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DEVELOPMENT AND VALIDATION OF NOVEL STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF PITOLISANT IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:
A new simple, accurate and precise stability-indicating RP-HPLC analytical method has been developed and validated for the quantitative analysis of Pitolisant as bulk drug and in tablet dosage form. The objective of this study was to explore Pitolisant degradation behavior under ICH recommended stress conditions, employing a newly designed stability indicating RP-HPLC method and validating it. The method was developed on Agilent eclipse C8 (150 mm × 4.6 mm) column with Methanol: 0.05 M Phosphate Buffer (pH 5) (65: 35, v/v) as mobile phase using simple gradient elution technique. Detection was carried out at 222 nm using a photodiode array detector at ambient temperature. The retention time was found to be 3.6 min. The developed procedure was successfully validated as per ICH Q2 (R1) guidelines. The developed method was found to be linear within the range of 5-30 µg mL^{-1} (R^2 =0.993), precise as % R.S.D. for inter-day and intra-day precision were found to be < 2 %, accurate as % recovery values in accuracy study was found to be in range of 98-102 % and sensitive as limit of detection and limit of quantitation were found to be 0.28 µg mL^{-1} and 0.85 µg mL^{-1}, respectively.

***Keywords**: Pitolisant, Stress degradation, Stability indicating, RP-HPLC*

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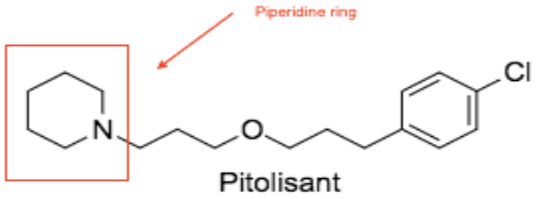
INTRODUCTION:

Pitolisant (chemically 1- [3 – [3 - (4 – chloro phenyl) propoxy] propyl] piperidine; hydrochloride) [1] (Figure 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.



Pitolisant

Literature survey revealed that very few methods were reported for the estimation of Pitolisant in pharmaceutical dosage form by RP-HPLC method and efficient economic methods were less. One reverse phase-high performance liquid chromatography (RP-HPLC) method involving method optimization and validation reported for Pitolisant using Inertsil octa decyl silane C18 column (250 × 4.6 mm, 5 µm) column with photo diode array (PDA) detector consisting of acetonitrile and 0.1 % formic acid (90: 10, v/v) as mobile phase at a flow rate of 1.0 mL min^{-1} at 268 nm with retention time of 4.358 min [3]. A single liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) method is also reported for Pitolisant [4].

Stability indicating RP-HPLC method for determination of pitolisant in bulk and pharmaceutical dosage form was proposed by Venkateswara Rao P *et al*. [5] which involved only details of method development and validation but stability related data was not reported in the research paper. The less amount of literature provides the need for developing a new suitable, selective, sensitive and economic stability indicating RP-HPLC method for determination of pitolisant. So, an economically newer and more sensitive stability indicating RP-HPLC method for estimation of pitolisant in tablet formulation was undertaken in this study by subjecting the drug to different stress conditions viz. hydrolysis, oxidation and thermal and photolysis as specified by ICH [6, 7].

MATERIALS AND METHODS:

Chemicals and reagents

Active pharmaceutical ingradient (API) Pitolisant was obtained as gift sample from Lupin Labs India Pvt. Ltd. (Pune, India). The pharmaceutical tablet dosage form Wakix labelled to contain 18 mg Pitolisant was procured from local market. Methanol (HPLC Grade) was obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

HPLC instrumentation

The samples were analyzed using HPLC system (JASCO), model PU 2080 plus pump with Rheodyne sample injection port (20 µL). The study was performed using Agilent eclipse C8 (150 mm × 4.6 mm) column with photo diode array (PDA) detector (MD 2010) with Borwin chromatography software (version 1.5) consisting of Methanol: 0.05 M Phosphate Buffer (pH 5) (65: 35, v/v) as mobile phase at a flow rate of 1.0 mL min^{-1} employed in gradient mode at 222 nm.

Selection of analytical wavelength

A solution of 10 µg mL^{-1} was prepared from standard stock solution (1000 µg mL^{-1}) and scanned over 200-400 nm in UV Spectrophotometer. The maximum absorbance was shown at 222 nm. Hence it was selected as analytical wavelength.

