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RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION FOR CYCLAM CONTENT IN THE PLERIXAFOR BY RPHPLC

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ABSTRACT

Plerixafor is a bicyclam derivative. Cyclamis the key starting material for the synthesis of Plerixafor, hence the detection and quantification of cyclam in the Plerixafor is very essential. A reverse phase HPLC method is developed and validated for the separation and quantification of cyclam content in Plerixafor. Separation was achieved on inertsil ODS -3V (250mm*4.6mm*5µm) column with gradient elution. Sodium perchlorate mono hydrate(0.01mM) with pH 2.0 and methanol (in the ration of 97:3:: v/v) was employed as mobile phase-A, whereas methanol and water (in the ratio of 90:10::v/v)was employed as mobile phase-B with run time 45min.The flow rate was 1mL/min and detection wave length was 200nm.Method validated with reference to the ICH guidelines. Method is identified as linear form the range 0.0025mg/mL to 0.01mg/mL, with injection volume 20µL.Minimum quantification level achieved as 0.0025mg/mL, where as the minimum detection level for the cyclam was 0.0005mg/mL.

Key words: Plerixafor, Cyclam, RPHPLC, Variable wavelength detector, Validation.

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INTRODUCTION

Plerixafor, chemically named as 1, 1'-(1, 4-Phenylenebis (methylene))-bis-1, 4,811-tetraazacyclotetradecane (http://en. wikipedia.org/ wiki/chromatography and Chandran *et al.,* 2007.). It is a hematopoietic stem cell mobilizer. It is used to stimulate the release of stem cells from the bone marrow in to the blood in patients with non-Hodgkin lymphoma and multiple myeloma for the purpose of stimulating the immune system. The stem cells are collected and used in autologous stem cell transplantation to replace blood forming cells that were destroyed by chemotherapy (4). Several procedures are reported for the related impurities determination in the Plerixafor product, but no method is available for cyclam content determination in Plerixafor product (12). Somehow methods are available for cyclam content with RI detection, but no method is available with UV detection. Two molecules of cyclam will react with 1, 4-bis (bromo methyl) benzene in the presence of base and form a Plerixafor. Hence quantification of cyclam in the Plerixafor is much important to ensure the complete formation of plerixafor product.

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Experimental Section

Chemicals: Cyclam Sigma Aldrich AR grade used as standard;Sodium hydroxide with rankem grade was procured; Acetonitrile was procured as Merck grade (Mumbai, India); Sodium perchlorate mono hydrate with Merck grade was purchased (Mumbai, India);Methanol purchased with rankem grade; Per chloric acid with Merck grade was purchased (Mumbai, India).

Instrumentation and chromatographic conditions: RPHPLC instrument with make Agilent Infinity 1260 series and sample and standard weights are taken by using analytical balance with make Sartorius and model MSA 225S-100-DA.Mobile phase solution were filtered by using 0.45 µm PTFE filters and sonicated well and degassed. Empower -3 software was used for processing the chromatographic data. Chromatographic conditions for quantification of cyclam content in the plerixafor were column temperature 30°C, flow rate 1.0 mL/min, detector wave length 200nm, injection volume 20µL, sample concentration 5.0mg/mL, diluent was Methanol: 0.1N NaOH solution (1:1::v/v) and run time is 45 min(Sethi, 2001 and Lauren Veltri *et al.,* 2015).

Sample preparation: Plerixafor standard wasprepared with concentration of 5.0 mg/ mL by dissolving in the diluent.

Cyclam standard stock solution was prepared with 0.5mg/mL by dissolving in the 0.1N NaOH solution. Spiked solution of plerixafor at different concentration of cyclam were prepared by adding the adequate amount of cyclam standard stock solution in the plerixafor standard solution.

