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ANALYTICAL CHEMISTRY | RESEARCH ARTICLE

Ultra performance liquid chromatographic method for simultaneous quantification of plerixafor and related substances in an injection formulation

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Abstract: Plerixafor (PLX) injections are administered to patients with cancers of lymphocytes (non-Hodgkin's lymphoma) and plasma cells (multiple myeloma). The main objective of the current study was to develop a short reverse phase chromatographic method for the simultaneous quantification of PLX and its impurities, in an injection formulation, to reduce the time required for these quality tests. Furthermore, the present work describes the role of nonalkyl branched nonquaternary ion pair reagent in improving the peak shape and reducing column equilibration time. The separation of PLX and its related substances is pH dependent (optimum pH = 2.50) and was achieved on an octadecylsilyl (C18) column. The method was validated for its intended purpose in accordance with the current regulatory guidelines for validation. The proposed method can be applied for quality control, release, and stability analyses of active pharmaceutical ingredient, PLX, as well as finished products, PLX injections.

Subjects: Analytical Chemistry; Organic Chemistry; Applied & Industrial Chemistry

Keywords: plerixafor; potential impurities; UPLC; stability-indicating assay; validation

1. Introduction

Patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) require mobilization of hematopoietic stem cells (HSCs) as a strategic treatment following high doses of chemotherapy

ABOUT THE AUTHORS

G. Venkata Narasimha Rao received the Bachelor of Science (BSc), Master of Science (MSc-Applied Chemistry) degrees from the Acharya Nagarjuna University of Guntur, Devi Ahilya University of Indore, India, respectively. During 2007-up to date, he is working as an analytical scientist with a designation of Associate Director, in Mylan Laboratories Ltd. He is presently perusing his research in developing shorter chromatographic methods for various pharmaceutical drug products, especially cancer therapeutic drugs, by exploring the supercritical fluid chromatography and associated techniques. His research interests are aimed at reducing the analysis time and the solvent consumption for a better waste management and safeguard of the environment.

PUBLIC INTEREST STATEMENT

For a medicinal drug to be proven safe and effective, it must be tested for various quality attributes before releasing into the market. Since most of the drugs are synthesized chemically and contain certain impurities which are clinically not safe for human body. Different drug regulations require identification and quantification of these impurities and control below a safety threshold limits. The techniques associated with these estimations involve intensive testing and take time to release the product into the market and in the process eliminate lots of organic wastes.
This research was aimed to develop new shorter procedures of testing on the same technique for a quick testing of the product at the quality control lab and release into the market. In this research, Plerixafor a cancer drug can be tested in 9 min for critical quality attributes like amount of main drug and impurities. As a result of reduced time, solvent usage is also reduced.

(Montgomery & Cottler-Fox, 2007). Currently, mobilization is initiated using granulocyte colony stimulating factor (G-CSF), which promotes the proliferation and differentiation of HSCs (Klocke, Kuhlmann, Scobioala, Schabitz, & Nikol, 2008).

Several days of treatment are required with placebo + G-CSF for adequate mobilization (Devine et al., 2008). Studies have shown that patients with NHL and MM exhibited a rapid increase (a 2.5-fold increase compared with only G-CSF) in peripheral blood CD34+ cells after subcutaneous administration of Plerixafor (PLX, direct antagonist of CXCR4/SDF-1) at a dose of 240 μg kg^{-1} (Hess et al., 2007). PLX is an on-demand, well-tolerated HSC mobilizer with mild adverse effects (Calandra et al., 2008; DiPersio, Micallef, et al., 2007; DiPersio, Stadtmauer, et al., 2007; Flomenberg et al., 2005).

PLX is a symmetrical bicyclam derivative with molecular formula $C_{28}H_{54}N_8$ and molecular weight 502.78 g mol^{-1}. The structural formula of PLX and its impurities are depicted in Figure 1. PLX is a white to off-white, hygroscopic, crystalline solid. The PLX injection formulation is a sterile, preservative-free, clear, and colorless to pale yellow isotonic solution for subcutaneous injection. Each single-use vial is filled to deliver 1.2 mL of the sterile solution containing 24 mg of PLX and 5.9 mg of sodium chloride in water. Mozobil is the brand name of the innovator (CHMP Assessment Report, 2009; Drug Bank, 2010; Drugs at FDA, 2008; Mozobil, 2016; Mozobil, Genzyme Corporation, 2015).

PLX and its injection formulation are not official monographs in any of the pharmacopeia (USP, EP, BP, JP, and IP). It has an orphan drug status, approved by the FDA in the USA and in the EU. Hence, no official methods have been reported for the estimation of PLX and related substances. A literature survey revealed several publications regarding the pharmacodynamics, pharmacokinetics, and therapeutic efficacy studies on PLX (DiPersio, Stadtmauer, et al., 2007; Gerlach, Skerlj, Bridger, Schwartz, 2001; Hatse, Princen, Bridger, De Clercq, & Schols, 2002; Hendrix et al., 2000; Hübel et al., 2004; Lack et al., 2005; MacFarland, Ewesuedo, Badel, & Calandra, 2007; Rosenkilde et al., 2004).