Stock solution and working standard preparation

An accurately weighed 10 mg of drug transferred to 10 mL volumetric flask, and the volume was made up to 10 mL with methanol to get standard stock solution of 1000 µg mL^{-1}. From the standard stock solution, working standard solution having concentration 100 µg mL^{-1} was prepared using mobile phase as final diluent.

Preparation of sample solution

20 Pitolisant (Wakix -18 mg) film coated tablets were weighed, finely powdered using mortar and pestle and a 10 mg equivalent was taken into a 100 mL clean volumetric flask and diluted with methanol to obtain a sample solution having concentration 100 µg mL^{-1}. The solution was filtered through Whatman paper No. 41. One mL of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the methanol to get 10 µg mL^{-1} sample solution. After setting the chromatographic conditions, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of drug present per tablet was estimated from the respective calibration curve. The % assay was found to be 99.22 ± 1.28 (mean ± S.D.).

Stress degradation studies

The bulk drug was subjected to stress conditions like acid, base, oxidation, heat, and photolysis. The peak areas obtained were analyzed and observed for any possible degradations. The studies were carried out at 20 µg mL^{-1} concentration. The hydrolytic studies

were carried out by treatment of stock solution of drug separately with 1 N HCl and 0.1 N NaOH at room temperature for 1 h. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 20 µg mL^{-1}. The oxidative degradation was carried out in 9 % H_2O_2 at room temperature for 30 min and sample was diluted to obtain 20 µg mL^{-1} solution. Thermal stress degradation was performed by keeping drug in oven at 105ºC for period of 6 h. Photolytic degradation studies were carried out by exposure of drug to UV light.

RESULTS AND DISCUSSION:

Method optimization

To develop an appropriate method for the estimation of Pitolisant, different mobile phases were tried to attain the better separation and resolution. The development was initiated through use of mobile phase which contained acetonitrile and water in diverse ratios like 50: 40, 30: 70, 60: 40, v/v that showed no peak for the drug. Then development performed by replacing methanol with acetonitrile in the similar proportions which showed the improper peak shape. Finally solvent mixture composing methanol: 0.05 M Phosphate buffer (pH 5) (65: 35, v/v) was selected as an optimum mobile phase. The mobile phase utilized offered excellent resolution along with sharp and well resolved peak without any tailing. The retention time was found to be 3.6 ± 0.04 by the use of the proposed method. The optimized chromatographic conditions are summarized in Table 1. The representative chromatogram of the standard solution is shown in Fig. 1.

Table 1: Summary of optimized chromatographic conditions

Parameters	Conditions used
Stationary Phase	Agilent eclipse C8 (4.6×150mm×5µm)
Mobile Phase	Methanol and 0.05 M Phosphate Buffer (65: 35, v/v)
Flow Rate	1 mL min^{-1}
RT (min)	3.6 ± 0.04
Asymmetry	1.63
Plates (N)	>2649

