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Method Development and Validation for the Estimation of Plerixa for by RP-HPLC Method in Bulk Drug and Pharmaceutical Dosage form

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Abstract : A simple, precise and accurate RP-HPLC method was developed for the determination of Plerixafor in bulk and pharmaceutical dosage forms. The estimation was carried out on Xterra RP 18 (4.6 x 250mm, 5µm) column using a mixture of Methanol: Water (50:50% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 215nm.The method was validated for linearity, accuracy, precision, specificity, limit of detection and limit of quantification and robustness as per ICH norms. The retention time of the Plerixafor was 5.481 min. The method produce linear responses in the concentration range of 10-50mg/ml with correlation coefficient (r2) of 0.999. The proposed method is useful for the estimation of plerixafor in its pure and injection dosage forms.
Keywords : Plerixafor, RP-HPLC, validation.

Introduction

Plerixafor (PRX) belongs to the class anticancer drug.[1] It is used to stimulate the release of stem cells from the bone marrow into the blood in patients with non-Hodgkin lymphoma and multiple myeloma[2]. These stem cells are then collected and used in autologous stem cell transplantation to replace blood-forming cells that were destroyed by chemotherapy.[3] Plerixafor has orphan drug status in the United States and European Union; it was approved by the U.S. Food and Drug Administration on December 15, 2008. Plerixafor inhibits the CXCR4 chemokine receptor and blocks binding to the marrow compartment of its cognate ligand, SDF-1alpha, which play a role in the trafficking and homing of human hematopoietic stem cells[4-6]. Plerixafor[7] is chemically 1,1'-[1,4-phenylenebis(methylene)]bis[1,4,8, 11- tetraazacyclotetradecane]. (Fig 1). Literature survey revealed estimation of plerixafor by only one HPLC method and no other methods have been reported for the determination of PRX in pharmaceutical dosage forms. In this present study an attempt was made to develop rapid and economical RP-HPLC method for the determination plerixafor in pharmaceutical formulation with better accuracy, precision and sensitivity using C18 column.

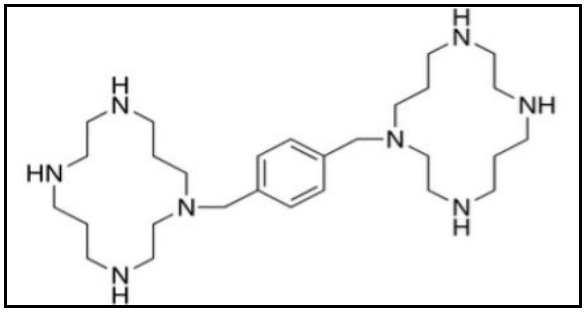

Fig1: Structure of plerixafor

Materials and Methods

Chemicals and Reagents:

Plerixafor standard (purity ≥ 99.0%) was obtained as gift sample from Sura Labs, Hyderabad. All chemicals and reagents used were HPLC grade. Acetonitrile (HPLC grade), methanol(HPLCgrade) were obtained from Merck (India). Plerixafor is available as single vial with brand names MOZOBIL, MOZOBIL DS. Chromatographic separation was achieved by using a WATERS Alliance HPLC system equipped with 2695 seperation module, software:Empower 2, 996 photodiode array detector with C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column maintained at 25 ºC. The overall run time was 10 min. and the flow rate was 0.8 mL min-1. 10 µL of sample was injected into the HPLC system.

Preparation of Mobile phase , Standard solution and Sample solution

Mobile phase

Accurately measured 500ml (50%) of Methanol, 500 ml of Water (50%) and were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the diluent.

Preparation of standard stock solution:

Stock solution was prepared by transferring 10mg of plerixafor into 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make the volume upto the mark with diluent. Further pipette out 0.3ml of above plerixafor stock solution into 10ml volumetric flask and dilute to the mark with diluent. Standard chromatogram is shown in fig 3 and result in table 1(a)

Preparation of sample solution:

Take average weight of injection sample and weigh equivalent to 10mg of plerixafor and transfer into 10ml volumetric flask,diluent was added to it and sonicate to dissolve and make the volume with diluent. Further pipette out 0.3ml of above plerixafor stock solution into 10ml volumetric flask and dilute to the mark with diluent. Sample chromatogram is shown in fig 4and result in table 1(b)

Chromatographic conditions:

Chromatographic separation was performed at ambient temperature on a reverse phase Waters ODS(C18) column, 250 mm × 4.6 mm i.d., 5 µm particle size. The mobile phase used in this analysis consists of a mixture of methanol and water in the ratio of 50:50. The mobile phase was filtered, degassed before use. The flow rate was adjusted to0.8ml/min, the detector wavelength was set at 215nm. The injector volume of standard and sample was 10µl. the solution was injected and chromatograms were recorded. Calibration curve was constructed and regression equation was calculated for plerixafor.

