THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

BHAIRAM BHOOMAIAH**et al, The** ***Experiment, August.2012 Vol. .2 (2), 93-101***

RP-HPLC METHOD FOR THE QUANTIFICATION OF CLADRIBINE IN PHARMACEUTICAL FORMULATION

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Cladribine in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Zodiac C_{18} (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: Water in the ratio of 64:22:14%, v/v/v. The UV detection wavelength was 231 nm and 20µl sample was injected. The retention time for Cladribine was 5.530 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Cladribine in tablet dosage form and bulk drug.

KEYWORDS: Cladribine, RP-HPLC, UV detection, recovery, precise, 231 nm

INTRODUCTION

Cladribine is a drug used to treat hairy cell leukemia and multiple sclerosis. Its chemical name is 2-chlorodeoxyadenosine. As a purine analog, it is a synthetic anti-cancer agent that also suppresses the immune system. Chemically, it mimics the nucleoside adenosine and thus inhibits the enzyme adenosine deaminase, which interferes with the cell's ability to process DNA. It is easily destroyed by normal cells except for blood cells, with the result that it produces relatively few side effects and results in very little non-target cell loss. Cladribine (as injections) is indicated [approved] for the treatment of symptomatic hairy cell leukemia.

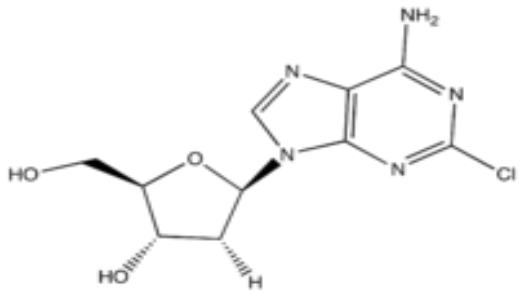


Figure 1: Structure of Cladribine

Cladribine is structurally related to fludarabine and pentostatin but has a different mechanism of action. Although the exact mechanism of action has not been fully determined, evidence shows that cladribine is phosphorylated by deoxycytidine kinase to the nucleotidecladribine triphosphate (CdATP; 2-chloro-2′-deoxyadenosine 5′-triphosphate), which accumulates and is incorporated into DNA in cells such as lymphocytes that contain high levels of deoxycytidine kinase and low levels of deoxynucleotidase, resulting in DNA strand breakage and inhibition of DNA synthesis and repair. High levels of CdATP also appear to inhibit ribonucleotide reductase, which leads to an imbalance in triphosphorylated deoxynucleotide (dNTP) pools and subsequent DNA strand breaks, inhibition of DNA synthesis and repair, nicotinamide adenine dinucleotide (NAD) and ATP depletion, and cell death. Unlike other antimetabolite drugs, cladribine has cytotoxic effects on resting as well as proliferating lymphocytes. However, it does cause cells to

accumulate at the G1/S phase junction, suggesting that cytotoxicity is associated with events critical to cell entry into S phase. It also binds purine nucleoside phosphorylase (PNP), however no relationship between this binding and a mechanism of action has been established.

Existing studies estimate that from 18 % to 42 % of patients will experience a fever after cladribine infusion. This is usually a transient fever which can be treated with acetaminophen (paracetamol). These fevers, which resolve in less than 48 hours, have no evidence of being related to infection.

Very few methods have been developed for the estimation of Cladribine in relative substances, in plasma by using hplc, and Spectroflurimetry. We develop a method of simple, sensitive and specific for the quantitative estimation of Cladribine in pharmaceutical dosage form using commercially available solvents and apply it to pharmaceutical dosage forms.

EXPERIMENTAL

Materials

Working standard of Cladribine was obtained from well reputed research laboratories. HPLC grade Water, Methanol, Acetonitrile was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample, PEAK LC software was used. UV 2301 Specophotometer was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Cladribine were scanned in the range of 200 -400 nm against mobile phase as a blank. Cladribine showed maximum absorbance at 238 nm. So the wavelength selected for the determination of Cladribine was 231 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Zodiac 100-5 C18. 250×4.6mm, manual injector Rheodyne valve) with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisted of a Methanol: Acetonitrile: Water in the ratio of 64:22:14%, v/v/v. Injections were carried out using a 20µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 231 nm with 10 min runtime.