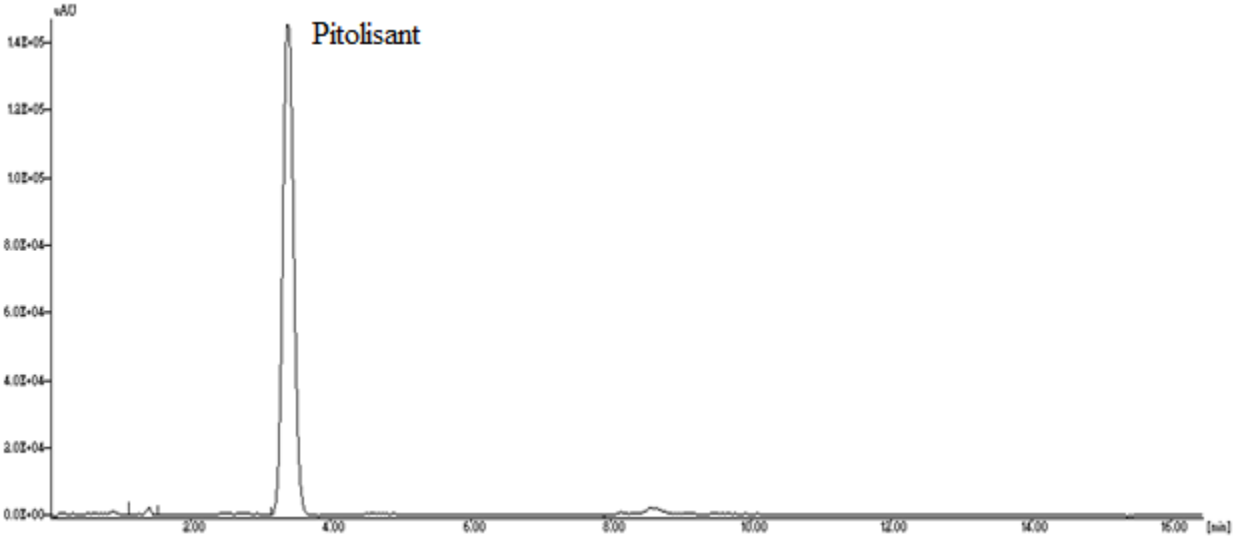


Fig. 1: Chromatogram of standard solution (20 µg mL^{-1}, RT = 3.6 ± 0.04)

Result of stress degradation studies

The stress degradation studies revealed susceptibility of Pitolisant to hydrolytic, oxidative stress conditions and stability under thermal and photolytic stress conditions. The drug showed marked degradation in hydrolytic conditions without appearance of degradants and oxidative stress conditions along with appearance of degradation product at RT 5.3 min. The method was found to be specific as degradation product was not interfering with retention time of drug. Figures 2-4 represents the chromatogram of acid, alkali and peroxide induced degradation. The findings of degradation studies along with % degradation and % of drug recovered are summarized in Table 2.

Table 2: Stress degradation studies

Stress conditions	% Recovery	% Degradation
Acid hydrolysis (1N HCl, Kept at RT for 1 h)	85.41	14.59
Base hydrolysis (0.1 N NaOH, Kept at RT for 1 h)	86.77	13.23
Oxidative degradation (9 % H_2O_2, Kept at RT for 30 min)	80.31	19.69
Thermal degradation (105º C for 6 h)	97.57	2.43
Photolytic degradation (UV light, 200-watt h square meter^{-1})	99.92	----