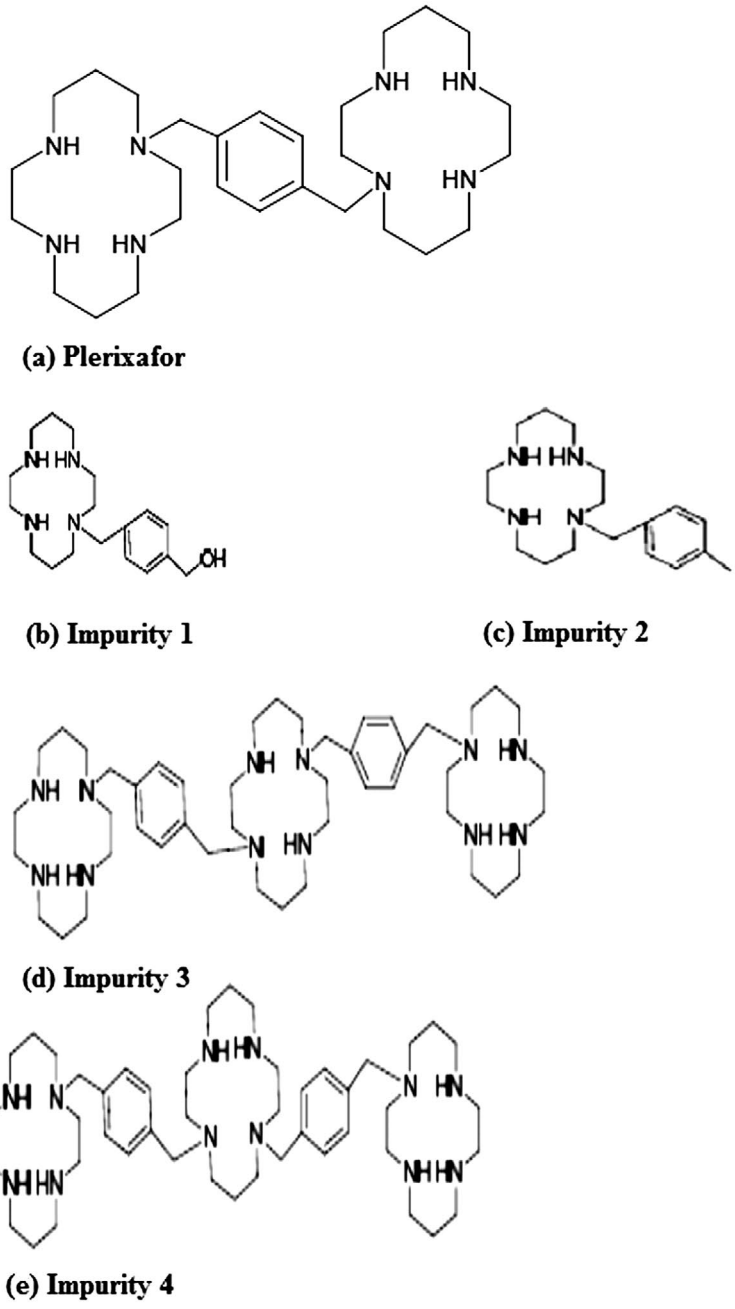
An HPLC method for the determination of PLX was reported by Mathrusri Annapurna, Sai Pavan Kumar, Goutam, and Venkatesh (2012). The method uses an isocratic elution mode using tetra butyl ammonium hydrogen sulfate (pH = 3.37) and acetonitrile mixed in the ratio 58:42 (v/v). The runtime was 10 min for PLX, and no impurities were addressed in the method.

An HPLC determination method was reported by Reddy et al., for PLX and its impurities in drug substance (Hanimi Reddy, Ravi Kumar, & Satyanarayana Murthy, 2015). In this method, three impurities and PLX were determined in 24 min using a gradient elution mode. The mobile phase was a complex mixture with a binary composition. The mobile phase A contained perchloric acid (1.0 mL) + heptane sulfonic acid (5 mM) (pH = 2.0) (buffer) and acetonitrile in the ratio 80:20 (v/v) and mobile phase B contained a mixture of buffer and acetonitrile in the ratio 20:80 (v/v). The formulation was separated on a phenomenex Luna phenyl–hexyl (L11) 100 mm × 4.6 mm, 3 μm column. The eluted components were detected at 210 nm. The method used long-chain alkyl sulfonates in the mobile phase, which required a longer equilibration time before analysis than do methods that do not use long-chain alkyl sulfonates (Fanali, Haddad, Poole, Schoenmakers, & Lloyd, 2013; Verpoorte & Baerheim, 1984).

Thus far, studies have been reported either on PLX determination or its impurities in active pharmaceutical ingredients. Studies on the estimation of PLX and its impurities in presence of excipients in an injection formulation were not available.

Thus, an attempt was made for developing an accurate reproducible method that uses a nonalkyl branched, nonquaternary ion pair reagent as a buffer for improving the peak shape for PLX and its impurities, in the presence of excipients.

Figure 1. Chemical structures of plerixafor and its potential impurities.



2. Materials and methods

2.1. Chemicals and reagents

Sodium perchlorate monohydrate (ACS grade) was used to prepare the buffer and obtained from Acros Organics. Perchloric acid (GR Grade), used for adjusting pH, was obtained from Merck specialties, India. Acetonitrile (HPLC grade) was used as a mobile phase component and was procured from Rankem India. Milli Q water was used for preparing the mobile phase. A standard working solution of PLX was prepared in house. Impurity standards were obtained internally. Small volumes of PLX injection samples and placebo mixtures were prepared in the laboratory.

2.2. Instrument and chromatographic conditions

The Integrated Acquity UPLC system used for the study was purchased from Waters Corporation, Milford, USA and equipped with Waters photodiode array detector (PDA). Data collection and analysis was performed using Empower software 2pro (Waters Corporation). The balance used for weighing the reference standards and samples was purchased from Metler Toledo. Separation was achieved on a Waters acuity CSH C18 column with dimensions 50 mm × 2.1 mm I.D and a particle size of 1.7 µm. A simple mobile phase consisting of sodium perchlorate buffer (0.02 M, pH 2.5) and acetonitrile (Mobile phase B) was pumped into the UPLC chromatograph using a gradient program with varying compositions (v/v) of B, T/B% 0/12, 2/15, 5/15, 5.50/70, 6.50/70, 7/12, 8/12, at a flow rate of 0.32 mL min^{-1}, with a column temperature of 35°C throughout the run. Sample volume of 3 µL was injected into the chromatograph and detected at 210 nm.

