Quantification of Pitolisant: A RP - HPLC study

Sowjanya Gummadi & Rajya Lakshmi Nimmagadda *

Department of Pharmaceutical Analysis, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh - 530045.

*Corresponding Author address

Rajya Lakshmi N
Department of Pharmaceutical Analysis,
GITAM Institute of Pharmacy, GITAM (Deemed to be University),
Rushikonda, Visakhapatnam, Andhra Pradesh – 530045.
Email: rlakshmi.pharma9@gmail.com
Mobile: 7288095994

Abstract

Objective
An attempt was made to develop a simple, isocratic and robust reverse phase high performance liquid chromatographic method for Pitolisant.

Material and methods
An Inertsil octa decyl silane column and photo diode array detector were employed for the quantification of Pitolisant at 268 nm using a mobile phase consisting of acetonitrile and 0.1 % v/v formic acid (90:10 % v/v) at a flow rate of 1.0 mL/min. for the efficient elution of Pitolisant.

Results
The developed method was validated for various parameters and the linearity range was observed at 25-150 µg/mL. The correlation coefficient, limit of detection and limit of quantification calculated from the calibration curve were 0.9998, 0.3 µg/mL and 1.0 µg/mL respectively. The mean percentage recovery was 99.9 %. The formulation was subjected to stress at various conditions resulting in minor degradants at different retention times.

Conclusion
An isocratic, fast, robust reverse phase – high performance liquid chromatographic method was developed which can be exploited for the estimation of Pitolisant in bulk and tablets.

Key words: *Pitolisant, High performance liquid chromatography, Photo diode array detector, Limit of detection, Degradation studies*

1. Introduction

Pitolisant (chemically 1- [3 – [3 - (4 – chloro phenyl) propoxy] propyl] piperidine; hydrochloride) [1] (fig. 1) is an antagonist/ inverse agonist of the human histamine H3 receptor, used for the treatment of narcolepsy with or without cataplexy in adults [2]. Pitolisant is soluble in alcohol, acetonitrile and has a dissociation constant of 9.67.

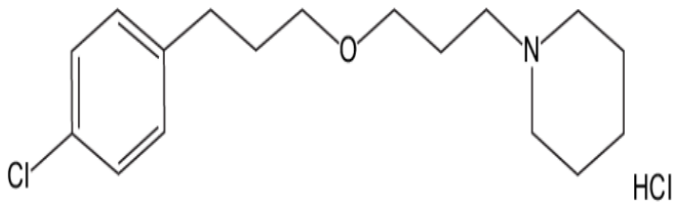


Figure 1. Chemical structure of Pitolisant

Literature review revealed that there is only one reverse phase – high performance liquid chromatography (RP - HPLC)[3] method was reported using waters X- bridge phenyl (150 mm × 4.6mm, 3.5 µm) column with photo diode array detector comprising of 0.1% of ortho phosphoric acid and acetonitrile (30:70 % v/v) as mobile phase at 1.0 mL/min. flow rate at 210 nm wavelength detection with 4.358 min. retention time and a single liquid chromatography – mass spectrometry/ mass spectrometry (LC-MS/MS)[4] method reported for Pitolisant. So, an economically newer method development was undertaken in this study.

2. Materials and methods

Chemicals and reagents

The chemicals and reagents formic acid (analytical grade) and acetonitrile (HPLC grade) utilized for the study were purchased from local market. Pitolisant standard was obtained from Glenmark pharmaceuticals private limited, Mumbai and sample tablets were obtained from Zydus Cadila health care limited, Secunderabad.

Instrumentation

The proposed work was executed on a waters autosampler high performance liquid chromatography (HPLC) equipped with Inertsil octa decyl silane C18 column (250 × 4.6 mm, 5 µm), photo diode array (PDA) detector operated with empower software. Shimadzu balance and smart ultrasonic bath were also used.