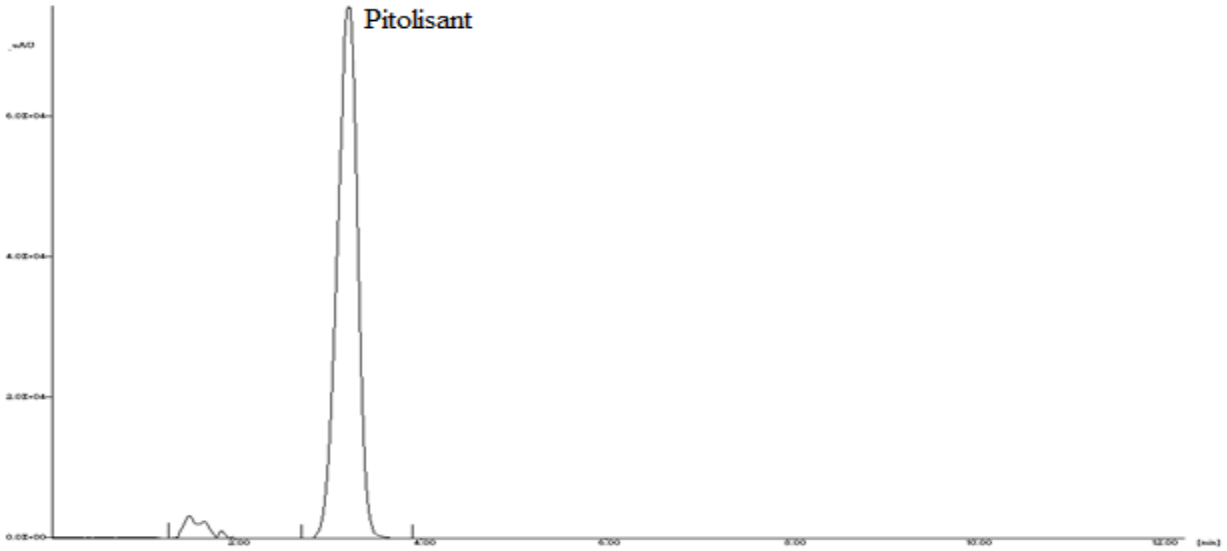


Fig. 2: Chromatogram after treatment with 1N HCl, Kept at RT for 1 h

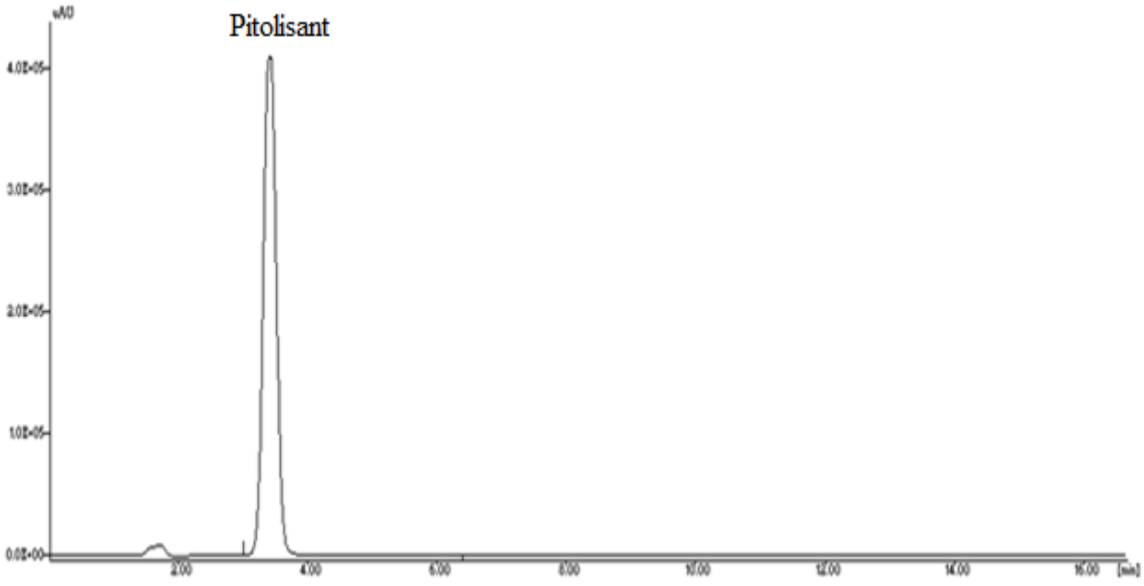


Fig. 3: Chromatogram after treatment with 0.1N NaOH, Kept at RT for 1 h

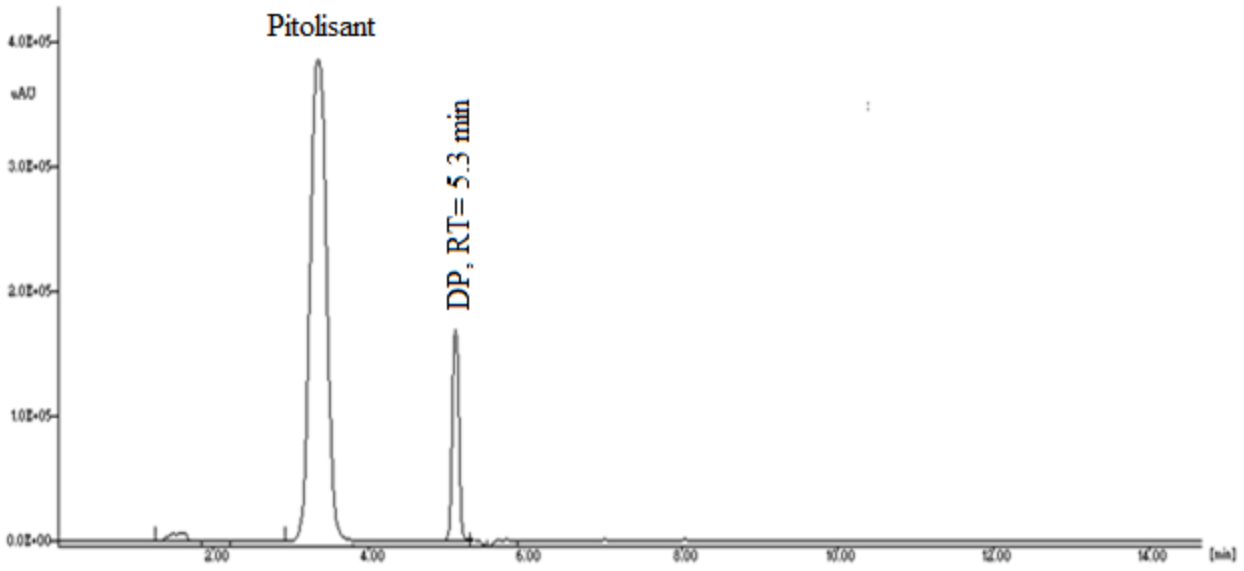


Fig. 4: Chromatogram of peroxide induced degradtion with degradation product (DP, RT= 5.3 min)

Analytical method validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH Q2 (R1) guidelines.

Linearity

From the standard stock solution (100 µg mL^{-1}), volumes of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mL each were pipetted into 6 labeled 10 mL volumetric flasks, dissolved and made up with diluent. Six replicates per concentration were injected and chromatograms were recorded. The peak areas of drug were recorded and calibration curve was plotted of peak area against concentration. Linear response was observed in the concentration range of 5-30 µg mL^{-1}. The correlation coefficient, slope and y - intercept were calculated from the curve (Fig. 5).

