INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CLOFARABINE IN BULK AND ITS FORMULATION

K. Kranthi Kiran*, B. Bhuvaneswari Bai, CH. Karishma, Anas Yousif Abbdelghne Ahmed, Mohammed Ezaldien Abdo Abdalrahiem, E. Triveni, M. Smiely, N. Anusha

Department of Pharmaceutical Analysis, Nova College of Pharmaceutical Education and Research, Jupudi, Andhra Pradesh, India

ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Clofarabine by RP-HPLC technique. Chromatographic conditions used are stationary phase Symmetry C_{18} column 150 mm x 4.6 mm, 3.5µ., Use Buffer 0.1% ortho phosphoric acid : acetonitrile (30:70v/v) and flow rate was maintained at 1.0 ml/min, detection wave length was 219nm, column temperature was set as ambient and diluent was mobile phase conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 10% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 1.2 for intermediate precision. LOD and LOQ are 0.10µg/ml and1.00µg/ml respectively. By using above method assay of marketed formulation was carried out 100.01% was present. Degradation studies of Clofarabine were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

Key Words: HPLC Clofarabine. Method development. ICH Guidelines

Author for correspondence
K. Kranthi Kiran,
Department of Pharmaceutical Analysis,
Nova College of Pharmaceutical Education and Research, Jupudi, Andhra Pradesh, India.
Email: kothapallikranthikiran@gmail.com

INTRODUCTION

The term chromatography (Greek: Khromatos-color and graphos- written) and its principles were first discovered by Mikhail Tswett. Rrichard Kehn and his co-workers applied adsorption chromatography as a preparative method for the separation at carotene in to its components. The second board type of chromatography involving partition between two liquids rather than absorption by a solid from a liquid was proposed by Martin and Synge. They used silica gel packed in column as a support for a stationary phase and were awarded Nobel Prize in1952 for his work (1). Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients along with a number of inert materials like diluents, disintegrants, colors and flavors. In order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components prior to quantitative analysis. Amongst

the most powerful techniques available to the analyst for the separation of these mixtures, a group of highly efficient methods called as chromatography are widely used. It is a group of technique, which works, on the principle of separation of components of a mixture, that depending on their affinities for the solutes, between two immiscible phases. One of the phases is an affixed bed other is a fluid, which moves through the surface of the fixed phase. The fixed phase is called stationary phase and the other is termed as mobile phase. Depending on the type of chromatography employed, the mobile phase may be a pure liquid or a mixture of solvents (eg. Buffer) or it may be gas (pure or homogeneous mixture).Chromatographic methods can be classified according to the nature of the stationary and mobile phases. HPLC is one among the most useful tools, available for quantitative analysis. High Performance Liquid Chromatography is a special branch of column chromatography, in which the mobile phase ids force through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially. Clofarabine is purine nucleoside antimetabolite marketed in the US and Canada as Clolar. In Europe and Australia/New Zealand the product is marketed under the name Evoltra.It is FDA-approved for treating relapsed or refractory acute lymphoblastic leukaemia(ALL) in children after at least two other types of treatment have failed. It is not known if it 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 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 ribonucleotide reductase, 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. Clofarabine is sequentially metabolized intracellularly to the 5'-monophosphate metabolite by deoxycytidine kinase and mono- and di-phosphokinases to the active 5'-triphosphate metabolite.

Clofarabine has high affinity for the activating phosphorylating enzyme, deoxycytidine kinase, equal to or greater than that of the natural substrate, deoxycytidine (2-5).

Aim is to develop and validate RP-HPLC method for the determination of Clofarabine dosage form and to develop and validate calibration curve method and derivative method for the determination of Clofarabine in bulk and tablet dosage forms.

MATERIALS AND METHOD

Determenation of Working Wavelength (λ_{max})

The wavelength of maximum absorption of the solution of the drug in acetonitrile were scanned using Photodiode array within the wavelength region of 200–400 nm against acetonitrile as blank. The spectra of drug shows at 219 nm, Thus 219 nm was selected as detector wavelength for the HPLC chromatographic method.

Preparation of Phosphoric acid Buffer pH-2.5 (6-7)

1ml Ortho Phosphoric acid was dissolved in 1lt Water and observes the P^H at 2.5.Buffer was filtered through 0.45µ membrane filter.

Preparation of Standard Solution and sample solution:

Clofarabine: Weigh accurately about 80 mg of Clofarabine working standard into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5mL to 50 mL

With the diluent from which different concentrations were prepared according to the linearity range. Diluent used was mobile phase.