Preparation of 0.1 % v/v formic acid

1.0 mL formic acid was pipetted to a 1000 mL volumetric flask, dissolved and made up with HPLC water.

Preparation of stock solution

25 mg of standard was weighed, transferred to a clean 25 mL volumetric flask. To this, a few mL of acetonitrile (ACN) was added, dissolved and made up with ACN (1000 µg/mL). All

further dilutions of working standard (100 µg/mL) and 25-150 µg/mL were prepared from this stock solution.

Preparation of sample solution

20 Pitolisant (Wakix -18 mg) film coated tablets were weighed, powdered finely using mortar and pestle and a 25 mg equivalent was taken into a 25 mL clean volumetric flask. To this a few mL of ACN was added, sonicated for 1 hour, made up with ACN and filtered using 0.45 micron filter. This solution was further diluted as required.

Method Development

Chromatographic method conditions

Few trials were executed to develop a simple, economic HPLC method for Pitolisant using various columns, flow rates, diluent ratios etc. A few trials were conducted with Waters X – bridge C8 column (150 × 4.6 mm, 3.5µ) using ACN: 0.1% v/v tri - fluoro acetic acid (TFA) (80:20 % v/v), Agilent C18 column (150 × 4.6 mm, 3.5µ) and Inertsil C18 column (250 × 4.6 mm, 3.5 µ)with ACN:0.1 % v/v TFA (70:30 % v/v) as mobile phase at a flow rate of 1 mL/min. Symmetrical peak shape, USP plate count and USP tailing were not satisfactory during the above trials. Then finally the method was optimized and developed using the Acetonitrile and 0.1 % v/v formic acid (90:10 % v/v) mixture at a flow rate of 1.0 mL/min. operated over a run time of 6 minutes and with an injection volume of 10 µg/mL.

Method validation

The method developed was validated[5] for parameters like system suitability, precision, linearity, limit of detection & limit of quantification, accuracy, specificity and robustness. The validation results obtained were compared with the acceptance criteria as per international council for harmonization (ICH) Q2 R1 guidelines.

System suitability

The suitability of the developed method and the HPLC system were tested with 100 µg/mL prepared from standard stock (1000 µg/mL).

Linearity

From the standard stock (1000 µg/mL), volumes of 0.25, 0.50,0.75, 1.0,1.25, 1.50 mL each were pipetted into 6 labeled 10 mL volumetric flasks, dissolved and made up with diluent. Each solution was poured into labeled vials, placed in HPLC vial tray and analyzed.

Limit of detection (LOD) & limit of quantification (LOQ)

The limit of detection and limit of quantification were calculated by S/N ratio.

Precision

Six different solutions of 100 µg/mL were prepared and analyzed. The peak areas obtained from the chromatograms during the same day and different days were noted and the % RSD was calculated.

Accuracy

The % recovery value was calculated at 3 levels (50 %, 100 %, 150 %) by standard addition method. To 1 mL sample stock taken into 10 mL volumetric flasks, 0.5 mL, 1.0 mL and 1.5 mL of 100 µg/mL standard was added at each level respectively (3 concentrations at each level)and made up with diluent. The solutions were transferred to clean labeled vials placed in a HPLC tray and analysed.

Specificity

The interference of the excipients, solvents, degradants and impurities in the formulation were observed by comparing the chromatograms obtained by injecting a blank, a standard (100 µg/mL) and a placebo.

Robustness

Any change in chromatographic parameters such as peak area, retention time, tailing factor and theoretical plates were observed by deliberate modifications of flow rate (± 5 mL/min.), mobile phase composition (± 5 % v/v) over the software command. A 1.0 mL standard stock (1000 µg/mL) was pipetted into a clean 10 mL volumetric flask, made up with diluents and analysed at the altered conditions.

Assay

The developed method was applied for calculating the amount of Pitolisant in tablet and compared with the labeled amount mentioned on the strip label. The sample solution as discussed above was further diluted and analyzed.