Results and Discussions

Method validation

System suitability

System performance parameters of HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N) , tailing factor, Retention time(RT) were determined. The results were shown in(table-1).From system suitability studies it is observed that %RSD values are within the limit i.e not more than 2 which indicates good performance of the system. The results are tabulated in table 2.

Linearty

A series of solutions were prepared using plerixafor working standard solution at *a* concentration levels from 10-50µg/ml and the peak area response of all solutions are measured. A graph was plotted against the Concentration(µg/ml)on X-axis versus area/response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. linearity graph is shown in Figure: 5, linearity results are tabulated in table:4

Specificity

It is the ability to asses unequivocally the analyte in the presence of components that may be expected to be present. Excipients that are commonly used were spiked into a pre weighed quantity of drugs. Appropriate dilutions were injected into chromatographic system and the quantities of the drugs were determined. The results are tabulated in table :3.

Precision

Precision studies were performed (Method, Day to day). The results are reported in term of Relative standard deviation. The repeatability studies were carried out by estimating response of 6 different concentrations of plerixafor and reported in terms of % RSD, % RSD was 0.3, for method precision it was 0.4 and for day to day precision it was 0.24. The results are tabulated in table :5,5a &5b.

Accuracy

Accuracy of the method was determined by calculating the recovery of plerixafor by the spiked method. Known quantity of plerixafor was added to a pre-determined sample solution and the amount of plerixafor was estimated by measuring peak areas. Mean % recovery values are within the limit(limit is 98-102%). Accuracy data was presented in table:6

LOD and LOQ Limits:

The level of detection(LOD) and level of Quantification (LOQ)were conducted on the basis of standard deviation of the response and the slope .The LOD and LOQ for plerixafor were found to be 1.03 and 3.14 respectively.

Robustness:

Robustness is a measure of its capacity to remain unaffected by small , but deliberated variation in the method parameters and gives an indication of its reliability during normal The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Plerixafor. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Plerixafor were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The method passed robustness test with well % RSD. .Robustness data was presented in table:7

Results and Discussion:

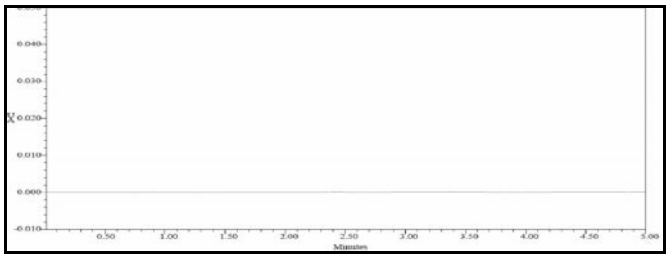

Fig2: Chromatogram showing bl

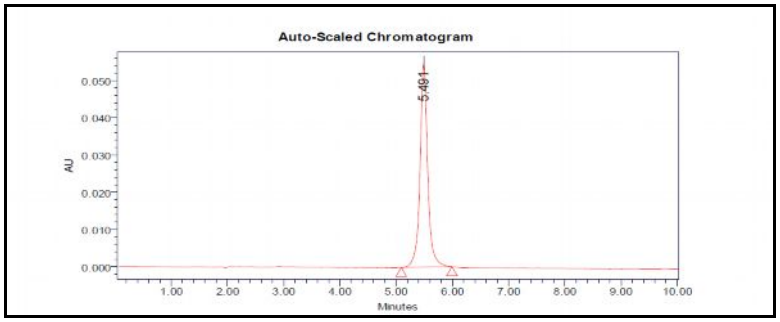

Figure3: Optimized Chromatogram (Standard)

Table1(a): Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Plerixafor	5.481	530529	55564	1.03	9222

Optimized Chromatogram (Sample)

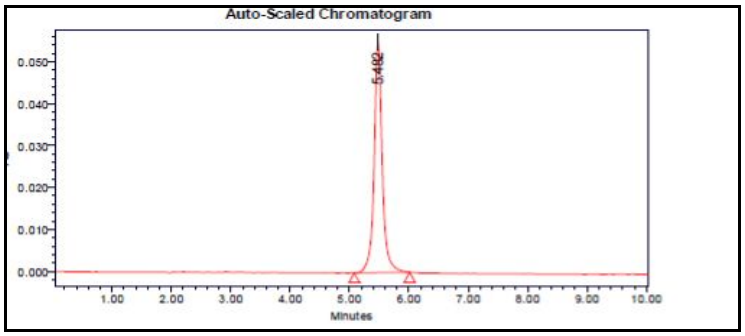

Figure 4: Optimized Chromatogram (Sample)