2.3. Preparation of standard and sample solution

A mixture of aqueous hydrochloric acid (0.01 M) and methanol in the ratio 90:10 was used as a diluent for preparing the solutions of standard and samples (diluent).

2.3.1. Standard stock solution

A standard stock solution was prepared by dissolving 50 mg of PLX working standard in 50 mL of the diluent.

2.3.1.1. Preparation of standard and sample solution for assay determination (0.1 mg mL^{-1}). The standard solution for assay of PLX was prepared by diluting the standard stock solution 10 times (5–50 mL) to obtain a concentration of 0.1 mg mL^{-1}. The vials of PLX injection were pooled and 2 mL of the sample was diluted to 100 mL and further diluted 5–20 mL to obtain the 0.1 mg mL^{-1} concentration, similar to that of the standard.

2.3.2. Preparation of standard solution for impurities determination (0.004 mg mL^{-1})

The standard solution for the determination of impurities was prepared by diluting 0.4 mL of the standard stock solution into 100 mL with the diluent to obtain a concentration of 0.004 mg mL^{-1}.

2.3.3. Preparation of sample and placebo solution for impurities determination

Sample solution was prepared by diluting 2 mL of the pooled PLX injection to 20 mL using the diluent to obtain a concentration of 2 mg mL^{-1}. Placebo equivalent to 2 mL of the sample was taken and diluted to 20 mL with the diluent and mixed.

2.3.4. Preparation of spiked sample solution for impurities determination

Stock solution of all impurities was prepared by dissolving an appropriate quantity of each impurity in the diluent to obtain a concentration of 1 mg mL^{-1}. An appropriate volume of impurity stock solution was diluted with sample solution to get a final concentration of 0.5% for each impurity.

3. Method development and optimization

PLX has ionizable amino groups, and hence, the retention time of the drug is highly dependent on the pH of the mobile phase. In the present study, the pH of the mobile phase was maintained acidic (pH = 2.5) by the addition of sodium perchlorate solution. The ion exchange interaction between positively charged amino groups on PLX and negatively charged silanol groups on column resulted in "mixed-mode retention." Such a mixed-mode retention effect is eliminated by the addition of acidic sodium perchlorate solution (0.1 M), which causes ion suppression or maintains the silanol groups in unionized form. This yields narrow and symmetrical peak.

Before initiating the development activity, information on impurities and their acceptable limits was collected to define sample concentration and the range of the method. The maximum daily dose of PLX is 40 mg/day. Based on the daily dose, the qualification threshold did not exceed 0.5%, and the identification threshold was 0.2%. Table 1 lists the chemical names and ICH limits for the specified impurities. Method development was targeted to cover a range of 50–150% of qualification

Table 1. Chemical names and limits based on ICH for impurities

Name of the impurities	Chemical names	ICH* limits QT**
Impurity-1 (Hydroxy impurity)	(4-((1,4,8,11-tetraazacyclotetradecan-1-yl)methyl)phenyl) methanol	NMT 0.50%
Impurity-2 (Methyl impurity)	1-[(4-methylphenyl)methyl]-1,4,8,11-tetraazacyclotetradecane	NMT 0.50%
Impurity-3	1,8-bis(4-((1,4,8,11-tetraazacyclotetradecane-1-yl)methyl) benzyl)-1,4,8,11-tetraazacyclotetradecane	NMT 0.50%
Impurity-4	1,11-bis(4-((1,4,8,11-tetraazacyclotetradecane-1-yl)methyl) benzyl)-1,4,8,11-tetraazacyclotetradecane	NMT 0.50%

*ICH The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

**QT Qualification Threshold based on maximum daily dose.

threshold for impurities and PLX for assay. A systematic approach was adopted for the method development.

3.1. Selection of detection wavelength

The sensitivity of a method that uses a UV detector depends on the proper selection of wavelength. An ideal wavelength is that which is maximally absorbed and provides an acceptable response for the drug, which should not interfere with other peaks.

UV spectra of the drug and its impurities were recorded by scanning between 200 and 400 nm.

The spectra of drug and its impurities were overlaid and the wavelength 210 nm was selected where the active analyte as well as impurities have sufficient response for detection and quantification. The ultraviolet scans of PLX and the four potential impurities are depicted in Figure 2.

Based on the physicochemical properties of the drug substance and the solubility (freely soluble in alcohols), the reverse phase chromatographic technique was selected for initial separation of the drug from its impurities.

The first development trial was initiated with sodium perchlorate buffer (pH 2.5), Waters Acquity HSS T3 (100 × 2.1 mm, 1.7 µm), column with linear gradient program using 100% Acetonitrile as the second component. The pump was maintained at a constant flow rate of 0.35 mL/min. The column was maintained at 35°C. Two microliter of spiked sample was injected into the chromatograph and the peak responses were monitored. All impurities were resolved from main peak; however, separation among impurities was not achieved. Figure 3 illustrates the chromatogram obtained from trial 1.

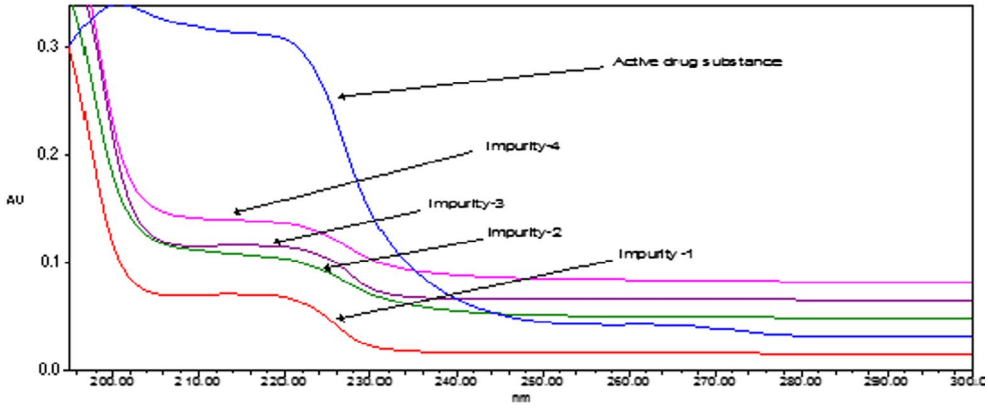


Figure 2. Ultraviolet scan of plerixafor and its impurities between 200 and 400 nm