Forced degradation study

The sample solution was subjected to stress conditions like acid, base, oxidation, hydrolysis, heat, photolysis[7]. The peak areas obtained were analysed and observed for any possible degradations. The efficacy of the method was also studied in the presence of degradants.

Acid degradation

A 1.0 mL of 1000 µg/mL sample solution and 1.0 mL 1N HCl were pipetted, transferred into a 10 mL volumetric flask, heated at 60 ^{0}C for 30 min., cooled and made up with diluent after neutralization with 1 mL 1N NaOH.

Basic degradation

To a clean 10 mL volumetric flask, 1.0 mL (1000 µg/mL) of sample solution and 1.0mL of 1N NaOH was added, heated for 30 min. at 60 ^{O}C, cooled, neutralized with 1 mL 1N HCl and made up with diluent.

Hydrolytic degradation

An aliquot (1.0 mL) of sample solution was pipetted and transferred into a clean 10 mL volumetric flask, 1.0 mL of HPLC water was added, heated at 60 ^{0}C for 30 min. cooled and made up to 10 mL with acetonitrile.

Thermal degradation

To a clean 10 mL volumetric flask, 1.0 mL of sample solution was added, heated at 105 ^{0}C for 30 min. and made up with diluent after cooling.

Photolytic degradation

In a clean 10 mL volumetric flask, 1.0 mL sample stock was taken, kept in UV chamber for 30 min. and made up with diluent.

Oxidation degradation

1.0 mL of sample stock was taken into 10 mL clean volumetric flask, 1.0 mL of 3 % v/v hydrogen peroxide was added, cooled after heating for 30 min. at 60 ^{0}C and made up with diluents.

All the degradation solutions made up were poured into labeled vials, placed in vial tray and 20 µL of this solution was injected into the HPLC column at specified intervals of time.

Solution stability

The stability study [6] was conducted with a 100 µg/mL sample solution at room temperature and 2-8 ^{0}C.

3. Results and discussion

Method development

The chromatographic method was optimized after the trials with the below conditions. Various combinations of C8 and C18 columns employing a variety of mobile phases were used for the method development. Finally an optimized peak (fig. 2) satisfying all the system suitability parameters is obtained. The conditions are mentioned in table 1.

