International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 1 | JAN-MAR| 2018 | 636-643
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

STABILITY INDICATING RP-HPLC METHOD FOR QUANTIFICATION OF PLERIXAFOR

Pedapanga Sandhya[1*], Pasupuleti laxmi prasanna[1], Pedapanga Sandhya[1], Pittala Geetha[2], Vallapatla M Anuhya[2]

[1]Asst.Prof, Department of pharmaceutical Analysis, Vikas college of pharmaceutical sciences, Rayanigudem, Suryapet(M), Suryapet (Dist), Telangana.508213

[2]Asst.Prof, Department of pharmaceutical Technology, Vikas college of pharmaceutical sciences, Rayanigudem, Suryapet(M), Suryapet (Dist), Telangana.508213.

***Corresponding Author Email: pharmabud@gmail.com**

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Plerixafor. Chromatography was carried out on a 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 retention time of the Plerixafor was 5.481 min respectively. The method produces linear responses in the concentration range of 10-50mg/ml of Plerixafor. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

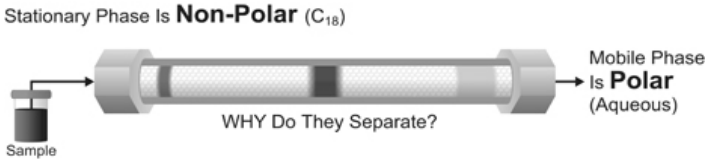
KEY WORDS

Plerixafor, RP-HPLC, validation.

INTRODUCTION

Plerixafor (PRX) is a hematopoietic stem cell mobilize [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- {[4-(1, 4, 8, 11-tetraazacyclotetradecan1-ylmethyl) phenyl] methyl}-1, 4, 8, 11- tetra aza cyclo tetra decane (Fig 1). No HPLC or spectrophotometric methods have been reported for the determination of PRX in pharmaceutical dosage forms. In the present work we have developed two simple, fast and precise liquid chromatographic and derivative spectrophotometric methods for the determination of Plerixafor (API).

Fig.No.1. Reversed-Phase Chromatography



MATERIALS AND METHODS

Chemicals and reagents Plerixafor standard (purity ≥ 99.0%) was obtained from Dr. Reddy's Labs, India. Acetonitrile (HPLC grade), sodium hydroxide and hydrochloric acid, tetra butyl ammonium hydrogen sulphate (TBAHS) and hydrogen peroxide were obtained from Merck (India). Plerixafor is available as single vial with brand names MOZOBIL, MOZOBIL DS (Label claim: 20 mg ml-1, 1.2 mL). All chemicals were of analytical grade and used as received. Instrumentation Chromatographic separation was achieved by using a Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column maintained at 25 ºC. A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Denver, Germany). Chromatographic conditions Isocratic elution was performed using tetra butyl ammonium hydrogen sulphate (10mM) (pH 3.37) and acetonitrile (58:42, v/v). The overall run time was 10 min. and the flow rate were 0.8 mL min-1. 20 µL of sample was injected into the HPLC system.

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Plerixafor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Mobile Phase Optimization [9]:

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water (50:50) and to, in proportion 60:20v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. X Terra RP 18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA 996 detector

Mobile phase ratio : Methanol: Water (50:50% v/v)

Column : X-Terra RP18 (4.6×250mm) 5µ

Column temperature : 35ºC

Wavelength : 215nm

Flow rate : 0.8ml/min

Injection volume : 10µl

Run time : 10min

VALIDATION

Preparation of mobile phase:

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

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Plerixafor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent [10]. (Stock solution)

Further pipette 0.3ml of the above Plerixafor stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Plerixafor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

PRECISION

Preparation of Plerixafor Product Solution For Precision [11]:

Accurately weigh and transfer 10 mg of Plerixafor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above Plerixafor stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Plerixafor working standard into a 10ml of clean dry volumetric

flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above Plerixafor stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents [12].

Preparation of Sample Solution:

Take average weight of the injection sample and weight 10 mg equivalent weight of Plerixafor sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3 ml of Plerixafor above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results [13].

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Plerixafor working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of the above Plerixafor stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions:

The sample was analyzed at 0.7ml/min and 0.9ml/min instead of 0.8ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio and 45:55, 55:65 instead of 50:50, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Table1: peak results for trail 1

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Plerixafor	7.553	263569	7627	1.87	2403

Observation:

The trial doesn't show proper baseline and peak and shows more void peaks in the chromatogram. so more trials were required for obtaining peaks good peaks.

Trail 2:

Table2: peak results for trail 2

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Plerixafor	4.396	266634	10526	1.37	1671

Observation: This trial show more void peaks and less plate count in the chromatogram, so more trials were required for obtaining proper peaks.

Trail 3:

Table3: - peak results for trail 3

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Plerixafor	2.608	15742	1523	1.73	570

Observation: This trial show very less plate count and improper baseline in the chromatogram, so more trials were required for obtaining good peaks.

Trail 4:

Table4: peak results for trail 4

S.No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Plerixafor	4.161	471920	21641	1.9	2057

Observation: This trial does not show Proper peak in the chromatogram. So it's required more trials to obtain good peaks.