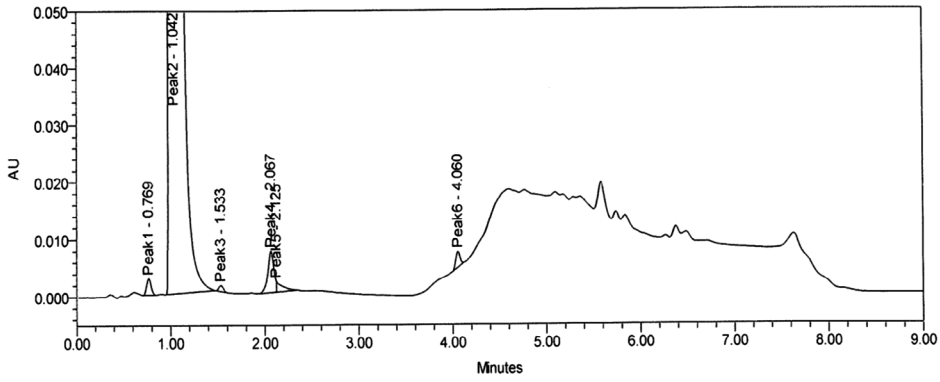


Figure 3. Chromatogram obtained from trial 1.

Another attempt was made by changing the gradient program and keeping other parameters unchanged. The patterns obtained from both trials did not differ significantly. The pattern obtained for trial 2 is depicted in Figure 4.

The next trial was made by changing the column to Waters CSH C18, 50 × 2.1 mm, 1.7 µm. The remaining chromatographic parameters were unchanged. All impurities were appropriately separated from each other. The resolution between impurity 1 and the PLX is further improved by modifying the gradient program. The optimized chromatographic conditions are listed in section 2.1. A specimen chromatogram obtained from the final method parameters is illustrated in Figure 5.

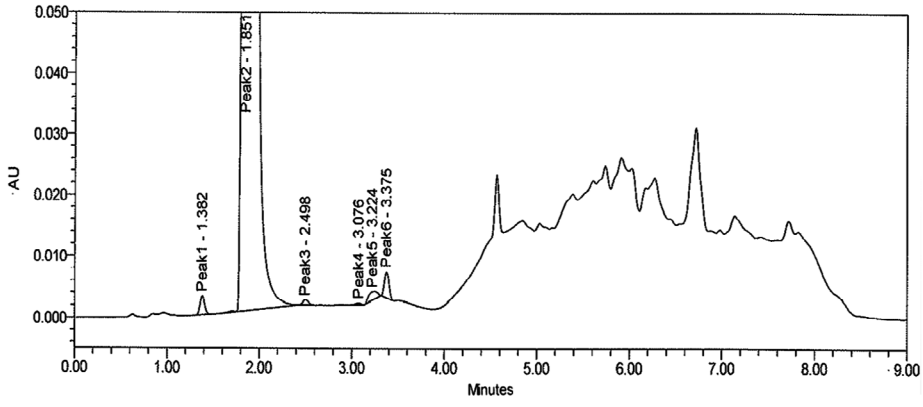


Figure 4. Chromatogram obtained from trial 2.

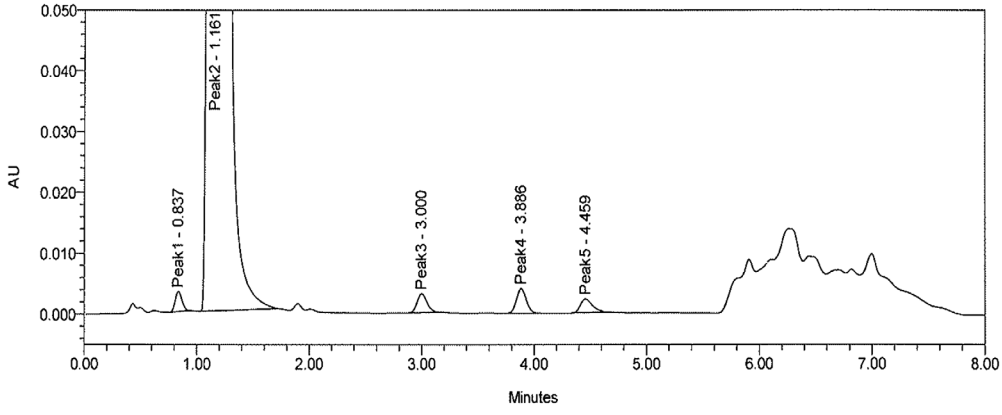


Figure 5. Specimen chromatogram from final method for spiked sample.

Table 2. Validation data of related substances of plerixafor

S. no	Compound	Retention time (mins) (RT ratio)	USP resolution (R_s)	USP tailing (T)	No. of theoretical plates USP tangent method (N)	Linearity (r^2)	M.P# %RSD	I.P# %RSD	LOQ %w/w (%RSD)	Accuracy			LOD$ (%w/w)	LOQ$ (%w/w)
										50% %w/w (%RSD)	100% %w/w (%RSD)	150% %w/w (%RSD)		
1	Impurity 1	0.837 (0.72)	–	1.14	8,074	0.998	1.67	1.50	87.5 (0.8)	91.1 (0.8)	93.6 (2.3)	87.7 (0.4)	0.0075	0.02421
2	Plerixafor	1.161 (1.00)	2.2	1.34	6,665	0.999	0.3	0.2	100.5 (1.5)	101.5 (0.8)	100.1 (0.9)	101.6 (0.7)	0.02	0.1
3	Impurity 2	3.000 (2.58)	10.8	1.20	5,655	0.997	1.22	1.15	88.3 (1.1)	106.4 (0.4)	99.4 (1.3)	100.0 (1.0)	0.0135	0.03216
4	Impurity 3	3.886 (3.35)	5.5	1.11	9,223	0.999	0.71	0.69	98.0 (0.9)	108.5 (0.5)	108.0 (0.8)	104.2 (0.2)	0.0150	0.02381
5	Impurity 4	4.459 (3.84)	3.2	1.40	7,717	0.997	0.57	0.50	101.6 (1.1)	89.2 (0.3)	86.8 (0.9)	87.9 (1.1)	0.0207	0.04600

#Method precision (M.P), intermediate precision (I.P).

