. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The development and validation of analytical methods [Spectrophotometric, High performance liquid chromatography (HPLC) & High performance thin layer chromatography (HPTLC)] for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing ones. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

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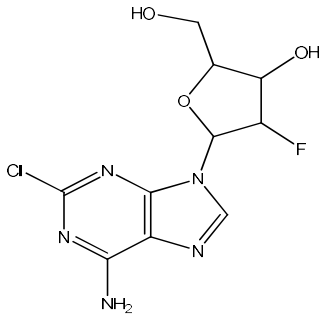
DRUG PROFILE:



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. This nucleoside analog also disrupts mitochondrial function and membrane integrity, resulting in the release of pre-apoptotic factors, including cytochrome C and apoptotic-inducing factors, which activate apoptosis.

MATERIALS AND METHODS:

Table 1: Reagents and Materials used

Sr. No	Chemicals	Specifications	Manufactures
1.	Clofarabine	Active Pharmaceutical	Sion pharmaceuticals Pvt Ltd
2.	Water	HPLC grade	Merck India
3.	Menthol	HPLC grade	Merck India
4.	Acetonitrile	HPLC grade	Spectochem
5.	Glacial acetic acid	HPLC grade	Merck India

Selection of Chromatographic condition:

- **Column:** Inertsil C_{18} (150mm×4.6mm) 5µm
- **Mobile Phase:** Buffer : Acetonitrile (90:10)V/V
- **Flow Rate:** 1.0 ml/min
- **Column Temperature:** 40°C
- **Detection Wavelength:** 263 nm
- **Run time:** 10 min
- **Injection volume:** 5 µl

Selection of mobile phase:

- Buffer: 1ml Glacial acetic acid →1000 ml water
- Mobile phase: Prepare a mixture of 90 volume of Buffer and 10 volumes of Acetonitrile. Filter through 0.45 µm filter and degas before use.

(A) Clofarabine standard preparation:

- Transfer an accurately weighed quantity of about 10 mg Clofarabine working standard in to 100 ml volumetric flask, dissolve and diluted up mark with Mobile phase (100µg/ml)

(B) Preparation of working standard solution:

- From the above prepared stock solution of drug (100 µg/ml Clofarabine) , take 1.5 ml of that solution and dilute u to 10 ml with mobile phase. This gave concentration of 15 µg/ml Clofarabine.

(C) Preparation of Sample standard stock solution of Clofarabine

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- Weighed accurately Clofarabine (10 ml) were transferred into 100 ml volumetric flask and dissolved in mobile phase to give a stock solution 100 µg/ml of Clofarabine. Stock solution (1.5 ml) was transferred in 10 ml volumetric flask and diluted up to mark with mobile phase to obtain working standard solution 15 µg/ml of Clofarabine and this solution was used to prepare standard solution for linearity.

RESULT AND DISCUSSION

Identification of Drugs:

Melting Point Determination

Melting point of the APIs were determined by using melting point apparatus. The observed melting points of APIs were compared with the reported melting point.

Table 2: Melting point determination

Name of Drug	Reported Melting Point	Observed Melting Point
Clofarabine	216-256 °C	232-244 °C

Infrared Spectroscopy

IR spectrum of Clofarabine were taken by KBr

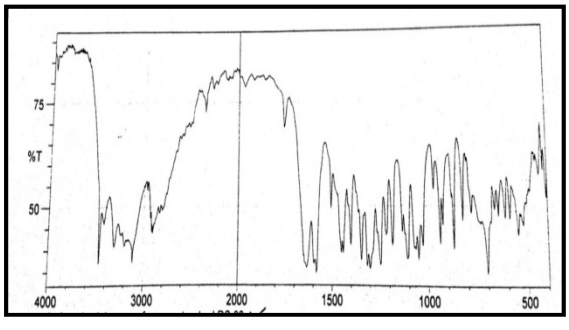

Fig. 1: Reference IR Spectra of Clofarabine

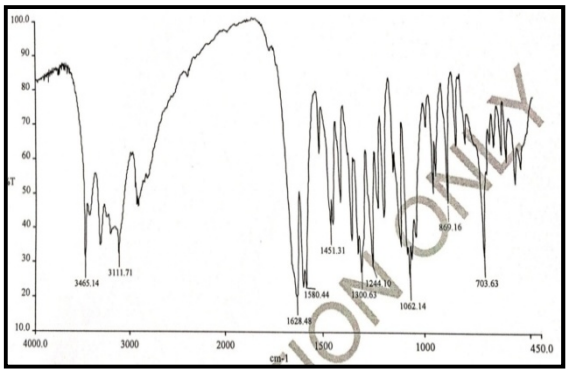

Fig. 2: IR Spectra Sample of Clofarabine

pellet method on FTIR and characteristic peaks were compared with IR spectrum of Reference standard given in Indian Pharmacopoeia.

Table 3: Interpretation of IR Spectra of Clofarabine

Sr. No	Functional group	Wave number (cm^{-1})	Mode of vibration
1	OH	3465.14	Stretching
2	NH_2	3111.71	Stretching
3	CH, CH2	<3000	Stretching
4	C=C	1628.48	Stretching
5	C=N	1580.44	Stretching
6	C-O	1062.14	Stretching
7	C-N	1244.10	Stretching
8	C-Cl	703.63	Stretching

Solubility Determination

The solubility of Clofarabine were checked in

Table 4: Solubility determination of Clofarabine

Sr No.	Drug	Reported	Observed
1	Clofarabine	**Water :** Slightly soluble in water **Methanol :** Sparingly soluble in Methanol **Dimethyl Sulphoxide :** Soluble in Dimethyl Sulphoxide	Complies with Reported solubility

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various solvents like distilled water, methanol, and Dimethyl Sulphoxide etc. The results are shown in Table 4.