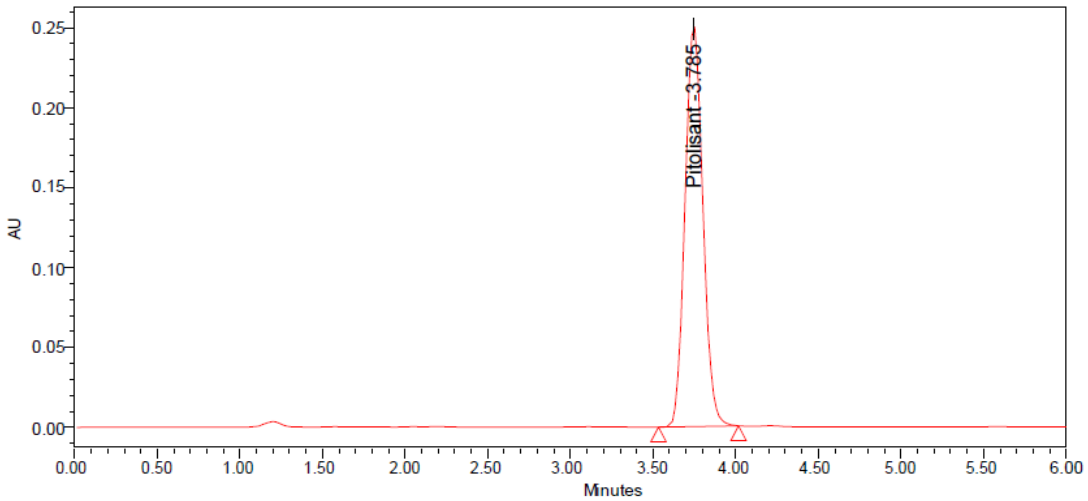


Figure 2. Chromatogram for standard (100 µg/mL) of Pitolisant

Table 1. Optimized chromatographic conditions of Pitolisant

Parameters	Value
Column	Inertsil ODS (250 × 4.6mm, 5µm)
Mobile phase	ACN: 0.1 % v/v formic acid (90: 10 % v/v)
Mode of elution	Isocratic
Flow rate	1.0 mL /min.
Detection wavelength	268 nm
Volume of injection	10 µL
Run time	6 min.
Retention time	3.680 ± 0.45 min.

ODS octa decyl silane, mm millimeter, µm micrometer, ACN Acetonitrile, % v/v percentage volume by volume, mL/min. milliliter/minute, nm nanometer , µL microliter, min. minute, ± plus or minus

Method validation

The results obtained by validation complied with ICH specifications.

System Suitability

The parameters like theoretical plates, tailing, and % RSD were within the acceptance criteria as given in table 2. indicates the suitability of the method.

Table 2. Results for System Suitability of Pitolisant

System suitability parameter	Acceptance criteria	Pitolisant
USP plate count	NLT 2000	4404
USP tailing	NMT 2.0	1.05
% RSD	NMT 2.0	0.79

USP United states pharmacopoeia, NLT not less than, NMT not more than, % RSD percentage relative standard deviation

Linearity

The relationship between concentration and peak area was observed using the calibration curve and the range was found to be 25 – 150 µg/mL. The correlation coefficient, slope and y - intercept were calculated from the curve (fig. 3) and table 3.

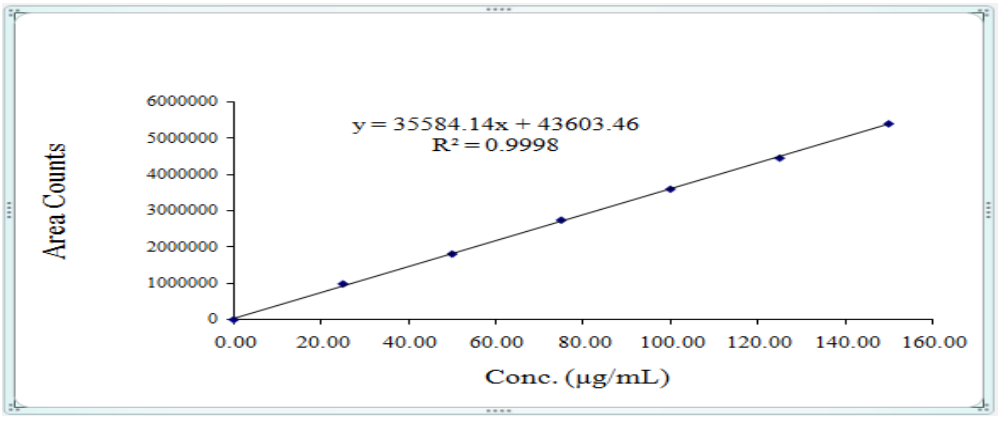


Figure 3. Linearity curve of Pitolisant

Table 3. Results for Linearity of Pitolisant

Conc. (µg/mL)	Area counts
25	985642
50	1810243
75	2745865
100	3596457
125	4452103
150	5396587

Conc. Concentration, µg/mL microgram/ milliliter

LOD & LOQ

The lowest amount detected and quantified was 0.3 µg/mL, 1.0 µg/mL respectively using S/N ratio.

Precision

The results obtained were precise as observed from the % relative standard deviation (0.79 for intraday and < 0.1 for inter - day study) which is in compliance with the ICH guidelines as in the table 4. (4A and 4B).