Table1(b): Optimized Chromatogram (Sample)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Plerixafor	5.482	522448	54873	1.06	9186

Table 2: System suitability:

S.No	peakname	RT	Area	Height (µV)	USP Plate count	USP tailing
1	Plerixafor	5.395	514884	54648	9011	1.07
2	Plerixafor	5.484	530529	55564	9222	1.05
3	Plerixafor	5.491	521608	54920	9148	1.04
4	Plerixafor	5.482	522448	54873	9186	1.06
5	Plerixafor	5.491	521608	54920	9148	1.04
Mean			522215.4			
Std. Dev.			5560.066			
% RSD			1.06			

Table3: Specificity:

Assay (Standard) :

S.No	Name	RT	Area	Height	Tailing Tailing	USP Plate Count	Injection
1	Plerixafor	5.427	530023	56127	1.03	9118	1
2	Plerixafor	5.430	531649	56299	1.05	9364	2
3	Plerixafor	5.443	533969	55991	1.05	9186	3

Assay (Sample):

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Plerixafor	5.453	534995	55722	1.05	9124	1
2	Plerixafor	5.462	532954	56050	1.03	9207	2
3	Plerixafor	5.466	533577	56095	1.03	9235	3

Linearity:

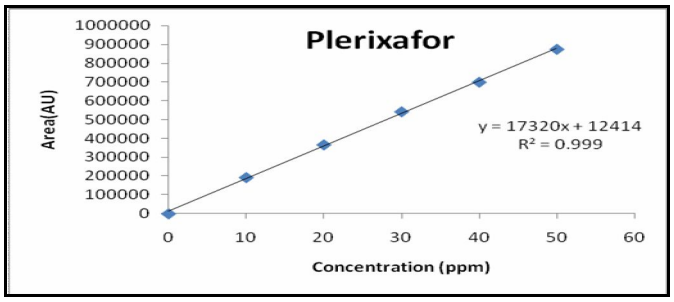

Fig 5: Calibration curve of plerixafor

Table 4:Linearity data

Concentration Level (%)	Conc.(mg/ml)	Avg Peak Area
33	10	192423
66	20	366108
100	30	541715
133	40	698851
166	50	873452

Table5: Results of Precision

S.No	PeakName	RT	Area	Height	USP Plate Count	USP Tailing
1	Plerixafor	5.352	516091	54804	9009	1.1
2	Plerixafor	5.346	518821	54903	9131.5	1.1
3	Plerixafor	5.293	519536	55996	9071.7	1
4	Plerixafor	5.284	519881	56012	9075.7	1
5	Plerixafor	5.319	519895	55577	8987.3	1
Mean			518844.8			
Std.dev			1599.873			
%RSD			0.3			

Intermediate precision:

Table5(a): Results of Intermediate precision

S.No	peakname	RT	Area	Height	USP Platecount	USPtailing
1	Plerixafor	5.352	516091	54804	9009.0	1.1
2	Plerixafor	5.346	518221	54903	9131.5	1.1
3	Plerixafor	5.306	519536	55996	9071.7	1.0
4	Plerixafor	5.284	519881	56102	9015.7	1.0
5	Plerixafor	5.319	519895	55577	8987.3	1.0
6	Plerixafor	5.306	522826	55808	9070.5	1.0
Mean			519408.3			
Std. Dev.			2216.8			
% RSD			0.4			

Table5(b): Results of Intermediate precision Day 2

S.No	Peak Name	RT	Area	Height	USP Platecount	USP Tailing
1	Plerixafor	5.274	518217	55506	8953.2	1.1
2	Plerixafor	5.306	518821	54903	9131.5	1.1
3	Plerixafor	5.306	518821	54903	9131.5	1.1
4	Plerixafor	5.274	518217	55506	8953.2	1.1
5	Plerixafor	5.352	516091	54804	9009.0	1.1
6	Plerixafor	5.319	519895	55577	8987.3	1.0
Mean			518343.7			
Std. Dev.			1262.452			
%			0.24			

Table 6 :Accuracy

%Conc.(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	269654.7	15	14.85	100%	99.90%
100%	529274	30	29.84	99.40%	
150%	794469.3	45	45.15	100.30%	

Robustness

Table7: Results for Robustness

Parameter used for sample analysis	Peak Area	RT	Theoretical plates	Tailing factor
Actual Flow rate of 0.8mL/min	530529	5.491	9222	1.03
Less Flow rate of 0.7mL/min	566441	5.599	9364	1.02
More Flow rate of 0.9mL/min	459187	4.576	7559	0.98
Less organic phase	24366	7.415	12009	1
More organic phase	93382	4.576	8274	1.07

Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Plerixafor in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Plerixafor was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water (50:50% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Plerixafor in bulk drug and in Pharmaceutical dosage forms.

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