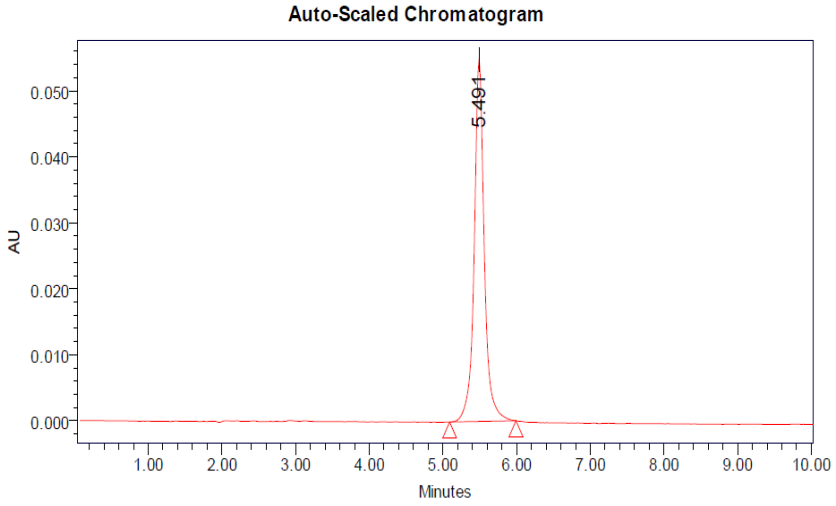


Figure: Optimized Chromatogram (Standard)

Table5: Optimized Chromatogram (Standard)

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

Observation: This trial shows proper plate count and tailing in the chromatogram. It's Pass the all system suitability parameters. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

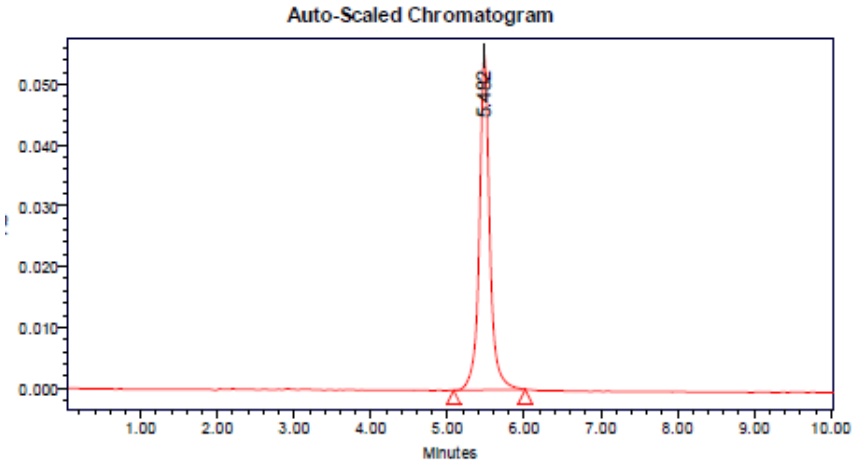


Figure2: Optimized Chromatogram (Sample)

Table6: Optimized Chromatogram (Sample)

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

VALIDATION

Blank:

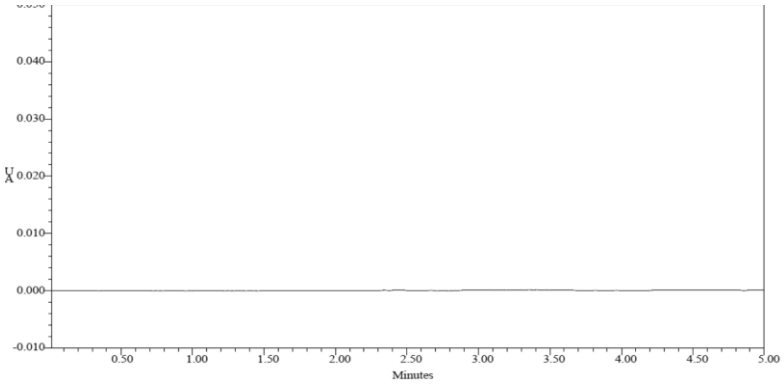


Fig3: Chromatogram showing blank (mobile phase preparation)

System suitability:

Table7: Results of system suitability for Plerixafor

S.No	Peak Name	RT	Area (µV*sec)	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			

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Plerixafor in drug product.

Assay (Standard):

Table 8: Peak results for assay standard

S.No	Name	RT	Area	Height	USP 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):

Table 9: Peak results for 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

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=533842/531880.3333×10/30×30/0.6075×99.7/100×1.2151/20×100 =100.0%

The % purity of Plerixafor in pharmaceutical dosage form was found to be 100.0%.

LINEARITY

Table 10: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

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

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table11: Results of repeatability for Plerixafor:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Plerixafor	5.352	516091	54804	9009.0	1.1
2	Plerixafor	5.346	518821	54903	9131.5	1.1
3	Plerixafor	5.293	519536	55996	9071.7	1.0
4	Plerixafor	5.284	519881	56012	9075.7	1.0
5	Plerixafor	5.319	519895	55577	8987.3	1.0
Mean			518844.8			
Std.dev			1599.873			
%RSD			0.3			

Intermediate precision:

Table 12: Results of Intermediate precision for Plerixafor

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
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			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Day 2:

Table13: Results of Intermediate precision Day 2 for Plerixafor

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USP Plate count	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

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
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			
% RSD			0.24			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 14: The accuracy results for Plerixafor

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	269654.7	15	14.85	100%	
100%	529274	30	29.84	99.4%	99.9%
150%	794469.3	45	45.15	100.3%	

LIMIT OF DETECTION FOR PLERIXAFOR

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: = 3.3×5454.719/17320

= 1.03 µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: = 10×5454.719/17320

=3.14 µg/ml

Robustness

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.

Table 15: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	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	1.00
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 derivatization 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.

REFERENCES

1. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., P ublication, (2007), PP 15-23.
5. Chromatography, (online). URL: http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
9. Detectors used in HPLC (online) URL:http://wiki.answers .com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online) ,URL:http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
13. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf

***Corresponding Author:**
Pedapanga Sandhya
Email: pharmabud@gmail.com