Table 4A. Results for Precision (Intra – day) of Pitolisant

Conc. (µg/mL)	Area counts	*Area counts	% Assay (%w/w)	*% Assay ± SD, % RSD
100	3526451	3539257	99.6	99.9 ± 0.78, 0.79
	3520130		99.8	
	3535326		99.4	
	3524856		99.5	
	3596175		101.5	
	3532601		99.7	

µg/mL microgram/milliliter, * Mean of six determinations, % percentage, % w/w percentage weight by weight, ± plus or minus, SD standard deviation, % RSD percentage relative standard deviation

Table 4B. Results for precision (Inter- day)

Conc. (µg/mL)	Day 1		Day 2		Day 3	
	Area counts	*Assay (% w/w) ± SD,% RSD	Area counts	*Assay (% w/w) ± SD,% RSD	Area counts	*Assay (% w/w) ± SD,% RSD
Control	3530362	-	3538612	-	3523112	-
100	3525101	99.8 ± 0.03, 0.03	3523451	99.6 ± 0.08, 0.08	3516351	99.9 ± 0.05, 0.05
	3524504		3520130		3519630	
	3525165		3525326		3517226	
	3524856		3524856		3518856	
	3527175		3527175		3520854	
	3525722		3522601		3520401	

Conc. Concentration, µg/mL microgram/milliliter, * mean of 6 determinations, % w/w percentage weight by weight, ± plus or minus, SD standard deviation, % RSD percentage relative standard deviation

Accuracy

The percentage recovery (100.4 % w/w) calculated was satisfactory as mentioned in the table 5.

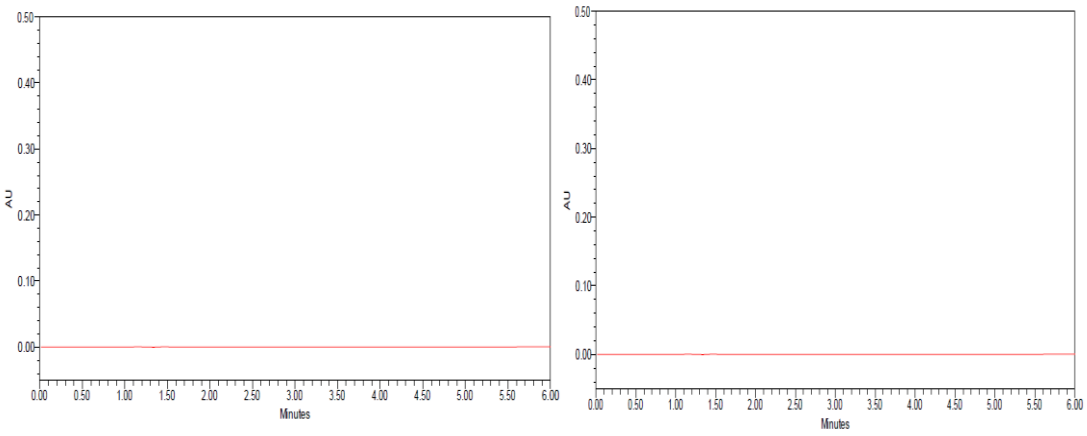
Table 5. Results for Recovery (%) of Pitolisant

% level	Area counts	*Peak area	Recovery (%)	*Recovery (%) ± SD, %RSD
50	5292415	5272527	149.8	149.2 ± 0.79, 0.79
	5282514		149.5	
	5242653		148.3	
100	7066421	7047733	199.9	199.4 ± 0.56, 0.56
	7024715		198.8	
	7052064		199.5	
150	8841754	8848177	250.2	250.1 ± 0.17, 0.17
	8842529		250.2	
	8831247		249.9	

*Mean of three determinations, % percentage, ± plus or minus, SD Standard deviation, % RSD percentage relative standard deviation

Specificity

There were no interferences due to the excipients, solvents, degradants and impurities at the retention time of the analyte as observed from blank, placebo (fig. 4) below.