$LOD, LOQ values are established by S/N ratio.

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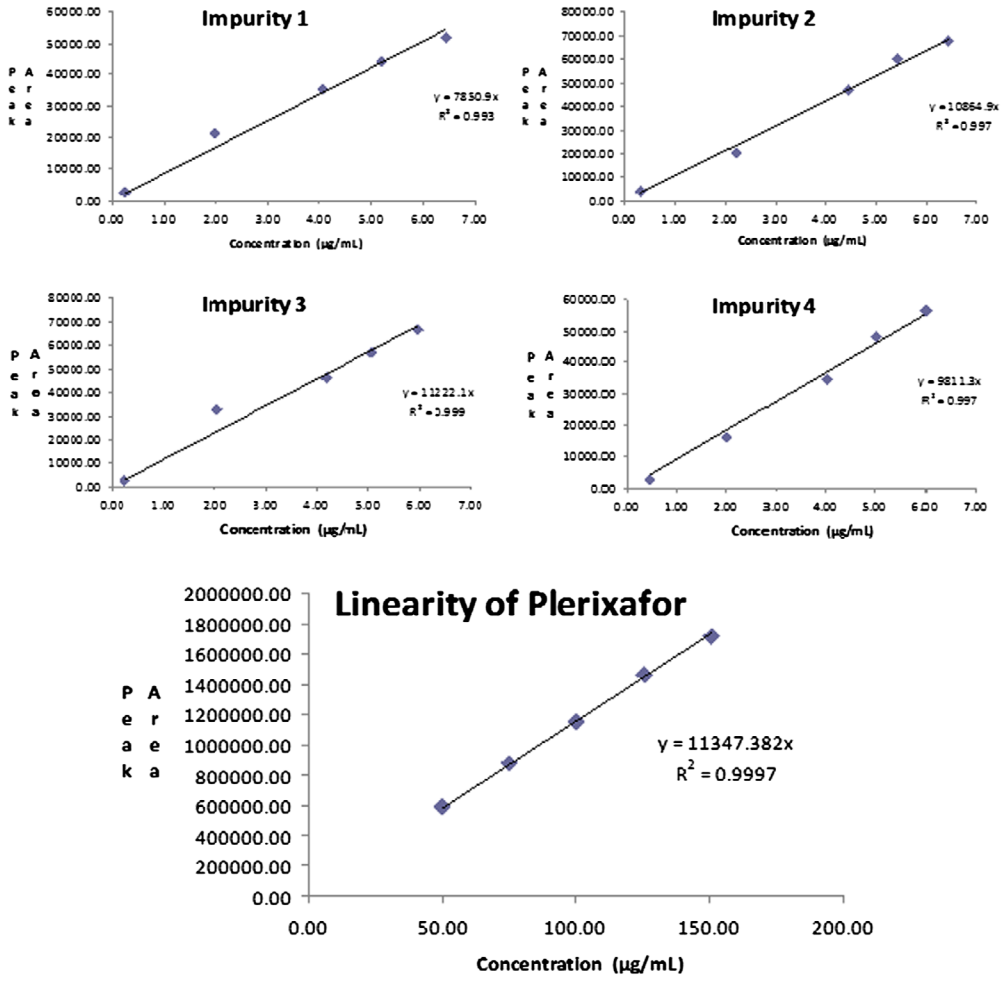


Figure 6. Linearity graph for impurities-1, 2, 3, 4 (related substances) and plerixafor (assay).

Table 3. Data on signal to noise ratio

Impurity name	Concentration (%w/w)	Signal (S) (µV)	S/N* ratio
Impurity 1	0.02421	1,024	12
Impurity 2	0.03216	654	10
Impurity 3	0.02381	724	9
Impurity 4	0.04600	816	10

*S/N signal to noise ratio.

4. Results and discussion

4.1. Method validation

The optimized method was fully validated for the assay of PLX and simultaneous determination of impurities, as per the current ICH guidelines (Q2A (R1) validation of analytical procedures, 2005).

4.1.1. System suitability

System suitability parameters were measured to verify the system performance for the intended analysis. Hence, system precision was determined on six replicate injections of standard

preparations and the relative standard deviation (% RSD) was evaluated. In addition to the % RSD, USP Resolution, USP tailing, and USP plate count were also evaluated and found to be satisfactory, as per current USP requirements for a chromatographic peak (USP General Chapter<621>). The system suitability results obtained are presented in Table 2.

4.1.2. Linearity

The linearity of the analytical method was tested to check its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. Hence, different concentrations of individual impurities and standard working solution of PLX were prepared and injected into the UPLC, and the chromatograms were recorded. The linearity of detector response was determined by plotting a graph of peak areas versus concentrations. Plots of linearity experiments are illustrated in Figure 6. The correlation coefficients, >0.997 for impurities and 0.999 for PLX, indicate that the method satisfies a linear relationship between the concentration and peak response. The linearity range is between the limit of quantification (LOQ) and 150% of the sample concentration for all impurities and between 50 and 150% of target sample concentration for assay determination. The linearity data for all four impurities and PLX are listed in Table 2. The signal-to-noise ratio for each impurity standard meets the criteria as per the ICH requirement and the data are presented in Table 3.

