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

P. Venkateswara Rao*, J. Leela Rani, M. Roja Kumari,

G. Kalyani, G. Sirisha and P. Naveen Reddy

Vikas College of Pharmacy, Vissannapeta,
Krishna District, Andhra Pradesh-521 215, India.

ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Pitolisant in pharmaceutical dosage form. Chromatographic separation of Pitolisant was achieved on Waters Alliance-e2695, by using Waters X-Bridge Phenyl, 150mm x 4.6mm, 3.5µm, column and the mobile phase containing 0.1% OPA& ACNin the ratio of 30:70% **v/v**. The flow rate was 1.0 ml/min; detection was carried out by absorption at 210nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Pitolisant was NLT 2000 and should not more than 2respectively. %Relative standard deviation of peak area of all measurements always less than 2.0**.** The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Pitolisant and study of its stability.

Keywords: HPLC and Pitolisant.

INTRODUCTION

Pitolisant is a selective antagonist or inverse agonist of the histamine H3 receptor that is used in the treatment of type 1 or 2 narcolepsy. Narcolepsy is a chronic neurological disorder that that affects 1 in 2,000 individuals and is characterized by excessive daytime sleepiness, abnormal REM sleep manifestations, sleep paralysis and hypnagogic hallucinations.[1,2,3] Pitolisant chemicaly1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine show in fig: with molecular formula $C_{17}H_{26}ClNO$ and molecular weight is 295.85. Pitolisant acts as a high-affinity competitive antagonist (Ki 0.16 nM) and as an inverse agonist (EC50 1.5 nM) at the human histamine H3 receptor subtype.[1] It is thought to bind to the antagonist binding site of H3 receptor, which is located within the trans-membrane core just below the extracellular loops. Piperidines forms a salt bridge with Glu206 in the membrane spanning segment and the hydroxyl of Tyr374 is H-bonded with the central oxygen of piperidine.[4] Pitolisant displays high selectivity for H3 receptors compared to other histamine receptor subtypes. Pitolisant also modulates acetylcholine, noradrenaline and dopamine release in the brain by increasing the levels of neurotransmitters but does not increase dopamine release in the stratal complex including nucleus accumbent.[4,5]

As per literature survey 2 LCMS methods are reported for the estimation of pitolisant[8,9]. The objective of the study is to develop accurate precise repeatable and reproducible method for simultaneous estimation of pitolisant and to validate to ICH guidelines and to perform the forced degradation studies[10, 11].

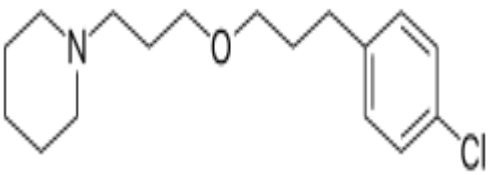

Fig. 1: Structure of pitolisant

MATERIALS AND METHODS

The reference sample of pitolisant was obtained as a gift samples and the tablet containing pitolisant 18mg was procured from local market. Water (HPLC grade) from Rankem and acetonitrile(HPLCgrade),orthophosphoricacid(ARgrade)methanol(Rankem),triethylamine (Rankem) from Merck Limited, 0.45 µm Nylon filter was from Phenomenex 87456 were used.

INSTRUMENTATION

Waters HPLC 2695 system equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer, PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbanceofpitolisant solution, Electronics Balance-Denver pH meter -BVK enterprises, India Ultrasonicator-BVK enterprises

CHROMATOGRAPHIC CONDITIONS

Waters Alliance-e2695, by using Waters X-Bridge Phenyl, (150mm x 4.6mm, 3.5µmS) column. Temperature was maintained ambient, mobile phase used was 0.1% OPA& ACN in the ratio of 30:70% **v/v**. flow rate was maintained at 1.0 ml/min. Diluent used throughout the method was water and ACN (50:50v/v) and the run time was 6 mins. All the samples and mobile phase were degassed for 30 mins and filtered by ultrasonic filtration by using 0.45 µm Nylon (N66) 47 mm membrane filter. Detection was carried out at 210nm using PDA detector with an injection volume of 10 µL. By using the above optimized conditions method was determined.

Preparation of Standard Solution

180µg/ml of Pitolisant is prepared by diluting with mobile phase. This solution is used for recording chromatogram.

Preparation of sample Solution

Two tablets (each tablet contains Pitolisant 18 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solution of Pitolisant (1800 µg/ml) was prepared by dissolving weight equivalent to 8 mg of Pitolisant and dissolved in sufficient mobile phase and sonicated for 5 min and dilute to 20ml with mobile phase. Further dilutions are prepared in 5 replicates of 180 µg/ml of Pitolisant was made up to mobile phase.