Preparation of Sample solution:

Take 5 injection bottles and weigh powder then take 4 bottles equivalent of sample into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 mL to 50 mL with the diluent. Filter through 0.45µ Nylon syringe filter.

Method Validation (8-9)

The validation of HPLC method for the determination of Clofarabine as per the protocol and to demonstrate that the method is appropriate for its intended use was studied for the following parameters. All the validation parameters were carried out according to ICH.

RESULTS AND DISCUSSION

Determenation of Working Wavelength (λ_{max})

The spectra of drug shows at 219 nm (Fig-1), Thus 219 nm was selected as detector wavelength for the HPLC chromatographic method.

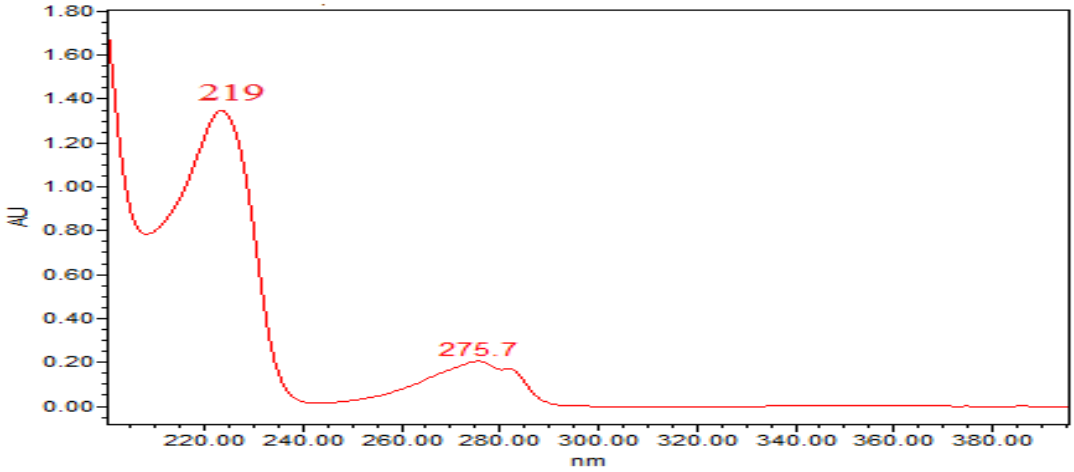

Fig-1 PDA-Spectrum of Clofarabine

Optimized chromatography

A sharp pin pointed peak was observed, so this trail was considered. The Clofarabine was observed at 3.4min with peak area 2599178, theoretical plates 5880 and tailing factor 0.99. Because of the satisfactory results, less retention time, this trial was optimized (Table-1).

Table-1 Optimized chromatography

	Name	Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Clofarabine	3.472	2599178	100.00		0.09	5880

Linearity

Linearity of an analytical method is its ability to elicit the test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between peak area Vs concentration of Clofarabine were in the range of 8-120µg/mL. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation. Correlation coefficient should be not less than 0.999 (Table-2 and fig-2).

Table-2 Results of linearity for Clofarabine

S.No	Clofarabine	
	Conc.(µg/mL)	Peak area
1	8	257486
2	20	614156
3	40	1237419
4	80	2593808
5	100	3241608
6	120	3925113
Regression equation	y = 32736x-25329	
Slope	32736.12	
Intercept	25328.68	
R^2	0.999	