4.1.3. Precision

Precision of the test method was evaluated by injecting six individual samples (assay concentration) and six individual samples spiked with all four impurities into the chromatograph. The % RSD values from the six individual test preparations were found to be 0.3 for assay determination and below 2% for all four impurities. The ruggedness (intermediate precision) of the method was determined using another system and column for the analysis in a different day. The precision data are listed in Table 2. The data indicate that a low % RSD, concluding that the method is precise for assay and impurities determination.

4.1.4. Accuracy

Accuracy of the analytical procedure expresses the degree of closeness of the obtained results with true values. Samples for accuracy were prepared in triplicate by spiking PLX and impurities at different levels in the placebo. The covered levels for assay were 50, 100, and 150% of target assay concentration (0.100 mg mL^{-1}). The covered levels for impurities were LOQ, 50% (1 mg mL^{-1}), 100% (2 mg mL^{-1}), and 150% (3 mg mL^{-1}) of the sample concentration (2 mg mL^{-1}). From the response of the analyte peaks, the amounts recovered (in %) and % RSD were reported. Accuracy results are summarized in Table 2.

The data indicate that the assay recovery results are between 100.1 and 101.5 with an RSD ranging from 0.8 to 1.5% for all three samples. The % recovery of impurities lies in the range of 85–110 with an RSD of between 0.3 and 2.3%. This indicates that the method is accurate and precise.

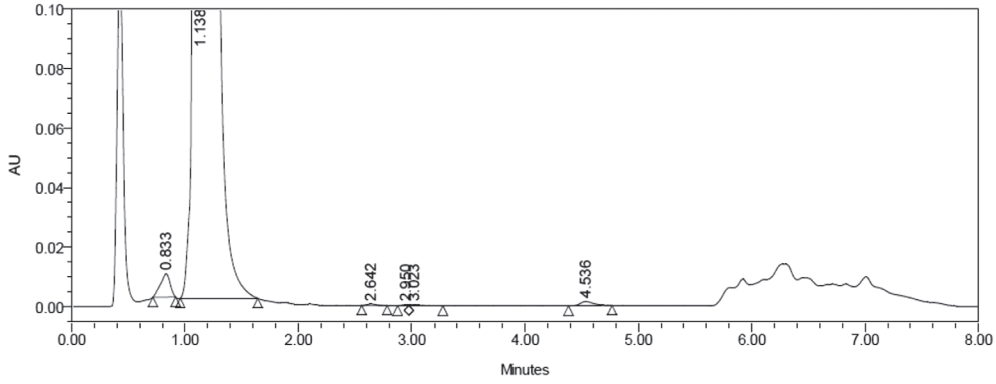
4.1.5. Specificity

The specificity of the method was determined by analyzing the diluent, standard solutions of PLX, placebo, and the impurities spiked sample. The chromatograms of the diluent and placebos solutions were evaluated for the interference of any peaks at the retention times of the analyte peaks. No interference was found. The samples of PLX injection were subjected to stress conditions, chemical conditions such as, acid hydrolysis, base hydrolysis, and 3% of oxidant treatment, as well as physical conditions, such as treatment with heat and light. A detailed forced degradation study has been detailed.

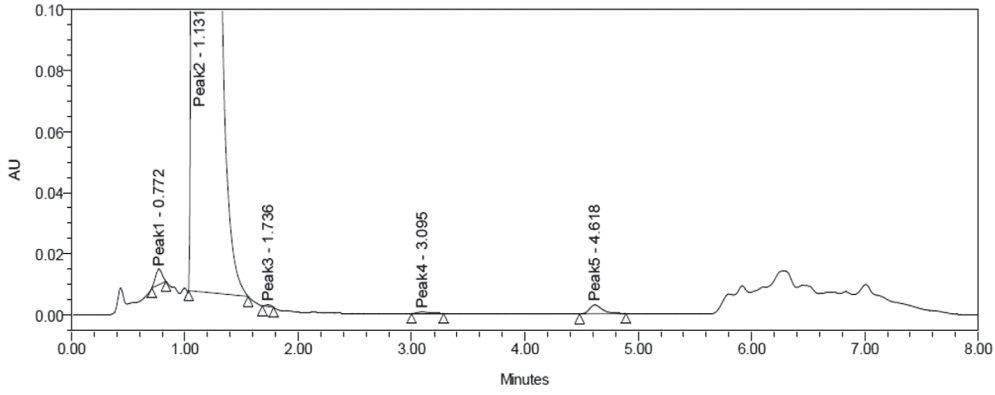
4.1.5.1. Forced degradation study. Forced degradation studies were conducted on samples and on the plain placebo to prove the specificity and stability-indicating power of the method. Specificity was determined by exposing test solution to oxidation by hydrogen peroxide, acid hydrolysis, base hydrolysis, heat and photolytic stress. A detailed procedure has been reported.

Oxidation stress studies were provided by adding 1 mL of 3% H_2O_2 to 2 mL of the sample and storing for 48 h on bench top. Acid hydrolysis was performed by adding 1 mL of 0.5 N HCl to 2 mL of the sample and storing for 24 h on the bench top. Base hydrolysis was performed by adding 1 mL of 0.5 N NaOH and storing for 24 h on the bench top. Heat stress was provided by exposing the sample to 70°C for 48 h. Photolytic studies were carried as per the current ICH requirements i.e. by exposing the sample to UV light (200 Watt h/m²), day light (1.2 million lux h) (Fanali et al., 2013). The stressed samples were then further diluted to 20 mL with the diluent and chromatographed as per the proposed method. The percentage assay and peak purity of PLX peaks were evaluated using the PDA