METHOD DEVELOPMENT

Selection of wavelength

Scan the standard solution and test solution on UV/Visible spectrophotometer, over the spectral range 200 to 400 nm. Use diluent as blank. The UV spectrum of the test solution should exhibit maxima at the same wavelength (±2 nm) as that of a standard solution. Clofarabine show reasonably good response at 263 nm.

A) Standard preparation

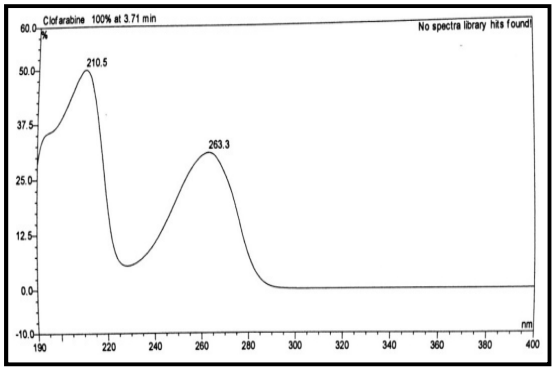

Fig. 3 Uv Spectrum of Clofarabine Standard solution showing selection of wavelength detection

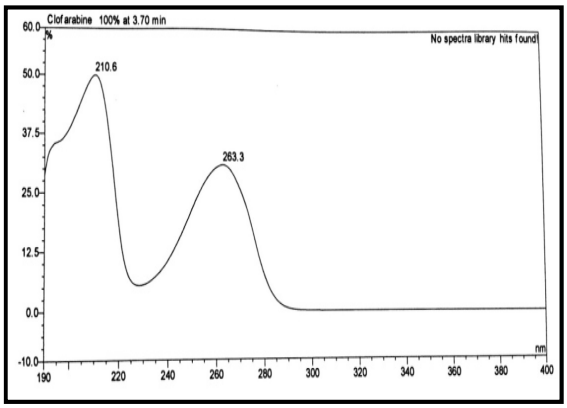

Fig. 4 UvSpectrum of Clofarabine Assay preparation showing selection of wavelength detection

Selection of Mobile Phase

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Clofarabine was obtained with a mobile phase Buffer (1 ml Glacial acetic acid in 100 ml of water) : Acetonitrile (85:15) at a flow rate of 1.0 mL/min

Development trials:

Sr. No.	Mobile Phase	Ratio	Retention Time (min)	Remarks
1	Water : Methanol	60 : 40	----	No peak was Observed
2	Buffer : Acetonitrile	65 : 35	2.945	Peak shape was not satisfactory and fronting observed.
3	Buffer : Acetonitrile	70 : 30	0.847	Peak shape was not satisfactory and Tailing observed.
4	Buffer : Acetonitrile	85 : 15	4.663	Peak shape was not satisfactory
5	Buffer : Acetonitrile	90 : 10	3.706	Peak was sharp and symmetric

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Trial 1

Table: 4 Trial in mobile phase Water: Methanol (60:40)%v/v

Trial	Mobile Phase	Ratio (%v/v)	Retention Time (min)	Remarks
1	Water : Methanol	60 : 40	----	No peak was Observed

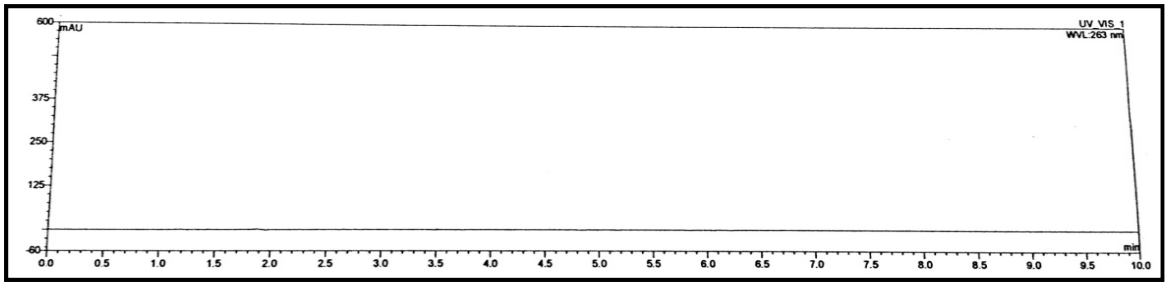

Fig. 5 Trial in mobile phase Water: Methanol (60:40)%v/v

Trial 2

Table: 5 Trial in mobile phase Buffer: Acetonitrile (65:35)%v/v

Trial	Mobile Phase	Ratio (%v/v)	Retention Time (min)	Remarks
2	Buffer : Acetonitrile	65 : 35	2.945	Peak shape was not satisfactory and fronting observed.