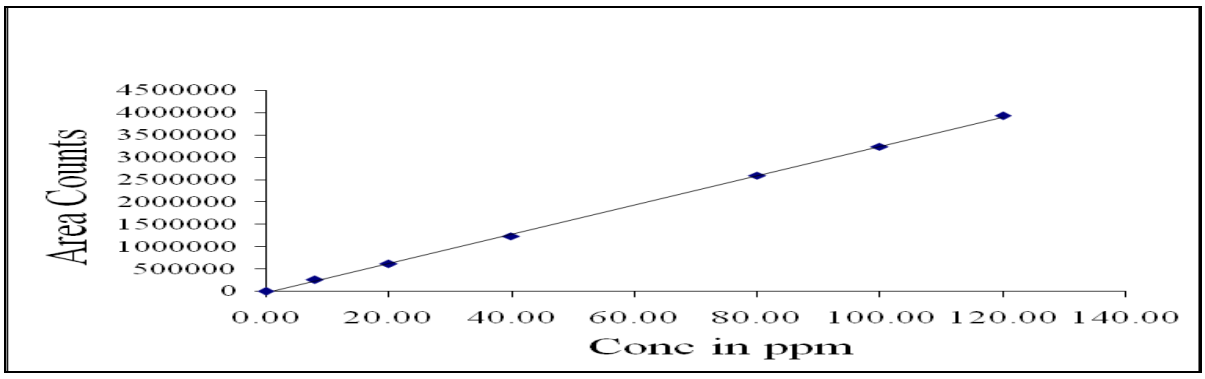

Fig-2 calibration curve for Clofarabine at 219 nm

Accuracy

The accuracy experiments were carried out by the standard addition method at 50%,100% and 150% levels of linearity and the recoveries obtained were 100.02 to 100.30% for Clofarabine. The mean %recovery at each level should not be less than 98%-102% (Table-3).

Table-3 Accuracy results of Clofarabine by RP-HPLC method

S.NO	% Level of Std	Conc. Of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	% R.S.D
1	50	0.41	1390583 1398385 1394635	100.51	100.2	100.25	0.0462
2	100	0.82	2570991 2523255 2510832	100.04	99.9		
3	150	1.23	3800434 3831334 3804702	100.94	100.04		

Stability Study

80µg/ml Clofarabine was prepared and stability study was carried out at different time intervals and the results were recorded (Table-4).

Table-4 Results of stability study

Time period (hours)	**Clofarabine**			
	Retention time	Peak area	Tailing factor	Plate count
Initial	3.472	2562536	1.10	5821
6 Hrs	3.493	2530638	1.11	5704
12 Hrs	3.476	2501037	1.12	5713
18Hrs	3.486	2475204	1.11	5851

CONCLUSION

An attempt has been made to develop a new stability indicating validated RP-HPLC method for the estimation of Clofarabine in bulk and in dosage form. As the literature survey revealed that only two methods are available for estimation of Clofarabine in bulk and in dosage forms so there is a need for a simple, economical and proper method of estimation of Clofarabine bulk and in dosage form. Symmetry C18, 150mm x 4.6mm, 3.5µm column with PDA detector with an injection volume of 10 µL was injected and eluted with the mobile phase containing ACN: Buffer (70: 30v/v). This is pumped at a flow rate of 1mL/min and detected by PDA (219nm) detector. The peak of Clofarabine was eluted at retention times of 3.4 min respectively. After method was developed, it was validated according to ICH guidelines for system suitability, specificity and linearity, sensitivity parameters, precision, accuracy and robustness studies. the validation results were found well within the limits(%RSD of areas were<2 for assay and recoveries in the range of 98%-102% for assay,r^2>0.999) indicating that the developed method is simple, rapid, accurate, precise, specific, robust and economical and less time consuming. Hence, the developed chromatographic method for Clofarabine is said to be rapid, simple, precise, accurate, specific and cost effective that can be effectively applied for the routine analysis.

REFERENCES

1. M. Kobylinska, and k. Kobylinska J. of Chrom. B, *Analytical sciences and applications* 7, 1207 (2000).
2. M. Kobylinska, and k. Kobylinska J. of Chrom. B, *Analytical sciences and applications* 7, 1207 (2000).
3. Venkata Narasimha Rao.G et al., Development of supercritical fluid (carbon dioxide) based ultra performance convergence chromatographic stability indicating assay method for the determination of clofarabine in injection, *Analytical Methods*, Issue 24, 2013.
4. Xiaowei Tu, Youming Lu et al., A sensitive LC–MS/MS method for quantifying clofarabine triphosphate concentrations in human peripheral blood mononuclear cells . *Journal of chromatography* B, vol 964, Pages 202-207, 2014.
5. Rie Nishi, Takahiro Yamauchi et al., Determination of Clofarabine Triphosphate Concentrations in Leukemia Cells Using Sensitive, Isocratic High-performance Liquid Chromatography. International *Journal of cancer research and treatment*, 34 (4) 1657-1662, 2014.
6. ICH, Q2A *Text on validation of analytical procedures*, international conference on Harmonization, Oct. 1994.
7. ICH, Q2A *Text on validation of analytical procedures*, international conference on Harmonization, Oct. 1994.
8. ICH, Q3B *validation of analytical procedures: methodology*, international conference on Harmonization, Nov. 1996.
9. ICH, Q3B *validation of analytical procedures*: methodology, international conference on Harmonization, Nov. 1996.