METHOD VALIDATION

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific

SYSTEM SUITABILITY

Tailing factor for the peak due to pitolisant in Standard solution should not be more than 2.0 Theoretical plates for the pitolisant peak in Standard solution should not be less than 2000.

Formula for Assay

$$\%\,\boldsymbol{Assay} = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where
AT= average area counts of test (sample) preparation.
AS= average area counts of standard preparation.
WS= Weight of working standard taken in mg.
DS= Dilution of working standard in ml.
DT= Dilution of test (sample) in ml.
WT= Weight of test (sample) taken in mg.
P= Percentage purity of working standard
LC= Label Claim mg/ml.

Linearity

The linearity of the proposed method was determined by quantitative dilution of the standard solution of pitolisant to obtain solution in concentration range of 18.00µg/ml-270.00µg/ml. A graph of peak area versus concentration in µg/ml was plotted for the drug. The slope, intercept, and correlation coefficient of regression line.

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

The LOD and LOQ represent the concentration of analyte that would yield to signal-to-noise ratio of 3 for LOD and 10 for LOQ. LOD and LOQ were calculated using following formula,

LOD=3.3 σ/S
LOQ= 10 σ /S

where, σ = standard deviation of response (peak area) and S = average of slope of the calibration

Method precision

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 180ppm of pitolisant).

System precision

System precision is checked by using standard chemical substance to ensure that the analytical system is working properly. In this peak area and % of drug of six determinations is measured and % RSD should be calculated.

Accuracy

The accuracy of this method was performed at three different levels (50%, 100%, 150%), by the addition of a known amount of standard to the sample at each level. Each level was repeated three times (n=3).

Robustness

Robustness is the measure of optimized method capacity to remain unaffected by small, but deliberate variations in method parameters such as mobile phase flow rate (±0.2 mL/min), wavelength nm (±1 nm), and column oven temperature (±1°C).

Solution Stability

The solution stability of pitolisant in diluents was determined by storing sample

Solution in tightly capped volumetric flask at room temperature for 24hrs. The amount of pitolisant was measured at different time intervals like 6,12,18 and 24 hrs and results obtained were compared with freshly prepared of pitolisant solution

RESULTS AND DISCUSSION

Chromatographic conditions

X-Bridge phenyl (150mm x 4.6mm, 3.5µm) Waters column. Temperature was maintained ambient, mobile phase used was 0.1% OPA & ACN in the ratio of 30:70% **v/v**. And flow rate was maintained at 1.0 ml/min. Diluent used throughout the method was water: acetonitrile (50:50 v/v) and the run time was 6 mins. All the samples and mobile phase were degassed for 30 mins and filtered by ultrasonic filtration by using 0.45 µm Nylon (N66) 47 mm membrane filter. Detection was carried out at 268nmnm using PDA detector with an injection volume of 10 µL. By using the above optimized conditions method was developed. Shown in Fig. 2 and Table 1.

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific**.**

Linearity

The linearity of the proposed method was determined by quantitative dilution of the standard solution of pitolisant to obtain solution in concentration range of18.00to270.00µg/ml. A graph of peak area versus concentration in µg/ml was plotted for the drug. The slope, intercept, and correlation coefficient of regression line were determined. Shown in Table no2.

SYSTEM SUITABILITY

Tailing factor for the peak due to pitolisant in Standard solution should not be more than 2.0

Theoretical plates for the pitolisant peak in Standard solution should not be less than 2000.

The results are summarized in Table 3.

Method precision

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD

The precision of the instrument was checked by repeatedly injecting (n=6) .solutions of 180ppm of pitolisant). TableNo: 4

System precision

System precision was determined by measuring the peak area of six replicate injections of standard solution. The value of %RSD was found to be <2, which ensure the analytical system is working properly. The results of system precision are tabulated in 5.

Accuracy

The accuracy of this method was determined by calculating percent recovery of pitolisant in formulation at three different levels (50%, 100%, and 150%). The % recovery obtained was found to be in the range of 100.2 to 99.8%. The accepted limits of mean recovery is 100.1% and obtained results were within the

acceptable range, which indicate recovery values were good, affirming the accuracy of the developed method. The results are summarized in Table 6.

Robustness

The method was found to be robust when minor changes were made in optimized chromatographic conditions such as oven temperature (±5°C), mobile phase flow rate (±0.1 mL/min), and ratio of mobile phase (±5mL). It was observed that there was no marked change in analytical data of the drugs which indicates good reliability during normal usage. The results are shown in Tab; 7.

Solution Stability

The solution stability of pitolisant in diluents was determined by storing sample Solution in tightly capped volumetric flask at room temperature for 24hrs. The amount of pitolisant was measured at different time intervals like 6,12,18 and 24 hrs and results obtained were compared with freshly prepared of pitolisant solution

Acceptance Criteria: The %RSD values for the assay of the solution stability experiments were calculated and should not more than 2.0%.