Standard and sample solutions

A 10 mg amount of Cladribine reference substance was accurately weighed dissolved in10ml mobile phase in a 10 ml volumetric flask to obtain 1000ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations of 210 to 60ppm.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Cladribine was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 26 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 120 µg/ml.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Api Concentration	120 ppm
Mobile Phase	Methanol: Acetonitrile: Water in the ratio of 64:22:14%, v/v/v
Wavelength	231 nm
Column	C_{18} Column
P^H	5.3
Pump Pressure	11.2 MPa
Temperature	Ambient
Retention Time	5.53 min
Run Time	12min
Area	513263.7
Th. Plates	10318
Tailing Factor	1.23

Table.1 System suitability parameters

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

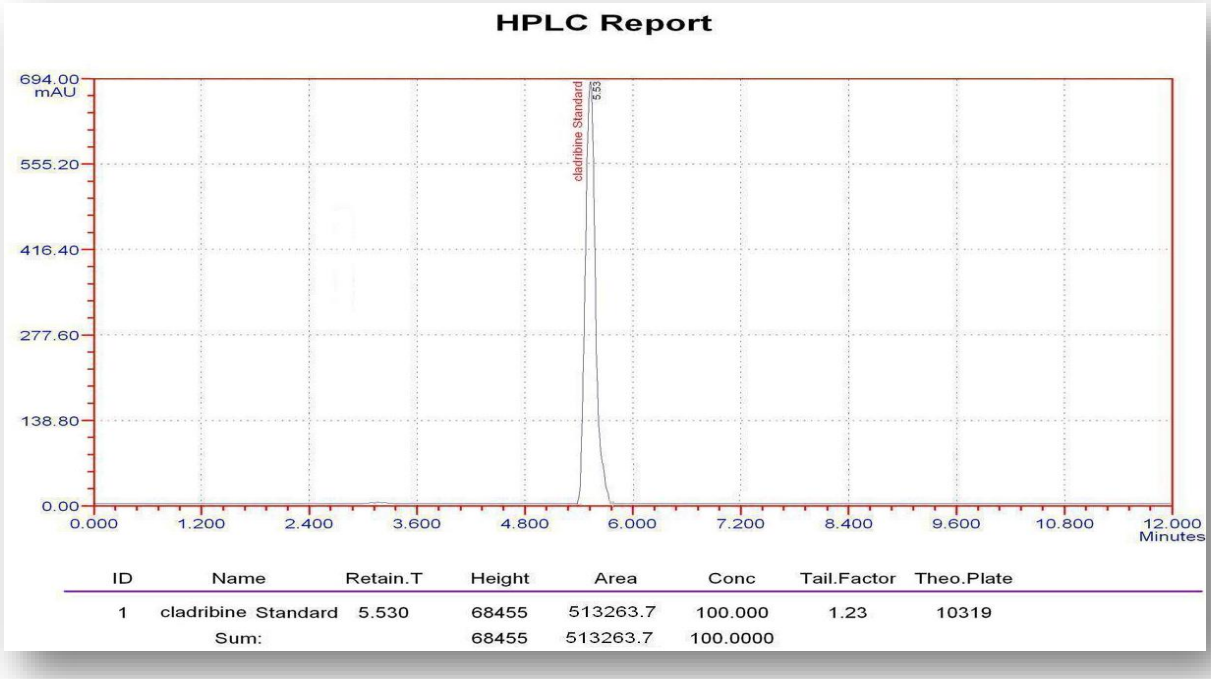


Figure.2: Standard Chromatogram

Range of linearity

Standard curves were constructed using seven standard concentrations in a range of 60, 90, 120, 150, 180, 210µg/ml for Cladribine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 6180.709+ 4200.982x (r= 0.9999). Linearity values can shown in Table: 2

