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Analysis of Forced Degradation Products in Rilpivirine using RP-HPLC and Peak Purity Evaluation

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ABSTRACT

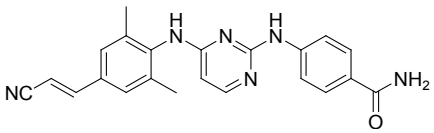
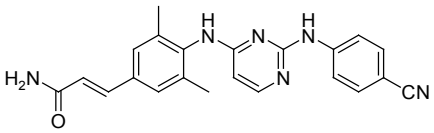
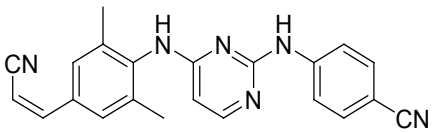
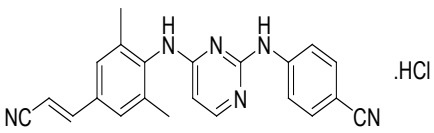
The primary objective of this research was to delve into the forced degradation products of Rilpivirine hydrochloride (RLP HCl), a crucial non-nucleoside reverse transcriptase inhibitor employed in management of epidemic disease named HIV-1. The investigation utilised the probable of RP-HPLC in tandem with peak purity assessment. In order to simulate conceivable degradation pathways, the study encompassed a *gamut* of stress conditions like acidic, alkaline, oxidative, thermal and photolytic environments. Authors used Agilent zorbax Eclipse XDB C18 column (150x2.1mm, 1.8 µm), RLP and impurities were separated. Buffer as pH of 3.0 and acetonitrile in gradient mode (68:32v/v), flow rate of 0.55 mL/minute. Volume injected is 3 µL and detection wavelength is 220 nm. Temperature is maintained at 55°C by 70:30v/v mixture of water and acetonitrile. System suitability was erect to be within the limits. The average percentage recoveries for impurities were 98% to 101%. The outcomes of this meticulous study unveiled the susceptibilities of RLP to a spectrum of stress factors, in the generation of impurity profile RLP-Amide A, RLP-Amide Band Z-RLP with peak purities. The forced degradation tests demonstrate that the peak of RP-HPLC is spectroscopically pure in all stressed conditions. All degradation products are separated from the main peak and do not interfere with main substance. This exploration not only augments the comprehension of RLP's stability profile but also underscores the pivotal role of analytical techniques in upholding the safety and efficacy benchmarks of pharmaceutical formulations.

Keywords: Rilpivirine HCl. Non-nucleoside, Gradient, Impurities, ICH, RLP-Amide A, RLP-Amide B and Z-RLP, Degradation products.

INTRODUCTION

Rilpivirine[1], (RLP) an aminopyrimidine which is a pyrimidine-2,4-diamine in that position of amino groups 2and 4 were interchangedby 4-cyanophenyl and 4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl groups in proper place to class of NNRTIs[2]. This should be utilized to manage HIV-1 infections individually or in combination with dolutegravir and cabotegravir. The remarkable potency and reduced risk of resistance, outstands RLP among other NNRTIs[3]. This Character arises from its adaptability within its binding site and its internal conformational flexibility.Its approval for various treatment regimens, including the pioneering Juluca, showcases its impact on HIV-1 management. The flexibility in dosing intervals further underscores its evolution in improving treatment options. The substance profile of drug is crucial task to the regulatory circumstances. Because of unknown foreign substances, unwanted solvents, at very less levels, may vary the consequence of efficiency drug along with side effects. Accordingly, profile of foreign substance of drug should be performedwith using the method for stability indicative.The literature review reveals that few methods were published for the analysis of RLP individually and in with other combinations using HPLC and LCMS[4,5]. But, no method is published till now for the impurity profiling of RLP by RP-HPLC with peak purity assessment. Separate a mixture of compounds to diagnose and compute into separateconstituentswith the help ofHPLC[6,7]. Different authors used various methods for the forced degradations studies and the results obtained are in the limit for the mobile phase, injection of the sample and wavelength[8-9]. The present work focussed to develop HPLC method to separate foreign substances and their degradation products[10-12] by taking everything in the mind limit ofspecification along with short run time and validated this process as per the rules and regulations given by ICH[13].

Table 1(a): Structures of RLP and its Impurities

Abbreviation	Chemical name	Structure
RLP Amide B	4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino] -2 pyrimidinyl] amino]benzamide	
RLP Amide A	(E)-3-(4-((2-((4-cyanophenyl)amino) pyrimidin-4-yl)amino) -3,5-dimethylphenyl) acrylamide	
Z-RLP	4-[[4-[[4-[(Z)-2-cyanoethenyl]-2,6-dimethylphenyl]amino] -2 pyrimidinyl]amino] benzonitrile	
RLP HCl	4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino] -2 pyrimidinyl] amino]benzonitrile monohydrochloride	

MATERIALS AND METHODS

Rilpivirine and related foreign substances are procured from clearsynth laboratory. Acetonitrile and methanol of HPLC standard are purchased from Rankem India Pvt. Ltd. Other chemicals used for the preparation of buffer were of analytical grade, pure milli-Q water is used from Millipore purification structure.