Limit of detection

This is the lowest concentration in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Limit of quantitation

This is the lowest concentration of analytic in a sample that can be determined with acceptable precision and accuracy.

Acceptance criteria

S/N Ratio value shall be 3-10 for LOD solution. S/N Ratio Value shall be 20-30 for LOQ solution shown in Table no: 8

Chromatographic conditions

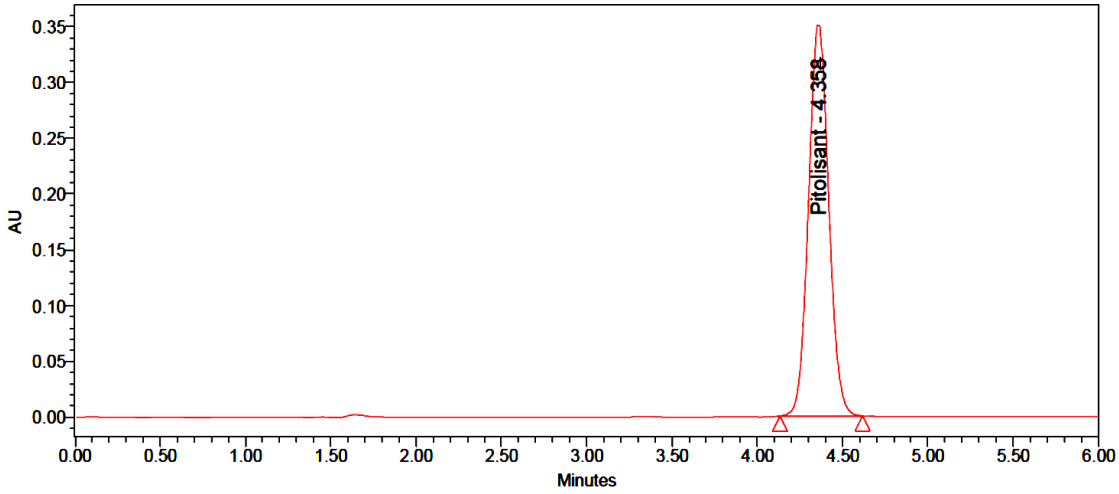

Fig. 2: Typical Chromatogram of pitolisant

Table 1: Optimized chromatographic conditions of pitolisant

S.NO	PARAMETERS	CHROMATOGRAPHIC CONDITIONS
1.	Mobile phase	0.1% OPA : ACN 30:70
2.	Column	X-Bridge phenyl 150mm x 4.6mm, 3.5µm
3.	Flow rate	1.0 ml/min
4.	Column temperature	Room temperature(20-25^{o}C)
5.	Sample temperature	Room temperature(20-25^{o}C)
6.	Wavelength	210nm
7.	Tablet volume	10 µl
8.	Run time	6 min
9.	Retention time	4.358 min Pitolisant

Linearity

Table No 2: Linearity of pitolisant

S.No.	Conc.(µg/ml) Pitolisant	Area Pitolisant	Acceptance criteria
1	18.00	293054	Squared co relation coefficient should be not less than0.999.
2	45.20	793514	
3	90.00	1416209	
4	180.00	2814505	
5	225.20	3318517	
6	270.00	4025617	