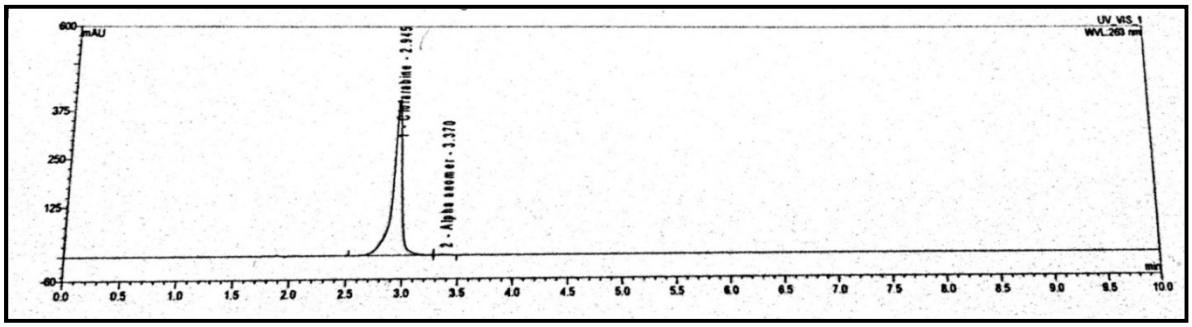

Fig. 6 Trial in mobile phase Buffer: Acetonitrile (65:35)%v/v

Trial:3

Table: 6 Trial in mobile phase Buffer: Acetonitrile (70:30)%v/v

Trial	Mobile Phase	Ratio (%v/v)	Retention Time (min)	Remarks
3	Buffer:Acetonitrile	70 : 30	0.847	Peak shape was not satisfactory and Tailing observed.

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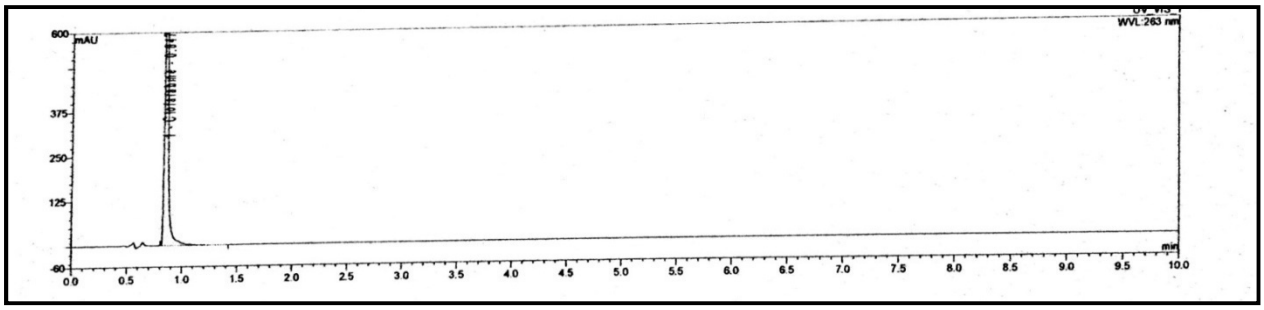

Fig. 7 Trial in mobile phase Buffer: Acetonitrile (70:30)%v/v

Trial 4

Table: 7 Trial in mobile phase Buffer: Acetonitrile (85:15)%v/v

Trial	Mobile Phase	Ratio (%v/v)	Retention Time (min)	Remarks
4	Buffer:Acetonitrile	85 : 15	4.663	Peak shape was not satisfactory

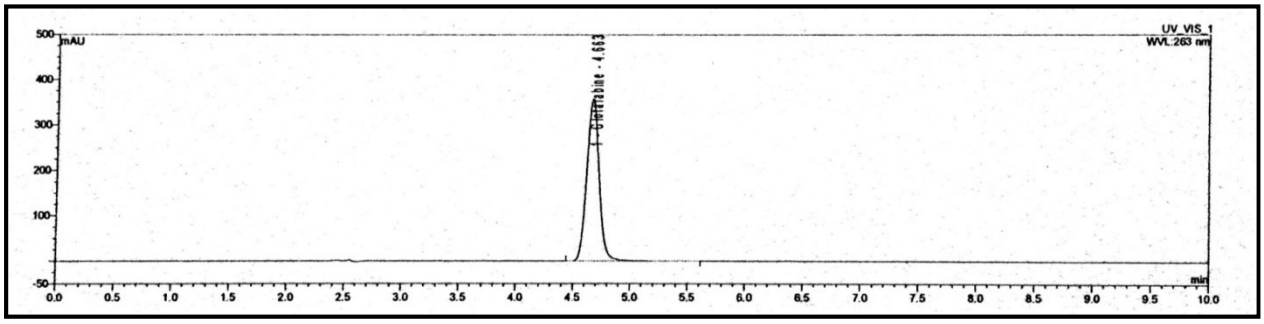

Fig. 8 Trial in mobile phase Buffer: Acetonitrile (85:15)%v/v

Trial 5

Table 8: Trial in mobile phase Buffer: Acetonitrile (90:10)%v/v

Trial	Mobile Phase	Ratio (%v/v)	Retention Time (min)	Remarks
5	Buffer:Acetonitrile	90 : 10	3.706	Peak was sharp and symmetric