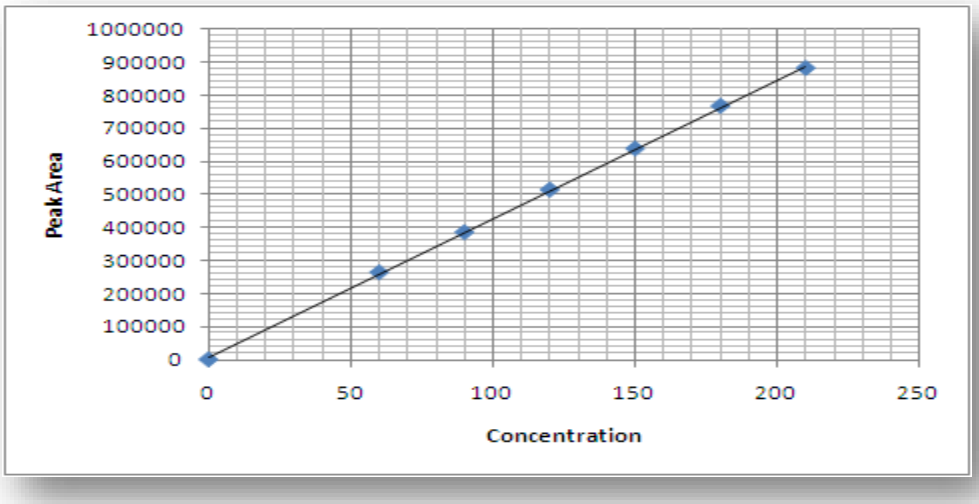
Level	**Concentration of Cladribine In ppm**	**peak area**
Level - 1	60	263547.1
Level - 2	90	384635.5
Level - 3	120	513263.7
Level - 4	150	637529.3
Level - 5	180	766321.8
Level – 6	210	880763.2
Range:60-210ppm	Slope Intercept CC	4200.982 6180.709 0.9999

Table.2: Linearity results of Cladribine

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE



Graph.1: Calibration curve

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as Intraday precision, Inter day precision.

Intraday precision

To study the Intraday precision, six replicate standard solutions (120ppm) of Cadribine were prepared and injected using the set chromatographic conditions. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.48, which is well within the acceptance criteria of not more than 2.0%. Results of Intraday system precision studies are shown in Table.

Intraday Precision:

Sample (µg/ml)	Area
1	513263.7
2	513921.4
3	512832.9
4	512936.5
5	519253.6
6	513292.5
RSD	**0.48**

Table.3: Intraday Precision results of Cladribine

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Interday Precision:

To study the Inter day precision, six replicates of standard solutions (120ppm) of Cadribine was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated for peak responses and it was found to be 0.56%, which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.

Sample (µg/ml)	Area
1	524965
2	518622
3	517249
4	520593
5	522965
6	522954
RSD	**0.56**

Table.3: Interday Precision results of Cladribine

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was diapered. After 0.4ppm dilution, Peak was not clearly observed. So it confirms that 0.4ppm is Limit of Detection for Cadribine using the current method and Limit of Quantification is 1.3ppm.

LOD	0.4ppm
LOQ	1.3 ppm

Table.4: LOD and LOQ Results of Cadribine

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	513264	……
2	Mobile Phase	69:17:14%	519513	1.22
		59:27:14%	521860	1.67
3	Wavelength	236nm	515627	0.46
		226nm	510604	0.52
4	Flow rate	0.8ml/min	520964	1.50
		1.2ml/min	517888	0.90

Table.5 Robustness results

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Recovery:

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. The standard addition method was performed at 50%, 100% and 150% level of 60ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.67 to 101.55% were obtained by the proposed method. This indicates that the proposed method was accurate.

% Recovery	Cadribine				
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	60	30	90	88.80	98.67
	60	30	90	89.21	99.12
150%	60	30	90	89.21	98.96
100%	60	60	120	118.90	99.09
	60	60	120	121.03	100.86
	60	60	120	120.81	100.68
150%	60	90	150	151.46	100.97
	60	90	150	152.32	101.55
	60	90	150	151.93	101.29

Table.6: Recovery results

Ruggedness:

Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentration of standard (120ppm) which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation

Sample (µg/ml)	Area
1	519437
2	517687
3	519491
4	526725
5	525325
6	527192
RSD	**0.81**

Table.7: Ruggedness results

FORMULATION ANALYSIS

A composite of 20 tablets (Movectro -10mg) was prepared by grinding them to a fine, uniform size powder. 10 mg of, Cladribine was accurately weighted and quantitatively transferred into a 10ml volumetric flask and the solution was sonicated for 15 min. The flask

was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 120µg/ml.