4A. Blank **4B. Placebo**

Figure 4. Chromatograms for Specificity of Pitolisant

Robustness

Deliberate modifications of flow rate and organic phase composition had little effect on USP plate count, USP tailing but much effect on the peak area as in the table 6. The method was found to be robust as the changes did not affect the assay and % RSD.

Table 6. Results for Robustness of Pitolisant

Parameter	Area counts	*Area counts	USP plate count	USP tailing	* % Assay ± SD, % RSD
Flow rate (+ 5 parts)	3784521	3776855	4361	1.06	100. 6 ± 0.2, 0.2
	3768594		4325	1.08	
	3777451		4396	1.08	
Flow rate (+ 5 parts)	3475826	3440900	4456	1.11	99.9 ± 0.92, 0.92
	3432658		4437	1.11	
	3414215		4438	1.12	
Organic mobile phase composition (+ 5% v/v)	3865748	3861527	4312	1.09	100.1 ± 0.91, 0.91
	3895687		4339	1.10	
	3823146		4313	1.09	
Organic mobile phase composition (- 5 % v/v)	3296587	3272707	4448	1.10	100.1 ± 0.67, 0.67
	3254581		4497	1.09	
	3266953		4361	1.10	

* Mean of three determinations, USP United states pharmacopoeia, SD Standard Deviation, % RSD Percentage relative standard deviation

Assay

The percentage assay of the analyte in tablets was calculated and compared with the assay specified for Pitolisant by the manufacturer (table 7. and fig. 5).

Table 7. Results for Assay of Pitolisant

Sample	Label claim (mg)	Amount found (mg)	% Assay (% w/w) ± SD
Wakix	18	18.1	100.4 ± 0.36

mg milligram, % percentage, % w/w percentage weight by weight, SD Standard deviation

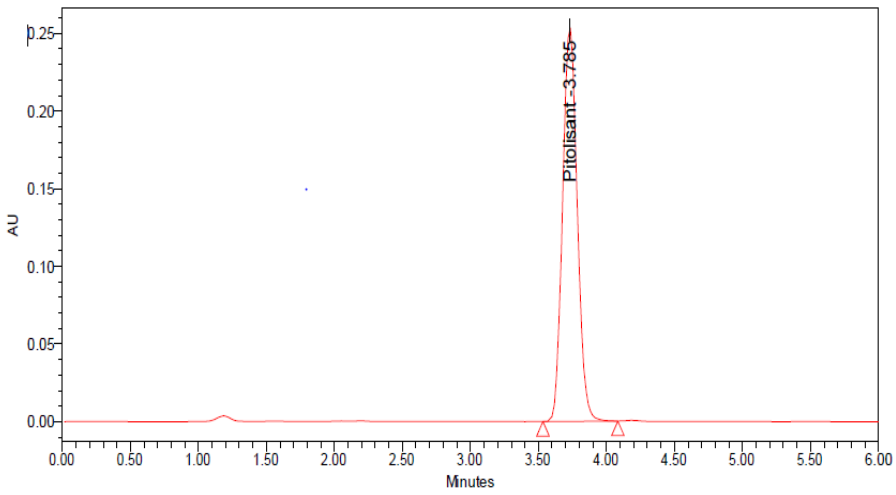


Figure 5. Chromatogram for Assay of Pitolisant

Solution stability

Stability study conducted showed negligible deviation up to 24 hr. both at room temperature and at 2-8 ^{O}C, the results are tabulated in table 8.

Table 8. Results for Solution Stability

Condition	Stability at RT		Stability at 2 – 8 ^{O}C	
	% purity	% deviation	% purity	% deviation
0 hr.	100.0	0.00	99.8	0.20
6 hr.	99.5	0.50	99.2	0.80
12 hr.	99.4	0.60	98.9	1.10
18 hr.	99.6	0.40	98.2	1.80
24 hr.	98.0	2.00	97.6	2.40

RT Room temperature, hr. hours, ^{O}C degrees Celsius, % percentage

Forced degradation studies

The degradation (%) at specified time intervals is provided in table 9. Major degradation occurred in acidic, alkali, oxidative conditions and minor degradation in thermal, photolytic hydrolytic conditions at 24 hr. as observed from the fig 6.