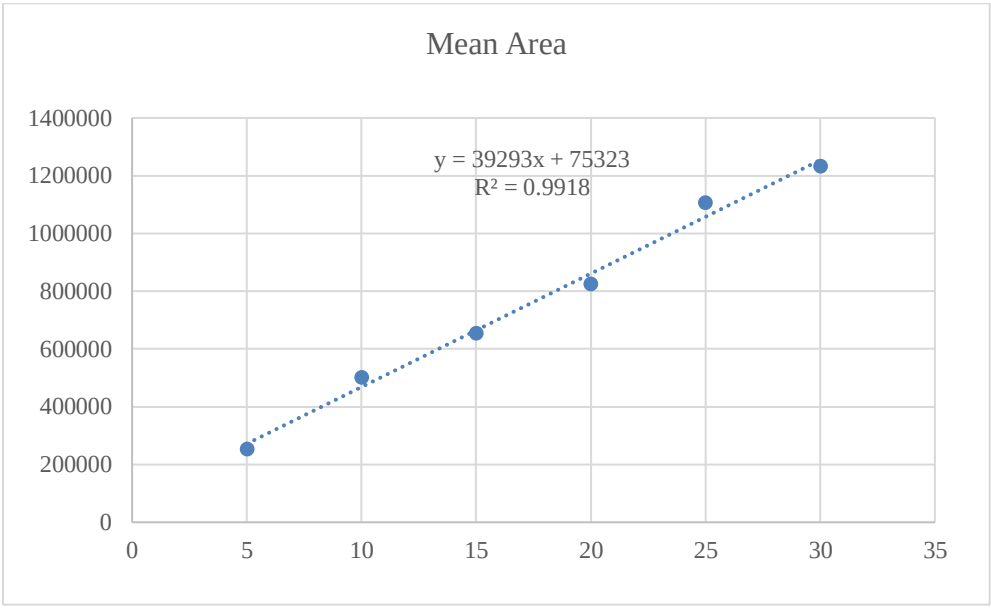


Fig. 5: Calibration curve for Pitolisant

Precision

Intra-day and inter-day variation studies were carried to find out precision of the method. Intra-day precision was determined by analyzing Pitolisant standard solutions at three different concentrations in linearity range for thrice on the same day. Each concentration was injected in triplicate and % R.S.D. was determined. Standard drug solutions at three different concentrations on different three days over a period of one week were analyzed and % R.S.D. was calculated for inter-day precision. The % R.S.D. values obtained for Intraday and interday variations were found to be < 2 which indicated that method is precise. The results obtained for intraday and inter-day precision studies are shown in Table 3 and 4, respectively.