Instrumentation and chromatographic conditions

Waters HPLC Model: 2695 furnished by 2996 photo diode array detector was utilized to developmentalong with validation of the method, byusing sample injector which is a automated. Agilent Zorbax Eclipse XDB C18 column (150 x 2.1mm, 1.8 µm) was used for the separation. 0.3%v/v solution of perchloric acid in water adjusted to pH 3.0 with 5% sodium hydroxide solution utilized as

a Eluent A and eluent B asAcetonitrile. Analysis wasperformedby gradient mode. The rate of flow is 0.55 mL/minute. 3µL volume is injected into the system. Temperature of the column is maintained at 55°C. 40 min is the runtime. The gradient program is represented in Table 1. At a wavelength of 220nm the data is identified. Final output signal is observed and also integrated with the help of Empower 2 software.

Table 1(b): Gradient Programme to separateforeign substancesin RLP

Time (Min)	% Eluent A	% Eluent B
0	68	32
7.0	60	40
21.9	20	80
22.0	68	32

Preparation of solutions

Diluent is prepared by mixing both water as well as acetonitrile in the proportions of 70v/v and 30v/v.

Preparation of sample solution: RLP HCl powder (about 20 mg) was weighed and treated as indicated and diluted to 50 mL with diluents (400 µg/mL). The obtained solutions were injected for Impurity profile. For assay determination and Peak purity each treated solution was diluted 5/20 with diluents(100µg/mL).

RESULTS AND DISCUSSION

This HPLC validation processis performed out to determine foreign substances in RLP as per the rules and regulations given by ICH for to demonstrating that this proposed process is a stability indicative topredetermined usage. Also forced degradation studies were done along peak purity assessment.

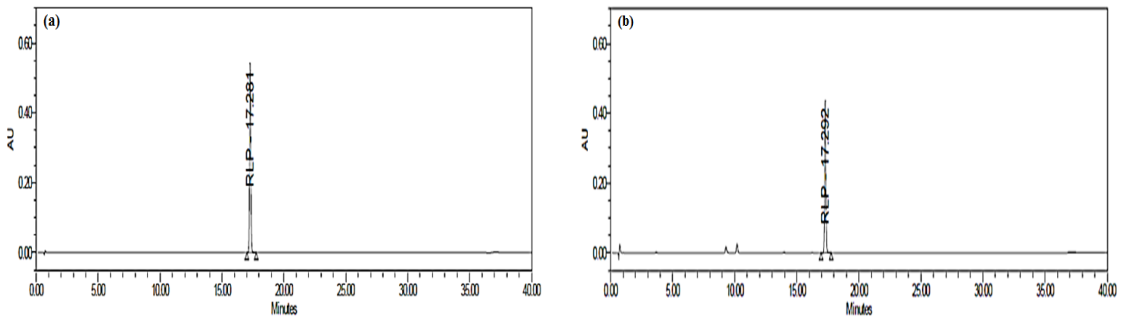


Fig. 1. Chromatogram of RLP(Standard and sample)

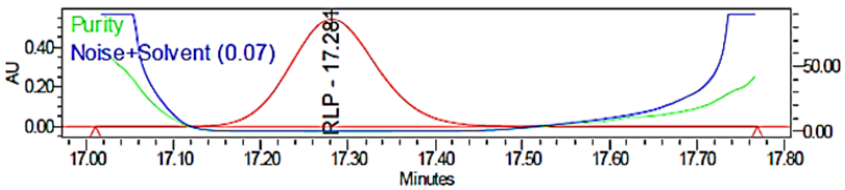


Fig. 2. Peak purity plot for unstressed sample of RLP

Table 2: Specificity experimental data

Name of the Sample	Retention time in Minute	Relative retention time
RLP amide B	9.298	0.54
RLP Amide A	10.181	0.59
Z-RLP	16.260	0.94
RLP.HCl	17.319	1
Blank	-	-

By this chromatogram, authors are observed that there is no interference because of diluent blank in retention times of RLP and its related foreign substances. All these foreign substanceswere apportioned by very good resolution.