Formulation	Dosage	Concentration	Amount found	% Assay
Movectro	10mg	120ppm	118.94	99.12

Table.8: Formulation results.

CONCLUSION

The proposed method for the assay of Cladribine in tablets or capsules is very simple and rapid. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFERENCES

1. Else M, Dearden CE, Matutes E, *et al.* (March 2009). "Long-term follow-up of 233 patients with hairy cell leukaemia, treated initially with pentostatin or cladribine, at a median of 16 years from diagnosis". *Br. J. Haematol.* 145 (6): 733–40.
2. Warnke C, Wiendl H, Hartung HP, Stuve O, Kieseier BC: Identification of targets and new developments in the treatment of multiple sclerosis – focus on cladribine. Drug Des Devel Ther. 2010 Jul 21;4:117-26.
3. Sigal DS, Miller HJ, Schram ED, Saven A: Beyond hairy cell: the activity of cladribine in other hematologic malignancies. Blood. 2010 Jul 15.
4. Khalid BA, Hamilton NT, Cauchi MN: Binding of thyroid microsomes by lymphocytes from patients with thyroid disease and normal subjects. Clin Exp Immunol. 1976 Jan;23(1):28-32.
5. Sampat K, Kantarjian H, Borthakur G: Clofarabine: emerging role in leukemias. Expert Opin Investig Drugs. 2009 Oct;18(10):1559-64
6. Kantarjian HM, Jeha S, Gandhi V, Wess M, Faderl S: Clofarabine: past, present, and future. Leuk Lymphoma. 2007 Oct;48(10):1922-30.
7. Van Den Neste E, Delannoy A, Vandercam B, *et al.* (1996). "Infectious complications after 2-chlorodeoxyadenosine therapy". *Eur. J. Haematol.* 56 (4): 235–40.
8. Saven A, Burian C, Adusumalli J, Koziol JA (1999). "Filgrastim for cladribine-induced neutropenic fever in patients with hairy cell leukemia". *Blood* 93 (8): 2471–7.
9. Nelson MC, Hogan DK (1995). "The role of cladribine in the treatment of lymphoid malignancies". *Oncol Nurs Forum* 22 (9): 1395–400.
10. Lauria F, Benfenati D, Raspadori D, *et al.* (1994). "Retreatment with 2-CdA of progressed HCL patients". *Leuk. Lymphoma* 14 Suppl 1: 143–5.
11. Yeung PK, Ferguson C, Jarrar A, King B, Li ML, Development and validation of a sensitive and specific HPLC assay of cladribine for pharmacokinetics studies in rats, J Pharm Pharm Sci. 2007;10(2):231-6.
12. James V. Weber, Keith Sampino, Richard Dunphy, David J. Burinsky*, Thomas Williams, Michael G. Motto, Characterization of cladribine and its related compounds by high-performance liquid chromatography/mass spectrometry, Journal of Pharmaceutical Sciences Volume 83, Issue 4, pages 525–531, April 1994.
13. Gajewska M, Pawiński T, Dzierzgowska-Szmidt A, Kazimierczuk Z, Spectrofluorimetric determination of 2-chloro-2'-deoxyadenosine (cladribine) in human plasma by photochemical reaction and chloroacetaldehyde derivatization, Pharmazie. 1995 Jul; 50(7):459-60.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

BHAIRAM BHOOMAIAH*[1], Dr.A.JAYA SREE[2]

Aurabindo Pharma Pvt Ltd, Hydrabad

Centre for chemical Sceiences and Technology, Jawaharlal Nehru Technological University, Hydrabad.

Corresponding Author: bhoom.analytical@gmail.com