Table 9. Results for Forced Degradation Studies of Pitolisant

Type of degradation	Area counts	Degraded (%)	Purity angle	Purity threshold
*Control	3534168	0.2	-	-
Acid	2671047	24.4	0.264	10.542
Alkali	2699532	23.8	0.264	10.541
Peroxide	2622810	25.9	0.265	10.505
Hydrolysis	3421054	3.4	0.219	10.547
Photolysis	3386578	4.4	0.253	10.533
Thermal	3145642	11.2	0.217	10.154

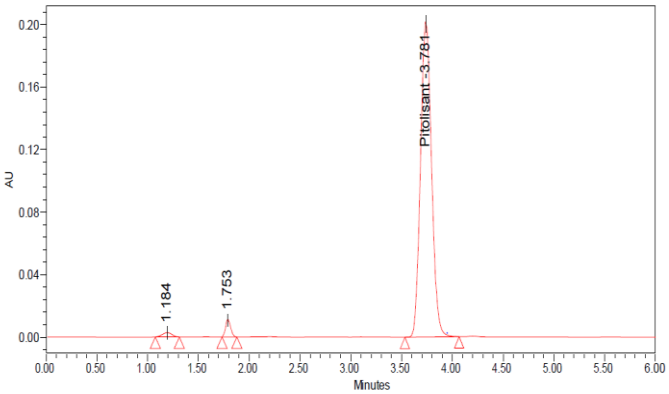


Figure 6A. Acid degradation

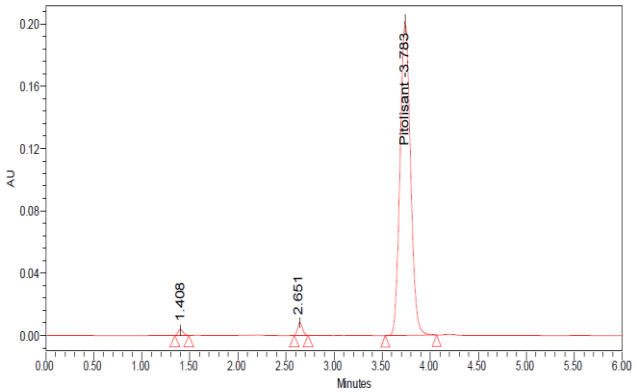


Figure 6B. Alkali degradation

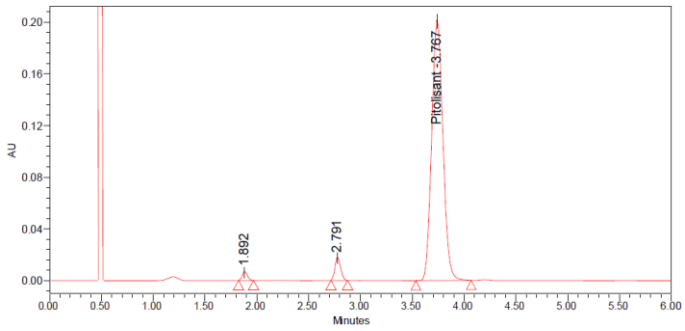


Figure 6C. Oxidative degradation

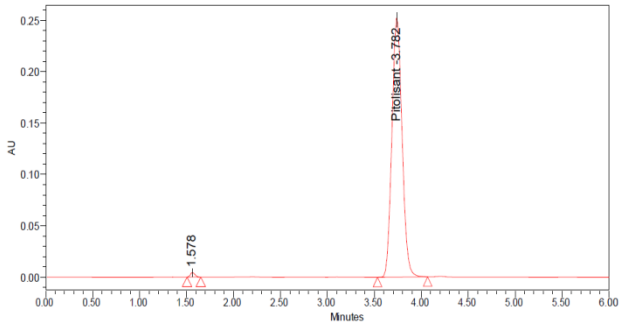


Figure 6D. Thermal degradation

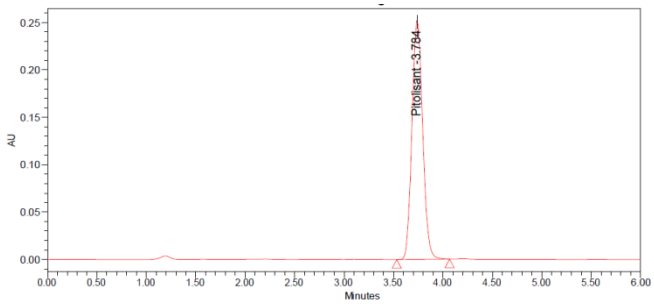


Figure 6E. Photolytic degradation

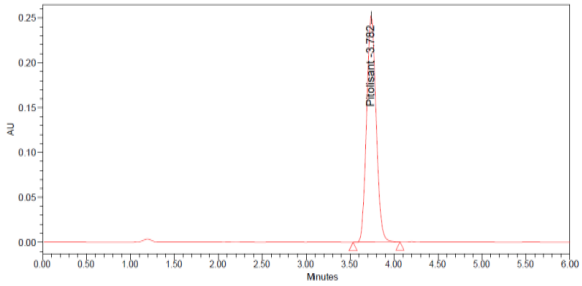


Figure 6F. Hydrolytic degradation

Figure 6. Chromatograms for Forced Degradation Studies

Conclusion

A simple, sensitive and economical isocratic RP-HPLC method was developed for quantification of Pitolisant and the results of validation were in accordance to ICH specifications. The proposed study can be considered as a rapid method as the analyte eluted at a less retention time than the previously reported method. The method was specific and stability indicating as few degradants were reported in all degradations. Thus, the present method can be routinely employed in the quality control testing of the analyte in active pharmaceutical ingredient and tablets.

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Ethics approval and consent to participate

Any kind of animals and humans were not used for the study

Availability of the data and material