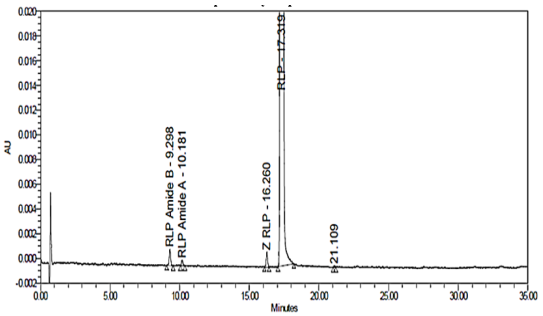


Fig. 3. Spiked sample chromatogram

Degradation studies

Preliminary SIM studies showed that Methanol as dilution solvent is participating in the degradation chemistry of RLPHCl under acidic and basic stress conditions. Therefore and due to RLP HCl poor solubility in organic solvents and water, DMSO was used as co solvent. These samples of RLP are stressed by using acid, base, oxidation, heat as well as humidity. Samples those are degraded are analysedwith a photo diodie array detector. Peak purity of RLP as well as its related impurities are recognized the forced degradation circumstanceswhich are denoted in Table 3 and the obtained results were specified in Table 4.

Impurity Profile by HPLC - Acid Degradation

The following degradation products were formed during degradation.

Table 3: Forced degradation conditions of RLP

Stressed condition	Solvent	Temp (ºC)	Exposed time in hours
Acid	0.5MHCl	70	48
Base	0.5M NaOH	RT	8.5
Oxidation	30% H_2O_2	RT	6
Photolytic Solution	Diluent	765W/m² at 35°C	1
Powder	-	765W/m², 35°C	6.9
Heat solution	Diluent	70	46
Powder	-	120	48
Humidity 80%	Diluent	-	-

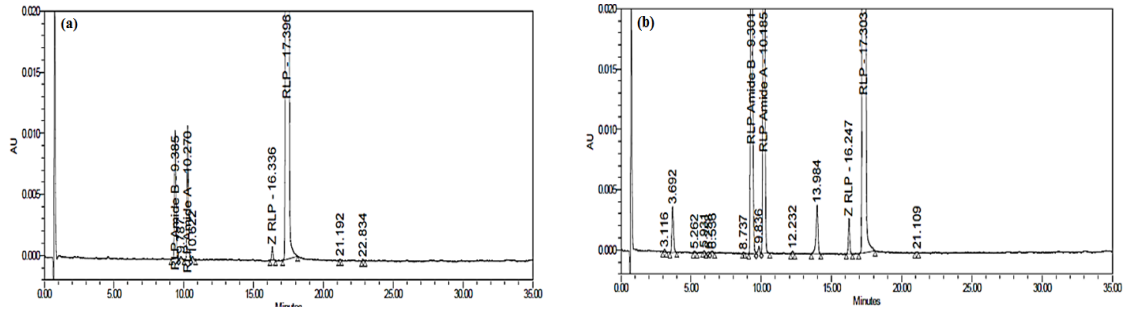


Fig. 4. Degradation with (a). Acid (b). Base

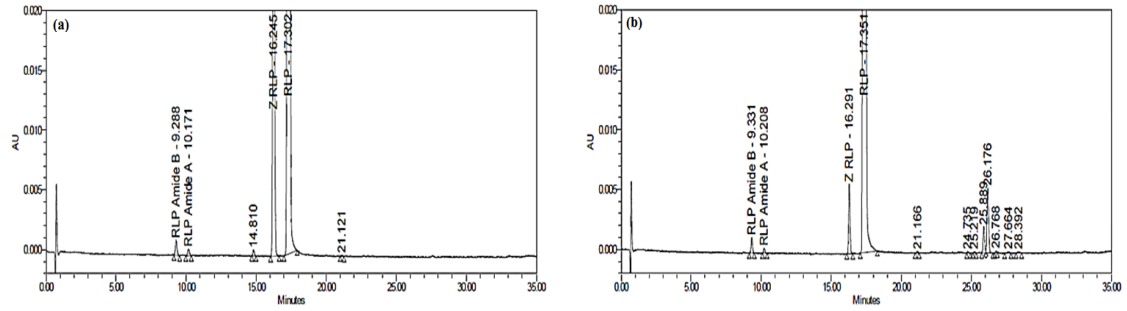


Fig. 5. Exposed toirradiation of xenon arc lamp (Z-RLP as degradant) (a). Solution (b). Powder