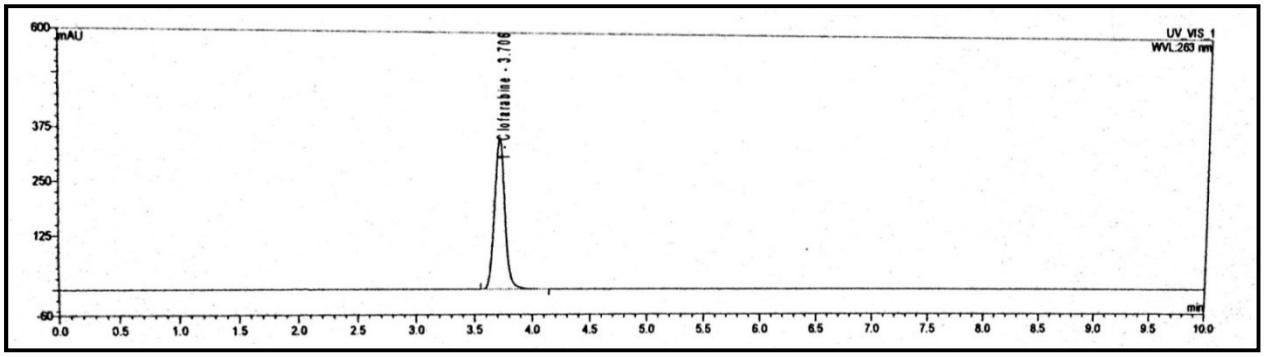

Fig. 9 Trial in mobile phase Buffer: Acetonitrile (90:10)%v/v

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Observation:

After considering the varying combinations of various mobile phases, Buffer: Acetonitrile (90:10)%v/v was finalized as it was showing good peak shapes and a significant amount of resolution.

FORCED DEGRADATION:

Acid Degradation:

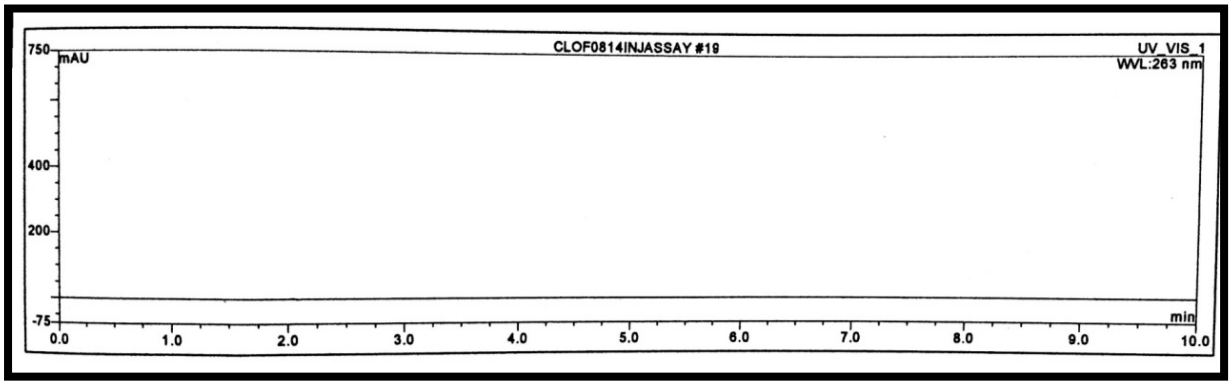

Fig. 10 Blank Chromatogram of Acidic Degradation

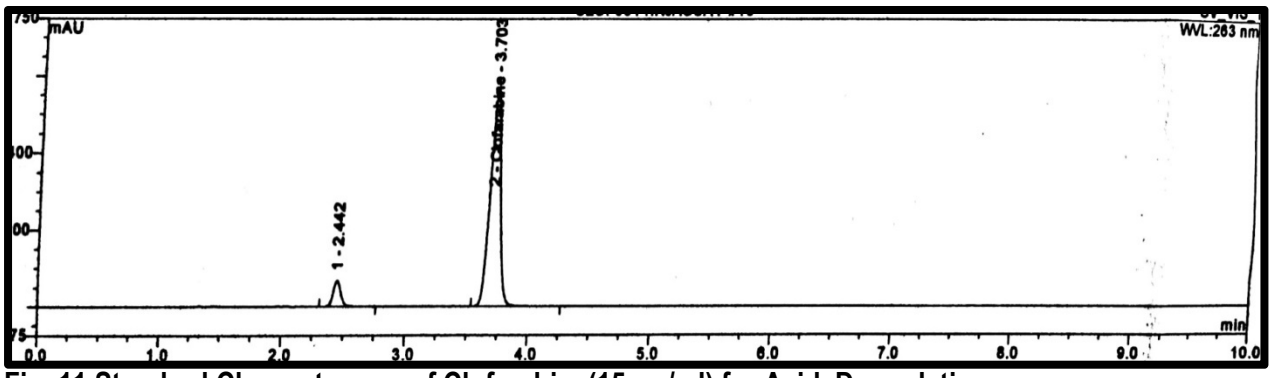

Fig. 11 Standard Chromatogram of Clofarabine(15 µg/ml) for Acid Degradation

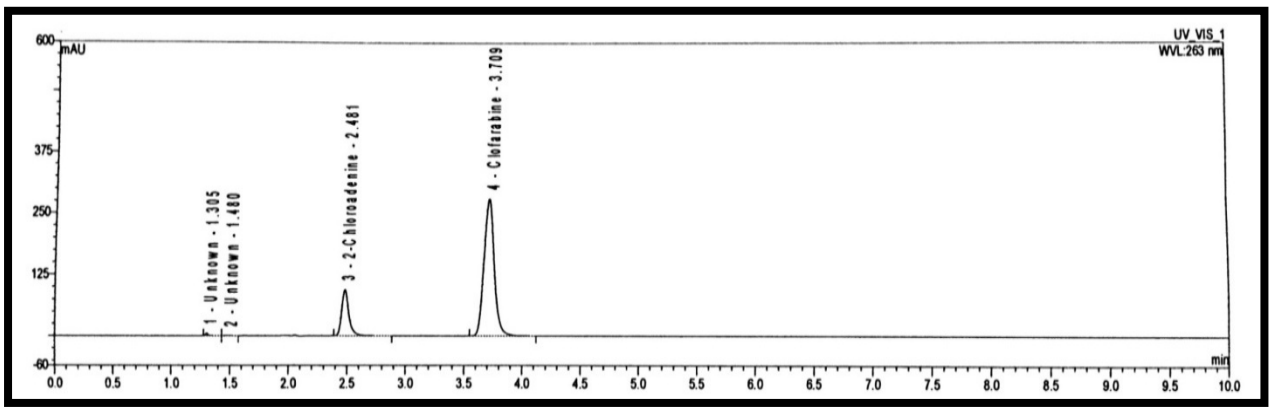

Fig. 12 Test Chromatogram ofClofarabine(15 µg/ml) for Acid Degradation

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Table 9 Acid Degradation

Degradation peaks	Retention Time(min)	Area	Tailing Factor	Theoretical Plates	Resolution
1	1.305	212.933	1.074	4618	2.687
2	1.480	214.958	1.708	4481	1.304
3	2.481	210.592	1.429	7096	6.424
4(CLO)	3.709	1698.681	1.489	3369	2.776

Basic Degradation:

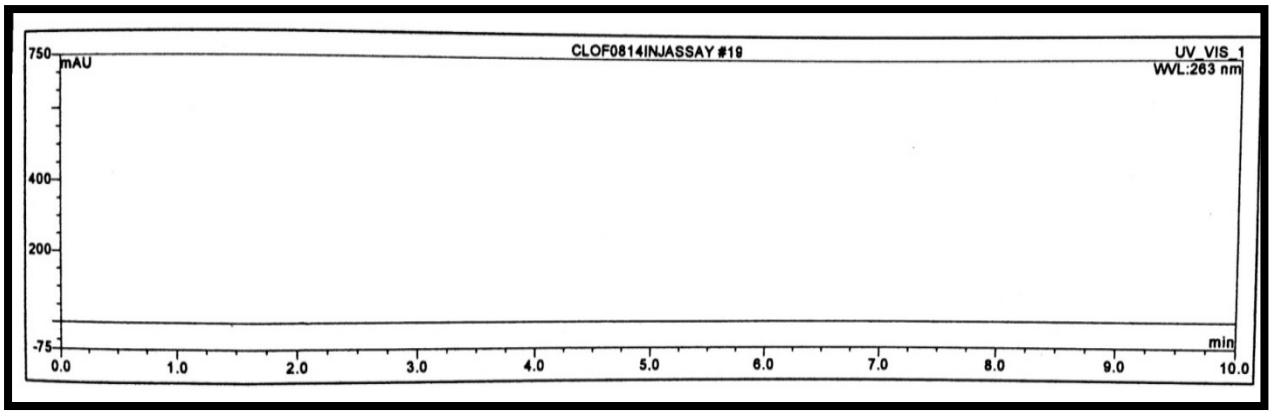

Fig. 13 Blank Chromatogram of Basic Degradation]