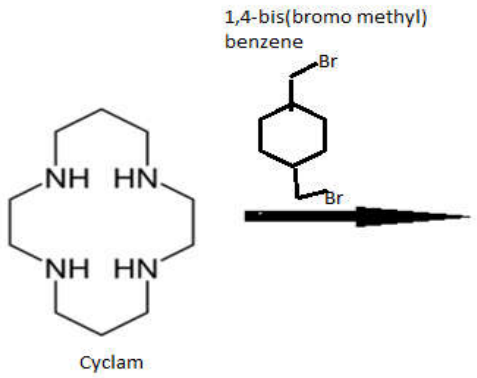


Figure A. Indicates Cyclam structure

Molecular formula: C10H24N4
Molecular Mass: 200.32g/mol

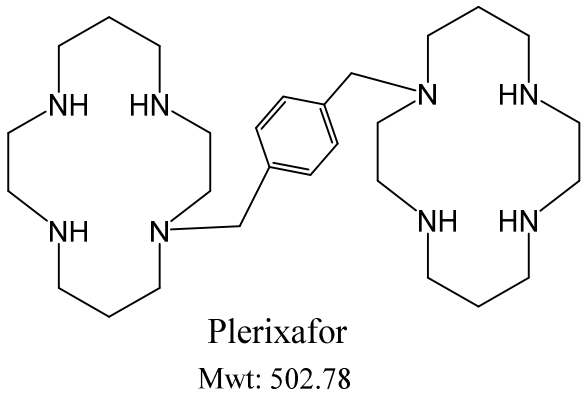


Figure B. Indicates the Plerixafor structure

Molecular formula: C28H54N8
Molecular Mass: 502.79g/mol

RESULTS AND DISCUSSION

To develop the quantitate HPLC method for cyclam content in the Plerixafor, several development trial are taken up by changing the concentrations of mobile phases, different columns and also different detector also (Luis G Alves *et al.,* 2017). Upon the several development trails, consistent and robust method was achieved on inertsil ODS -3V (250mm*4.6mm*5µm) with variable wavelength detector at wavelength 200nm.Peak purity for the cyclam in the Plerixafor was checked by photodiode array detector (Mihaela Vlassa et and Attila Bende, 2005; Dennis *et al.,* 2016 and Venkata Narasimha Rao *et al.,* 2017).

Method Validation: The described cyclam content in the plerixafor samples was validated as per the ICH guidelines (Hamini Reddy *et al.,* 2015).

Limit of detection and Limit of quantification: LOD and LOQ of cyclam content was established by preparing the solution form the known concentration of stock solution to achieve the signal-to-noise ratio as 3:1 and 10:1.Carried out the LOQ precision by preparing six individual solution of plerixafor with LOQ concentration of cyclam. Accuracy at LOQ was also carried out by preparing the Plerixafor recovery solutions with cyclam at LOQ level concentration. The results for the precision at LOQ and accuracy at LOQ are tabulated in the table-2.

Method precision: Method precision was carried out by preparing the six individual plerixafor solutions with spiking of 0.15 %(0.0075mg/mL) cyclam in each preparation. Ruggedness was carried out through performing the same experiment by different analyst, different column and different instrument in the same laboratory. Results are tabulated in Table-2.

Linearity: Linearity carried out by preparing the cyclam solutions at different concentration levels (i.e.LOQ, 50%, 75%, 100%, 125% and 150%)in the diluent. Calculated the values of correlation coefficient and %Y-intercept. Results are tabulated in Table-2.

Table 1.

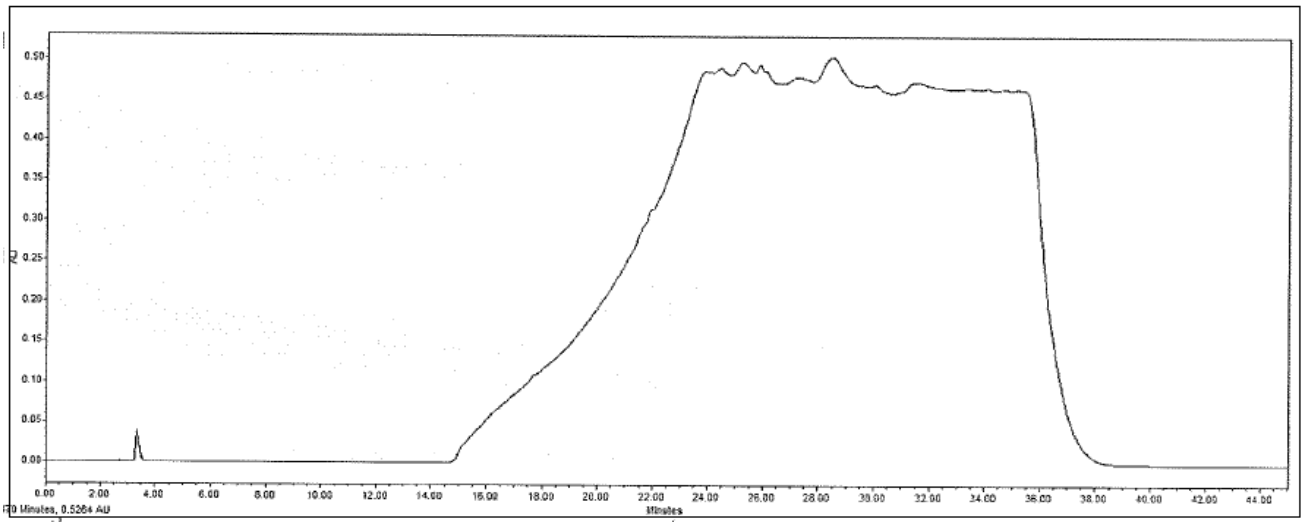
S.No	Change applied	Condition-1	Condition-2
1	Flow	1.2mL	0.8mL
2	Buffer pH	1.8	2.2
3	Mobile phase composition	MP-A →Buffer: Methanol(97.3::2.7)	MP-A →Buffer: Methanol(96.7::3.3)
4	Oven temperature	25°C	35°C

Table 2.