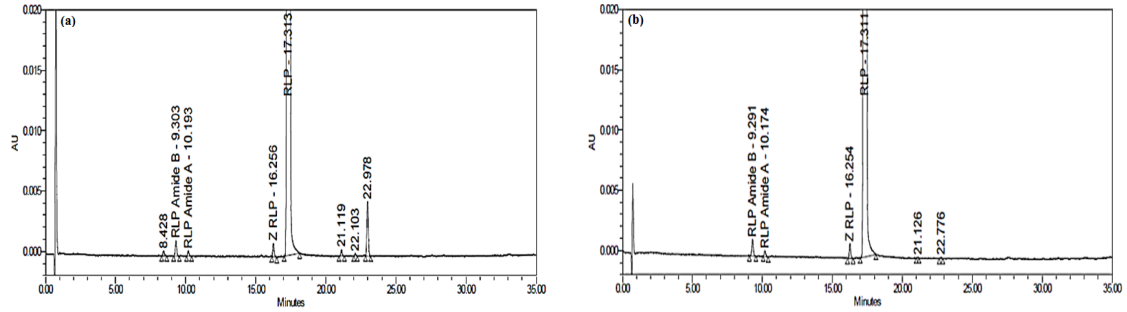


Fig. 6. RLP HCl solution under heat treatment degrades to minor degradation product at RRT 1.33 (a). Solution (b). Powder. No degradation products (equal or higher than 0.02% w/w) were observed

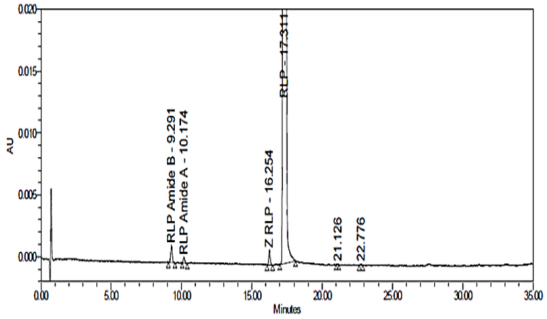


Fig. 7. Degraded under 80% humidity: No degradation products (equal or higher than 0.02% w/w) were observed

Table 4: Summary of Assay and Peak Purity of degraded RLP HCl on various conditions

Stress action	Conditions		Time interval	%Assay	Purity angle	Purity Thresh-hold	Match angle	Match Thresh-hold
Unstressed	NA	NA	NA	NA	0.044	0.083	0.00	0.087
Oxidation	1 mL H_2O_2 30%	RT	6 h	98.4	0.045	0.085	0.057	0.088
Acid	1 mL HCL 0.5M	70°C	48 h	99.6	0.039	0.095	0.062	0.103
Base	1 mL NaOH 0.5M	RT	8.5 h	90.2	0.069	0.225	0.076	0.147
Sun cabinet	Sun cabinet of solution	765W/m² 35°C	1 h	93.5	0.039	0.085	0.064	0.091
	Sun cabinet of powder	765W/m² 35°C	6.9 h	99.6	0.040	0.084	0.059	0.089
Heat	Heating of solution	70°C	46 h	100.2	0.043	0.096	0.055	0.103
	Heating of powder	120°C	48 h	101.9	0.044	0.065	0.051	0.091
Humidity	Exposure to humidity	RT	24 h	99.4	0.069	0.225	0.076	0.147

The forced degradation tests demonstrate that the peak of RLP HCl is spectroscopically pure in all stressed conditions, all degradation products are separated from the main peak and do not interfere with main substance. RLP HCl powder was found to be stable under heat treatment. RLP HCl solution was found to be stable under oxidation treatment. RLP HCl solution was found to be stable under acidic treatment without heat. Acidic degradation combined with heat: The main degradation products are RLP Amide A and RLP Amide B. Basic degradation: Main products of degradation were RLP Amide A as well as RLP Amide B. Exposure of RLP HCl solution to heat: RLPHCl solution main degradation product is peak at RRT 1.33. Exposure of RLP HCl solution to irradiation of xenon arc lamp: Main degradation product is Z-RLP.RLP HCl powder under irradiation of Xenon arc lamp 765W/m2, 35°C was decomposed to Z-RLP and some unknown impurities: RRT 1.49 and RRT 1.51.Exposure of RLP HCl powder to humidity no degradation product formed.

CONCLUSION

By theexperimentalvalues it was finalized that, this newly developed method to simultaneous estimation of related substances in RLP is identified as simple, more precise and highly accurate along with the high resolution. This present approach provides cost effective and should be furnished to routine analysis in pharma industry. The outcomes of this meticulous study unveiled the susceptibilities of RLP to a spectrum of stress factors, in the generation of impurity profile RLP-Amide A, RLP-Amide Band Z-RLP with peak purities. The forced degradation tests demonstrate that the peak of RLPHCl is spectroscopically pure in all stressed conditions. All degradation products are separated from the main peak and do not interfere with main substance. This exploration not only augments the comprehension of RLP's stability profile but also underscores the pivotal role of analytical techniques in upholding the safety and efficacy benchmarks of pharmaceutical formulations.

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Conflicts of Interest

There are no conflicts of interest among the authors.

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