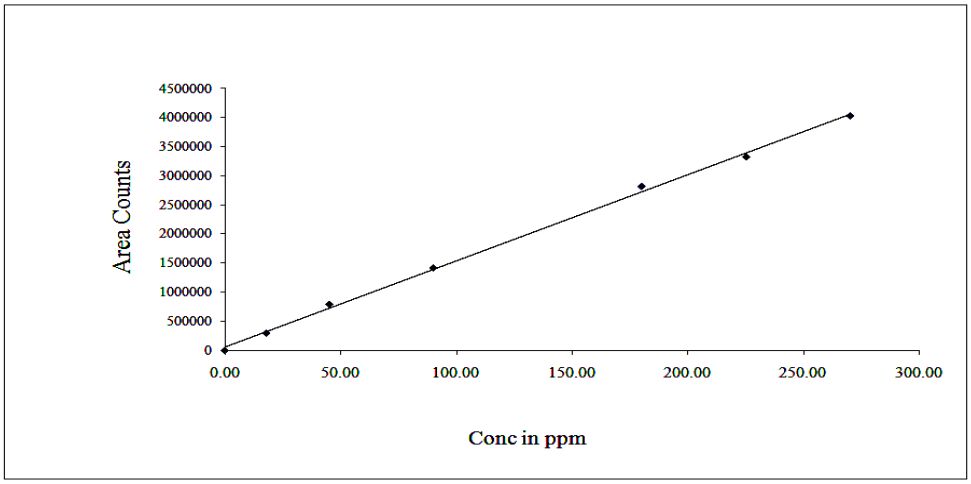

Fig. 3: Linearity graph of pitolisant

Table 3: System Suitability Results of Pitolisant

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor (TF)	Resolution
1	4.346	2839455	6527	1.04	-
2	4.348	2861501	6530	1.04	-
3	4.349	2844080	6412	1.04	-
4	4.356	2880398	6328	1.07	-
5	4.356	2881624	6324	1.07	-
6	4.358	2889900	6333	1.06	-
Mean		2866160			
SD		21108.496			
%RSD		0.74			

Table 4: Method precision results of Pitolisant

Pitolisant		
S.No.	**RT**	**Area**
1	4.347	2850839
2	4.345	2842476
3	4.344	2874127
4	4.350	2857333
5	4.339	2866629
6	4.348	2857396
Ave area		2858133
St dev		11202.83
%RSD		0.39

Table 5: System precision of Pitolisant

S.No.	Standard area of Pitolisant
1	2839455
2	2861501
3	2844080
4	2880398
5	2881624
6	2889900
Ave area	2866160
St dev	21108.449
%RSD	0.74

Table 6: Accuracy data of Pitolisant

Recovery level	Accuracy Pitolisant				
	Amount taken (mg)	Area	Ave Area	%Recovery	%RSD
50%	90	1525742	1525437	100.2	0.78
	90	1537252			
	90	1513317			
100%	180	2859312	2872186	100.1	0.42
	180	2883118			
	180	2874127			
150%	270	4181148	4174889	99.8	0.20
	270	4178082			
	270	4165437			

Table 7: Result for Robustness of Pitolisant

Parameters		Pitolisant % RSD
Flow Rate	1.2 ml/min	0.15
	0.8 ml/min	0.34
Organic Phase	63:37	0.84
	77:23	0.47

Table 8: LOD and LOQ Results of Pitolisant

S.No.	Sample name	LOD		LOQ	
		Conc. (µg/ml)	S/N	Conc. (µg/ml)	S/N
1.	Pitolisant	0.18	6	1.8	26

CONCLUSION

Development and validation of RP-HPLC method for the estimation of Pitolisant in bulk and pharmaceutical dosage forms with the facilities and the results are incorporated in this thesis. In conclusion a validated RP-HPLC method has been developed for determination of Pitolisant the bulk and tablet dosage form. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of Pitolisant tablet dosage form. Several analytical procedures have been proposed for the quantitative estimation of Pitolisant separately and in combination with other drugs. So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Pitolisant in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

REFERENCES

1. Wakix, INN-Pitolisant - European Medicines Agency. Europa EU.
2. WAKIX (Pitolisant) tablets. for oral use- FDA Label.
3. Chwartz JC. The histamine H3 receptor: from discovery to clinical

trails with pitolisant. Br J pharmacol. 2011;163(4):713-721.
4. Calik MW. Update on the treatment of narcolepsy. Clinical efficacy of pitolisant. Nat Sci Sleep. 2017;26;9;127-133.
5. Kotanska M, Kuder KJ, Szczepanska K, Sapa J and Kiec-Kononowicz K. The histamine H3 receptor inverse agoinst pitolisant reduces body weight in obese mice. Naunyn Schmiedebergs Arch Pharmacol. 2018;391(8):875-881.
6. Nirogi R, Ajjala DR, Kandikere V, Pantangi HR, Jonnala MR, Bhyrapuneni G, Muddana NR and Vurimindi H. LC-MS/MS method for the determination of pitolisant: application to rat pharmacokinetic and brain penetration studies. Biomed Chromatogram. 2013;27(11):1431-1437.
7. Ohayon MM, Priest RG, Zulley J, Smirne S and Paiva T. Prevalence of narcolepsy symptomatology and diagnosis in the European general population. Neurology. 2002; (12):1826-1833.
8. LinJs. Histamine H3 receptors and sleep-wake regulation. J Pharmacol Exp Ther. 2011;336(1):17-23.
9. Chan CC. Analytical Method Validation: Principles and Practices, in Pharmaceutical Manufacturing Handbook: Regulations and Quality. Edited by Shyne Cox Gad. John Wily and Sons. 2008.
10. ICH Q2A. Text on Validation of Analytical Procedures. Geneva Q2A, in 2005 Incorporated in Q2 (R1). 1994.
11. ICH Q2B. Validation of Analytical Procedures: Methodology. Geneva Q2B, in 2005 incorporated in Q2 (R1). 1996.