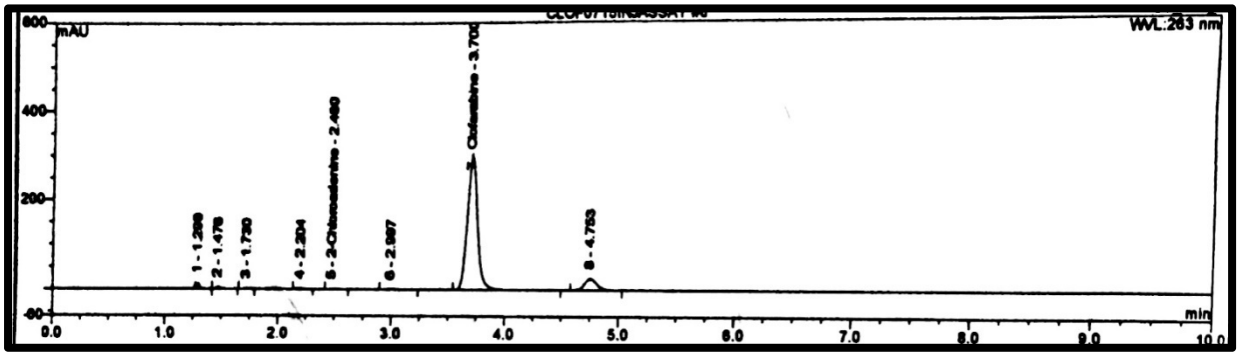

Fig. 14 Standard Chromatogram of Clofarabine(15 µg/ml) for Basic Degradation

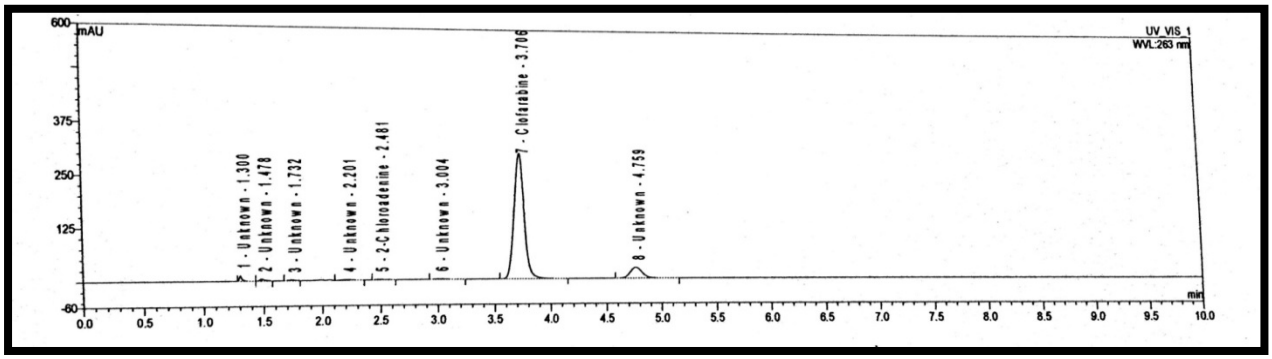

Fig. 15 Test Chromatogram of Clofarabine(15 µg/ml) for Basic Degradation

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Table 10: Basic Degradation

Degradation peaks	Retention Time(min)	Area	Tailing Factor	Theoretical Plates	Resolution
1	1.300	212.933	1.074	4618	2.687
2	1.478	214.958	1.708	4481	1.304
3	1.732	210.592	1.429	7096	6.424
4	2.201	205.867	1.489	3369	2.776
5	2.481	268.354	1.489	3369	2.776
6	3.004	267.214	1.340	7313	5.432
7(CLO)	3.706	1856.484	1.516	4538	1.831
8	4.759	248.157	1.447	3385	2.857

Oxidative Degradation*:*

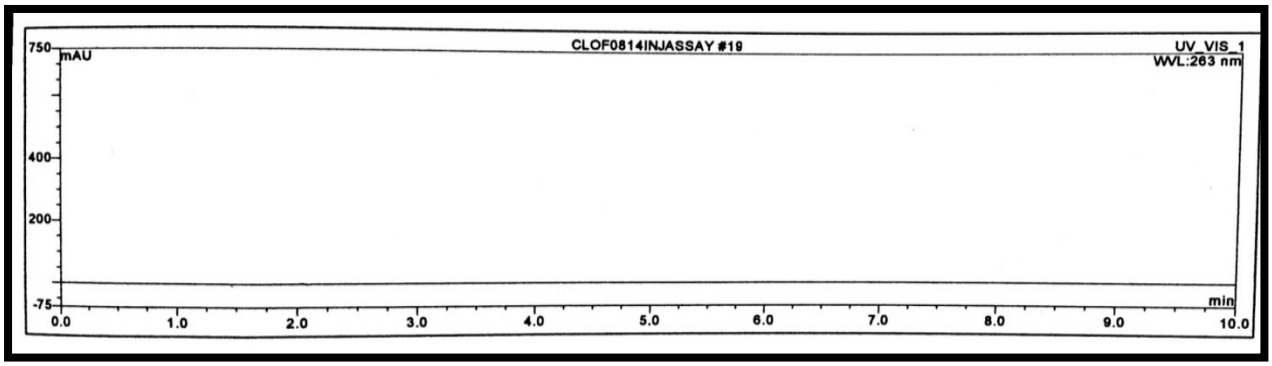

Fig. 16 Blank Chromatogram of Oxidative Degradation

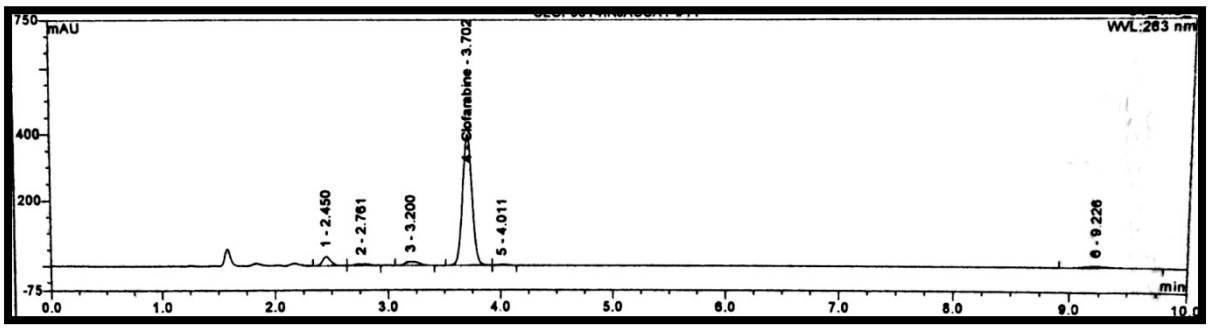

Fig. 17 Standard Chromatogram of Clofarabine (15 µg/ml) for Oxidative Degradation

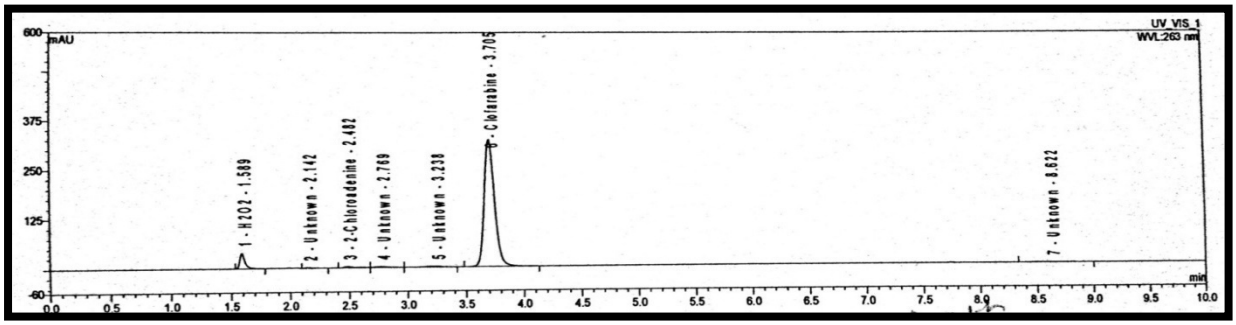

Fig. 18 Test Chromatogram of Clofarabine (15 µg/ml) for Oxidative Degradation

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Table 11: Oxidative Degradation