The data and material will be available on request.

Contribution

The authors shared equal contribution in the preparation of manuscript and approved the manuscript.

References

[1] Marta Kollb - Sielecka , Pierre D. , Joseph E. , Greg M., Tomas S., Manuel Haas. The European medicines agency review of Pitolisant for treatment of narcolepsy: summary of the scientific assessment by the committee for medicinal products for human use. Sleep medicine. May 2017; 33: 125-9.

[2] Magdalena K., Kamil K., Katarzyna S., Jacek S. et.al. The histamine H3 receptor inverse agonist Pitolisant reduces body weight in obese mice. Naunyn - Schmiedeberg's Archives of Pharmacology.2018 August; 391(10): 875-81.

[3] Venkateswara Rao P., Leela Rani J., Roja Kumari M., Kalyani G., Sirisha G. and Naveen Reddy P. Stability indicating RP - HPLC method for determination of Pitolisant in bulk and pharmaceutical dosage form. International Journal of Research in Pharmacy and Chemistry.2020; 10(29):137-143.

[4] Ramakrishna N. , Devender Reddy A., Vishwottam K., Hanumanth Rao P., Mahesh Reddy J., Gopinadh B., Nageswar Rao M., Himabindu V. LC-MS/MS method for the determination of Pitolisant: application to rat pharmacokinetic and brain penetration studies. Biomedical chromatography.2013 Nov; 24(11):1431-37.

[5] ICH Q2 R1. Validation of analytical procedures: text and methodology. Current step 4 version.1994 October; revised on 1996 November & 2005 November

[6] ICH Q1A R2. Stability testing of new drug substances and products. 2003 February.

[7] ICH Q1B. Photo stability testing of new drug substances and drug products. Current step 4 version, 1996 November 6.