Table 3: Intraday precision studies

Concentration (µg mL^{-1})	Average Area	Recovered concentration (µg mL^{-1})	% R.S.D.*
10	464239	09.89	0.24
15	658172	14.82	0.16
20	861590	20.03	0.65

* Average of three determinations

Table 4: Interday precision studies

Concentration (µg mL^{-1})	Average Area	Recovered concentration (µg mL^{-1})	% R.S.D.*
10	461957	09.83	1.69
15	657194	14.80	1.78
20	860620	19.98	0.43

* Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 0.28 µg mL^{-1} and 0.85 µg mL^{-1} respectively.

Accuracy

Recovery studies were carried out to confirm accuracy by applying the method to drug content present in sample to which known amount of standard was added at 80 %, 100 % and 120 % levels. The technique involves addition of standard drug solution to preanalysed sample solution. The resulting sample solutions were injected and chromatogram was recorded. The concentration of drug was determined from calibration curve. At each of the levels, three determinations were performed. Basic concentration of sample chosen was 10 µg mL^{-1} from tablet solution. The results of the recovery studies indicated that developed method is accurate for estimation of drug in tablet formulation.

Table 4: Recovery studies

Drug	Basic sample concentration (µg mL^{-1})	Concentration added (µg mL^{-1})	Concentration found (µg mL^{-1})	% Recovery±R.S.D.*
Pitolisant	10	8	17.81	98.99±0.79
	10	10	19.88	99.40±0.88
	10	12	21.97	99.91±0.60

*Average of three determinations, R.S.D. is relative standard deviation

Specificity

The specificity of method was confirmed by peak purity profiling studies. The peak purity values were found to be ≥995, indicating the no interference of any other impurity or matrix. The ability of developed method to separate the drug from excipients present in the formulation proved the specificity of method.

Robustness

As per the ICH, method robustness expresses its capacity to remain unaltered through small, deliberate variations in parameters of method. The parameters altered were change in flow rate of mobile phase (± 0.1 mL min^{-1}) and mobile phase composition (± 1% methanol). Not any of the variations produced a marked change in chromatograms as well as peak areas of drugs which demonstrated robust nature of the developed method.

CONCLUSION:

Stability-indicating RP-HPLC technique with no any interference from the excipients or degradant products have been developed as well as validated for the estimation of Pitolisant in tablet formulation. Compared with HPLC method reported [5], the established method is more sensitive as the linearity for the method developed is in the concentration range 5-30 µg mL^{-1} whereas for reported method, it was found to be in the concentration range 18.00-270 µg mL^{-1}. HPLC methods that have been reported involved use of acetonitrile in mobile phase which is costlier than methanol, in our method mobile phase is binary mixture of methanol and buffer. So, the developed method is simple, economic, sensitive, accurate and precise and has been validated as per the ICH guidelines. The proposed study can be considered as a rapid method as the analyte eluted at a less retention time than the previously reported method. It can therefore be concluded that use of the method can save much time and money and it can be used even in small laboratories and can also be utilized as quality control tool for the determination of Pitolisant as bulk and in its tablet formulation with very high accuracy and precision.

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REFERENCES:

1. Kollb-Sielecka M, Pierre D, Joseph E, Greg M, Tomas S, Haas M. 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 Med, 2017; 33: 125-129.
2. Magdalena K, Kamil K, Katarzyna S, Jacek S. The histamine H3 receptor inverse agonist pitolisant reduces body weight in obese mice. Naunyn Schmiedebergs Arch Pharmacol, 2018; 391(10): 875-881.
3. Gummadi S, Rajya Lakshmi N. Quantification of Pitolisant: A RP-HPLC study. YMER, 2022; 21(6): 896-907.
4. Ramakrishna N, Devender Reddy A, Vishwottam K, Hanumanth Rao P, Mahesh Reddy J, Gopinadh B, Nageswar Rao M. LC-MS/MS method for the determination of pitolisant: application to rat pharmacokinetic and brain penetration studies. Biomed chromatogr, 2013; 24(11):1431-1437.
5. Venkateswara Rao P, Leela Rani J, Roja Kumari M, Kalyani G, Sirisha G, 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(1): 137-143.
6. ICH Guideline Q2A (R1), Validation of analytical procedure, text and methodology Q2 (R1) (2005)
7. ICH Guideline Q1A (R2), Stability testing of new drug substances and products Q1A (R2) (2003)