Degradation peaks	Retention Time(min)	Area	Tailing Factor	Theoretical Plates	Resolution
1	1.589	292.235	1.248	4381	3.687
2	2.142	254.355	1.921	2486	2.304
3	2.482	235.557	1.348	6745	5.424
4	2.769	237.839	1.483	3462	4.776
5	3.238	2372379	1.804	1585	1.776
6(CLO)	3.705	1996.539	1.354	7153	3.432
7	8.622	237.456	1.842	4218	2.831

Thermal Degradation:

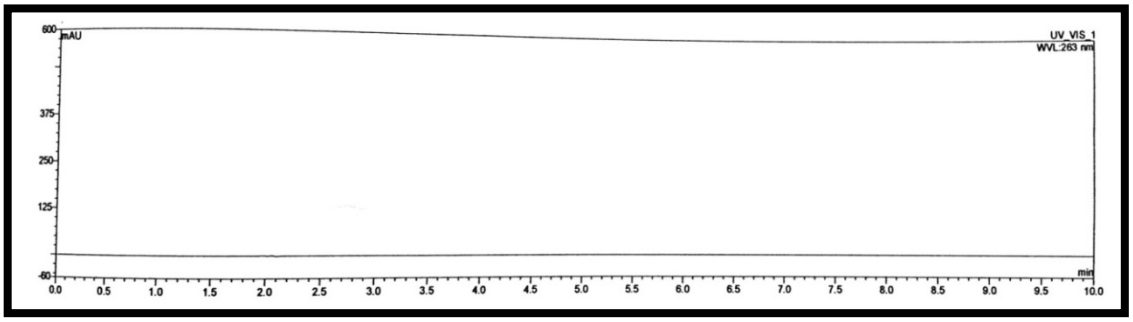

Fig. 19 Blank Chromatogram of Clofarabine for Heat Degradation

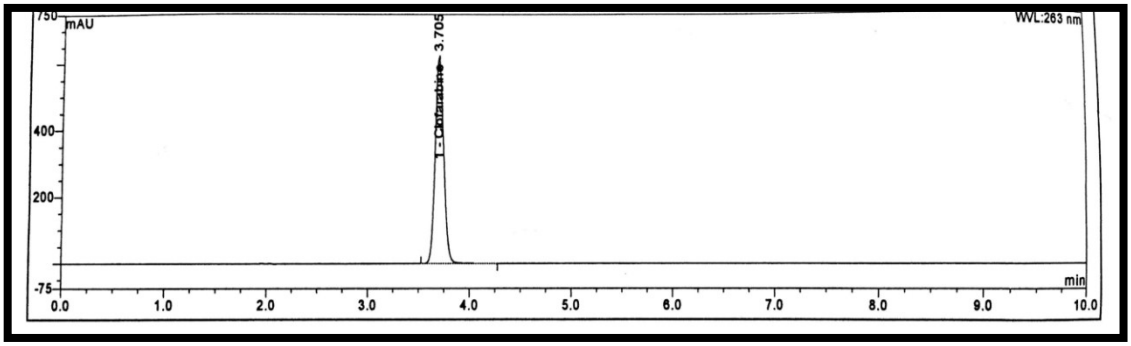

Fig. 20 StandardChromatogram of Clofarabine (15 µg/ml) for Heat Degradation

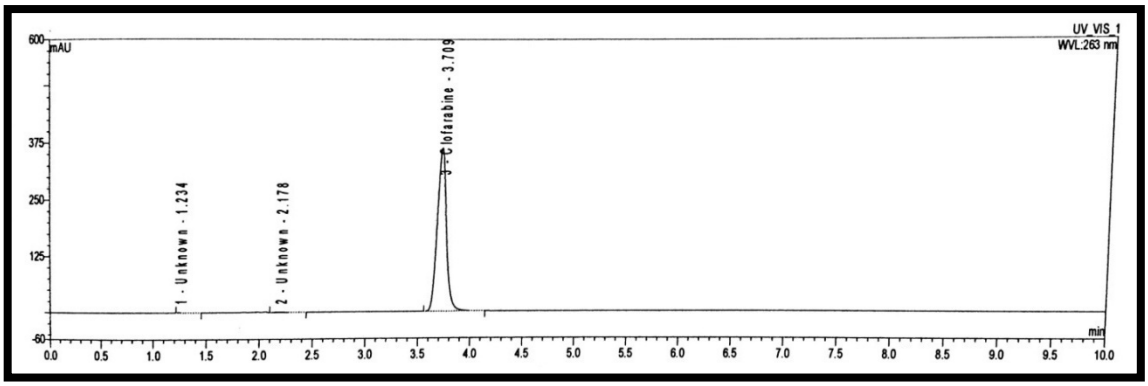

Fig. 21 Test solution Chromatogram of Clofarabine (15 µg/ml) for Heat Degradation

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Table 12: Thermal Degradation

Degradation peaks	Retention Time(min)	Area	Tailing Factor	Theoretical Plates	Resolution
1	1.234	235.645	1.348	3548	2.345
2	2.178	234.845	1.522	9423	1.842
3(CLO)	3.709	2191.691	1.354	3567	3.458

Photolytic Degradation:

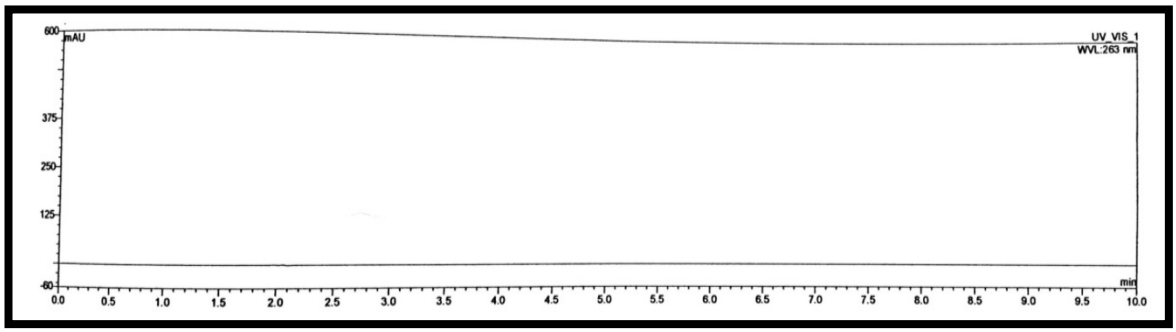

Fig. 22 Blank Chromatogram of Clofarabine for Photolytic Degradation

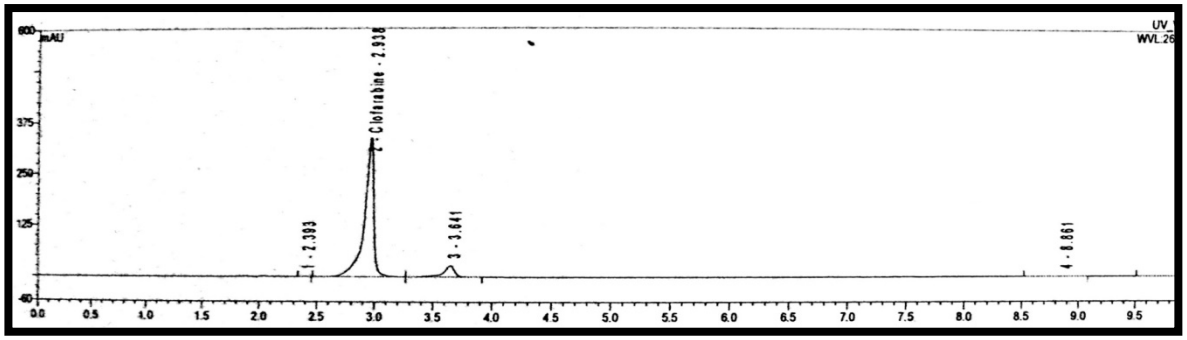

Fig. 23 Standard solution Chromatogram of Clofarabine (15 µg/ml) for Photolytic Degradation

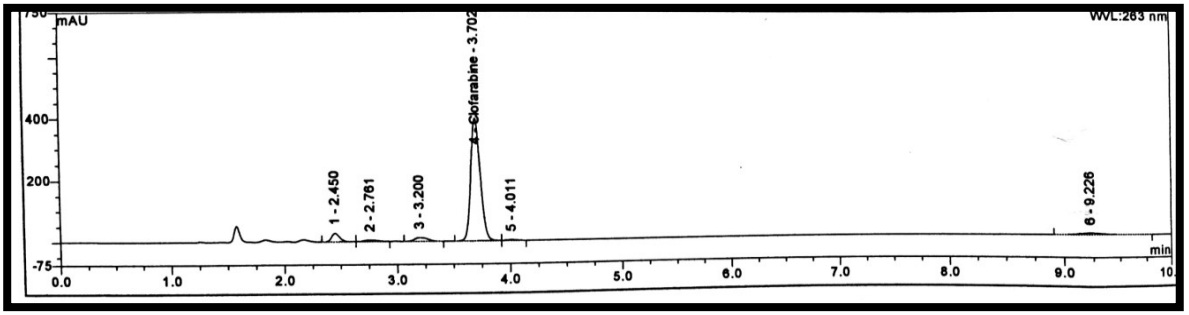

Fig. 24 Test solution Chromatogram of Clofarabine (15 µg/ml) for Photolytic Degradation

Table 13: Photolytic Degradation

Degradation peaks	Retention Time(min)	Area	Tailing Factor	Theoretical Plates	Resolution
1	2.450	282.235	1.364	2145	3.685
2	2.761	259.347	1.675	2467	2.545
3	3.200	248.348	1.654	2746	2.545
4(CLO)	3.702	1596.648	1.314	2987	3.456
5	4.011	231.347	1.875	2667	1.655

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Table 14 Data of Force degradation study of Standard Solution

Condition	Area	Tailing Factor	Theoretical Plates	%degradation
Acid degradation	1698.6815	1.41	7334	18.8%
Alkali degradation	1856.4843	1.21	3339	15.1%
Oxidative degradation	2567.5398	1.54	6066	17.1%
Heat degradation	1962.6915	1.74	4982	11.2%
Photolytic degradation	1896.6488	1.87	2667	15.4%

Table 15 Data of Force degradation study of Test Solution

Condition	Area	Tailing Factor	Theoretical Plates	%degradation
Acid degradation	2564.5625	1.34	2745	17.5%
Alkali degradation	1854.4655	1.15	5855	15.04%
Oxidative degradation	2547.7652	1.31	8771	16.21%
Heat degradation	2345.8782	1.51	3632	10.1%
Photolytic degradation	1356.3547	1.42	3656	16.6%

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