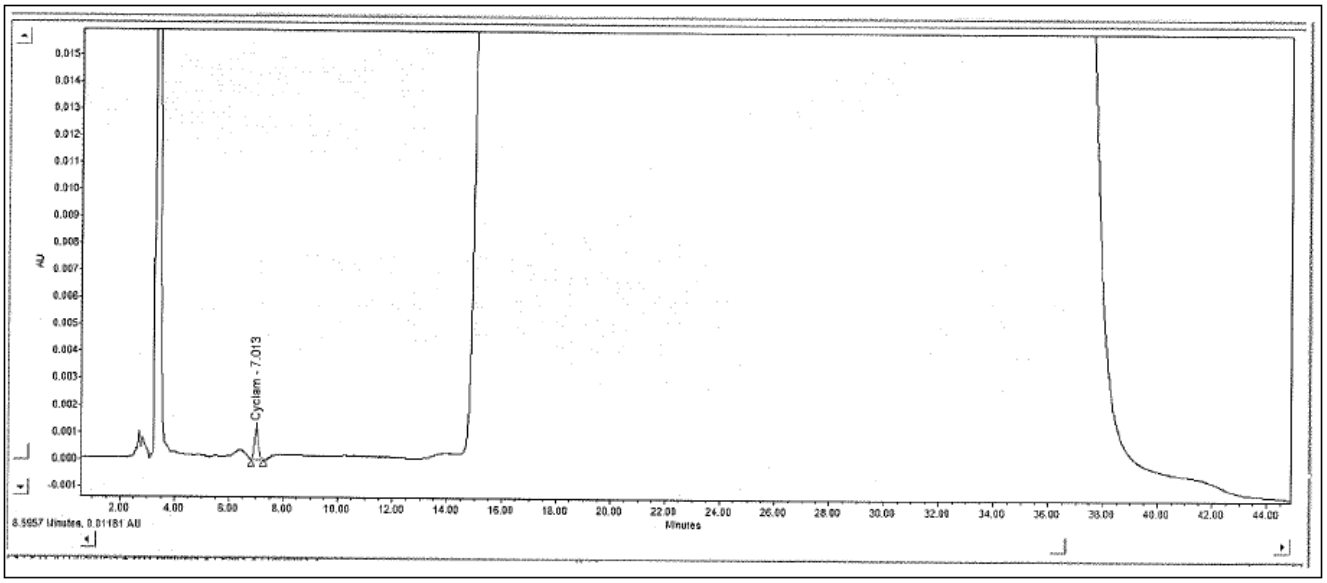
S.No	Validation parameter	Criteria	Result
1	LOD	Cyclam(mg/mL)	0.001
2	LOQ	Cyclam(mg/mL)	0.0025
3	Method precision(n=6)	%RSD for content	2.0
4	System precision(n=6)	%RSD for area	3.5
5	Intermediate precision(n=6)	%RSD for area %RSD for content	1.8 0.8
6	Rudeness(n=12)	%RSD for content	3.1
7	LOQ Precision(n=6)	%RSD for area	2.7
8	Accuracy at LOQ	%Recovery %RSD for content	100.1 1.8
9	Accuracy	%Recovery at 50% %Recovery at 100% %Recovery at 150%	102.3 104.0 105.1
10	Linearity	Correlation coefficient %Y-intercept Correlation	0.9998 0.73 0.9991

Reference chromatograms

Blank



Reference standard chromatogram



Accuracy: The accuracy of the method was carried out by triplicate preparation at three different concentration levels of cyclam in plerixafor (i.e.0.004mg/mL,0.008mg/mL and 0.012mg/mL).Calculated the recovery of cyclamen all the preparations and tabulated in Table-2.

Robustness: Robustness of an analytical method is the measure of its capacity to remains unaffected by small changes in terms of column oven temperature, mobile phase composition, Buffer pH and flow rate etc. To verify the robustness of the developed method, performed the original experiment with the following changes.For all above modified conditions the system suitability results were calculated and tabulated in the Table-2.

Solution and mobile phase stability: Solution stability was carried out for the plerixafor solution in presence of cyclam at specification level by keeping the solution in well-closed condition at room temperature on bench top of the laboratory for 48hrs(Sai baba *et al.,* 2017). Mobile phase stability was also perfomed in the similar manner, plerixafor spiked solution With known amounts of cyclam was prepared at different time intervals and verified the mobile phase stability for 48hrs.

Conclusion

The developed rapid HPLC method for the determination of cyclam in the plerixafor is precise, accurate, linear and specific. The method was validated as per the requirements of ICH guidelines, hence it can be used for the routine analysis of production plerixafor samples to check the cyclam content.

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