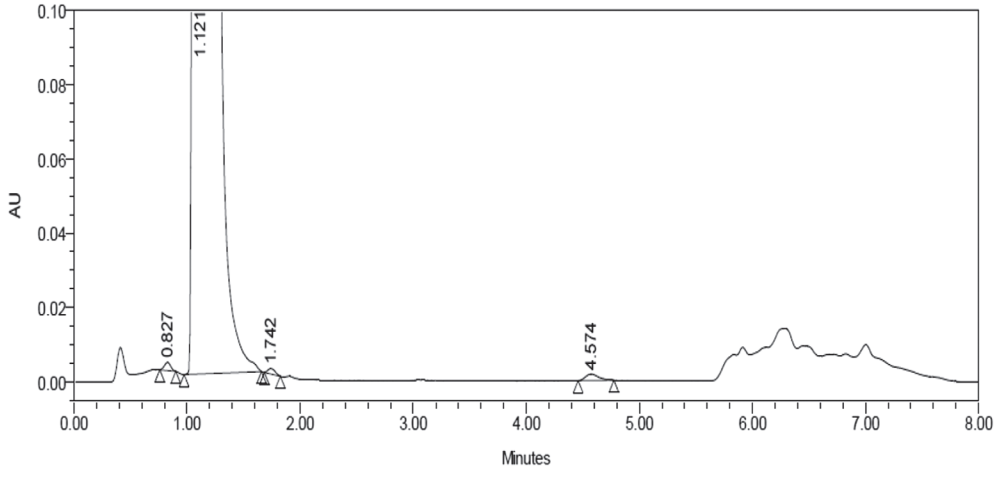
Figure 7. Forced degradation chromatograms of plerixafor.



(a) Peroxide degradation

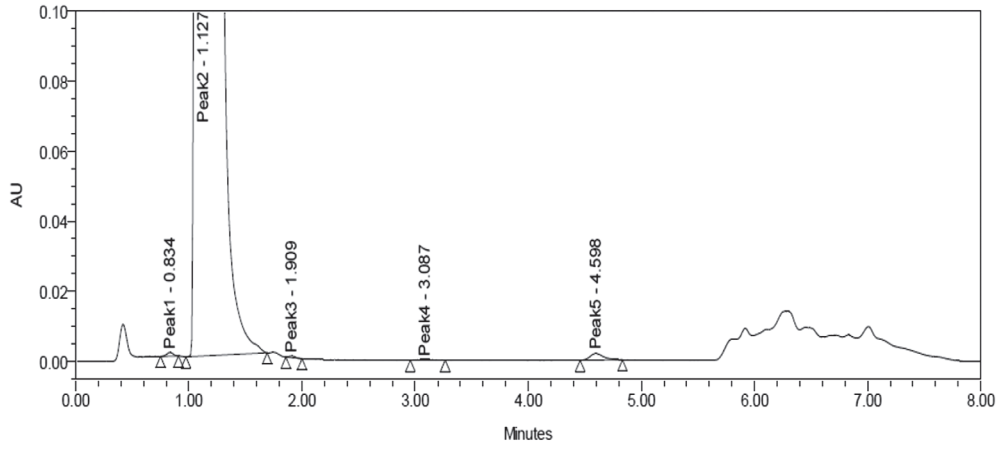


(b) Thermal degradation

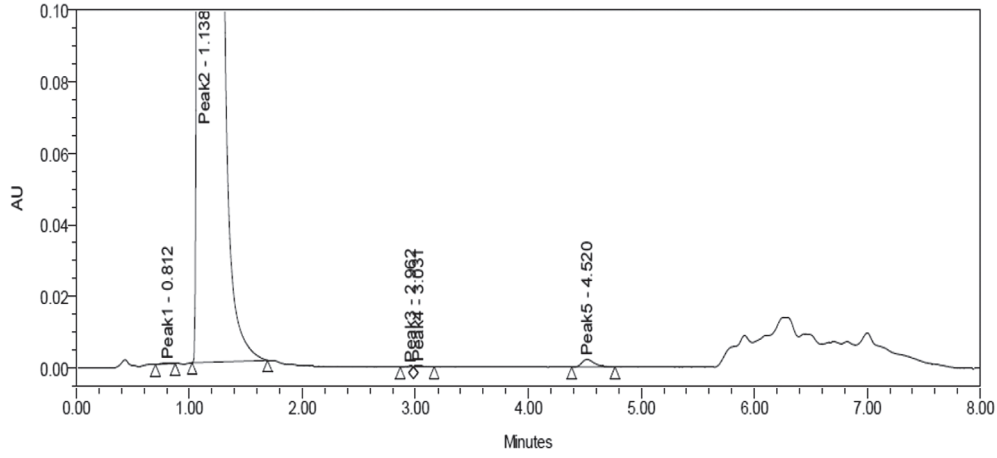


(c) Acid degradation

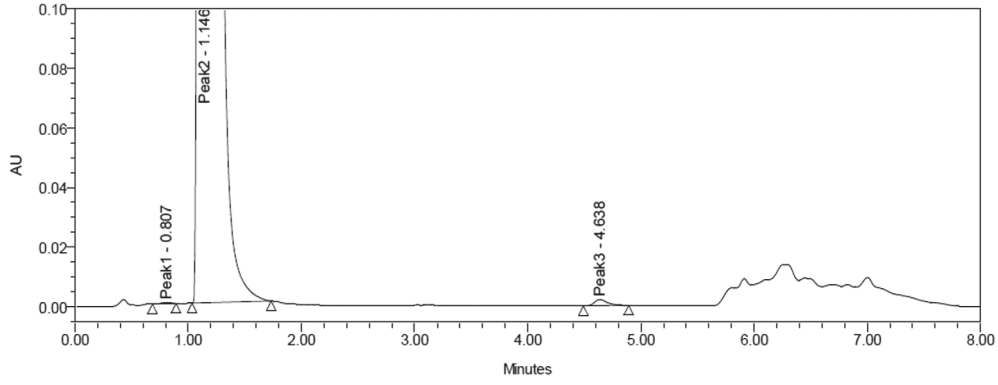
Figure 7. (*Continued*)



(d) Base degradation



(e) UV degradation



(f) Light degradation

detector. The forced degradation chromatograms are illustrated in Figure 7. Table 4 presents the results obtained from the stress studies.

The data in Table 4 indicate that the maximum degradation was observed in case of oxidation (5%), followed by base hydrolysis (0.8%). The molecule appears to be extremely stable under light

Table 4. Results of forced degradation studies

Stress conditions	% Degradation	Peak purity*			Purity flag**
		Impurity	Purity angle	Purity threshold	
Treated with 0.5 N HCl (1 ml) solution for 24 h on bench top	0.2	Impurity-1	1.120	1.328	No
		Impurity-2	12.528	12.697	
		Impurity-4	1.595	1.800	
Treated with 0.5 N NaOH (1 ml) solution for 24 h on bench top	0.8	Impurity-1	2.327	2.693	
		Impurity-2	9.083	11.358	
		Impurity-4	1.466	1.766	
Treated with 3% H_2O_2 (1 ml) solution for 48 h on bench top	5.0	Impurity-1	0.384	0.408	
		Impurity-2	5.558	8.645	
		Impurity-4	2.173	2.251	
Treated with heat at 70°C for 48 h	0.2	Impurity-1	1.695	2.591	
		Impurity-2	6.581	7.965	
		Impurity-4	1.480	2.014	
Exposed for sunlight 1.2 million lux h	0.1	Impurity-1	9.059	9.470	
		Impurity-2	9.666	14.499	
		Impurity-4	1.940	2.312	
Exposed for UV-light 200 W h $sq.m^{-1}$	0.1	Impurity-1	9.478	9.855	
		Impurity-2	8.427	9.989	
		Impurity-4	1.650	1.966	

Note: If it is shows "Yes" then peak is non homogenous.

*Peak purity passes if purity angle < purity threshold.

**Purity flag: It indicates homogeneity of all peaks.

Table 5. Results of stability of standard solution and sample at room temperature

Stability interval (about)	Plerixafor sample				
	Result			Difference	
	Standard	Sample-1	Sample-2	Sample-1	Sample-2
Initial	NA	100.1	100.0	NA	NA
24 h	1.00	99.0	99.2	1.1	0.8

stress conditions. According to Waters Empower software, the peak is homogenous if the purity angle is less than the purity threshold. The peak purity data indicated that all known impurities and PLX peaks were homogenous and free from interference, and their estimation was unaffected in presence of other degradant peaks. This confirms the stability-indicating power of the developed method.

4.1.6. Solution stability and mobile phase stability

The solution stability of standard and samples was determined by storing both the test solutions of sample and standard at room temperature for 24 h. The similarity factor was determined for the two standard solution responses, and %w/w concentrations were determined for assay of sample and impurities. Tables 5 and 6 present the solution stability for assay and related substances, respectively.

From Table 5, the similarity factor between the standard at zero hours and standard at 24 h is 1.00. This indicates that the standard solution is stable up to 24 h at room temperature.

The difference of % concentrations of impurities obtained between sample at zero hours and that obtained at 24 h indicates that the sample solution is stable up to 72 h.

4.1.7. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate changes in the method parameters. The robustness of the method was determined by deliberately varying pH, flow rate, and column temperature. The effect of mobile phase pH was studied at 2.3 and 2.7, the effect of flow rate was studied at 0.29 mL/min and 0.35 mL/min, and the effect of column oven temperature was studied at 30 and 40°C. The impact of column temperature on the assay of PLX was studied at 50 and 60°C. System suitability parameters, such as USP resolution, tailing % RSD, and retention time, of PLX were noted. The data are listed in Table 7. The data reveal that the method is sensitive to low pH and influences the resolution between impurity 1 and PLX. Furthermore, the flow rate affects the retention behavior of PLX.

Table 6. Results of stability of standard solution for impurities at room temperature

Name of the impurity	% of impurities (w/w) for sample-1			% of impurities (w/w) for sample-2		
	Initial	After 24 h	% Difference	Initial	After 24 h	% Difference
Impurity 1	0.061	0.058	4.9	0.070	0.068	2.9
Impurity 2	0.105	0.102	2.9	0.112	0.109	2.7
Impurity 3	0.051	0.050	2.0	0.050	0.050	0
Impurity 4	0.060	0.060	0	0.060	0.060	0
			Difference			Difference
Total impurities	0.358	0.312	0.046	0.301	0.298	0.003

Table 7. Robustness and method sensitivity data

S. no.	Condition	RT of plerixafor (min)	Assay of plerixafor in spiked sample (%w/w)	USP resolution for impurity 1	USP tailing of plerixafor	% RSD of standard	Method sensitivity*
1	Control (No change)	1.161	99.1	2.2	1.3	0.3	NA
2	pH (−)2.3	1.125	98.9	1.6	1.5	0.65	Yes
3	pH (+)2.7	1.105	98.9	2.2	1.3	0.44	No
4	Flow (+)0.35 mL/min	1.086	99.1	1.9	1.2	0.51	Yes
5	Flow (−)0.29 mL/min	1.341	99.3	2.3	1.4	0.52	No
6	Column oven temperature (+)40°C	1.122	99.0	2.2	1.2	0.44	No
7	Column oven temperature (−)(30°C)	1.163	99.0	2.0	1.2	0.48	No

*Based on the comparison of results from altered parameters with those of control from.

5. Conclusions

The rapid gradient reverse phase UPLC method, developed for the quantitative analysis of PLX and related substances in pharmaceutical dosage forms, is precise, accurate, linear, robust, and specific. Satisfactory results were obtained from validation of the method. The retention time (1.1 min) enables rapid estimation of the drug. This method exhibits an excellent performance in terms of sensitivity and speed. The method is stability-indicating and can be used for routine analysis of production samples, checking the stability of samples, and checking the stability of samples of PLX.

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