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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PITOLISANT HYDROCHOLRIDE API BY UV-SPECTROPHOTOMETRY

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ABSTRACT

Pitolisant Hydrochloride API a Non-Pharmacopeial drug which is used to treat Narcolepsy. A simple, precise, and cost-effective UV method was developed and validated for the estimation of Pitolisant Hydrochloride API. The absorbance and spectral measurements were done on a double-beam Labindia UV-visible spectrophotometer with the software UV Win. 1cm quartz cells at wavelength 221nm and the solvent system used is Methanol. The method is developed and validated for linearity, precision, robustness, stability, LOD, LOQ**.** The proposed UV-spectrophotometric method for estimation of Pitolisant Hydrochloride API and its validation was carried out as per ICH Q2(R1) guidelines.

Keywords: Pitolisant Hydrocholride API, UV, Non- Pharmacopeial, ICH Q2(R1), Narcolepsy, Method validation

INTRODUCTION

Narcolepsy is a chronic, debilitating, rare neurological disorder of sleep–wake state instability that is characterized by excessive daytime sleepiness (EDS), cataplexy, and other manifestations of rapid eye movement (REM) sleep dysregulation **[1, 2]**. This drowsiness might manifest as incessant, recurring "sleep attacks." During these instances, a person may unexpectedly nod off while eating, strolling, or operating a vehicle. Less than 1% of men and women suffer with narcolepsy, which usually manifests in adolescence and early adulthood and lasts a lifetime. It falls within the category of hypersomnia, a collection of sleep disorders characterized by excessive daytime drowsiness as its main symptom. People with narcolepsy still feel sleepy throughout the day even when they get enough sleep at night. This is not the result of inadequate sleep **[3]**.

Pitolisant Hydrocholride a Non Pharmacopeial Drug is a selective antagonist or inverse agonist of the histamine H3 receptor used to treat type 1 or 2 narcolepsy **[4]**. H3-receptors are primarily located in the cerebral cortex, hypothalamus hippocampus, and basal ganglia. Pitolisant's hydrocholride blockade of histamine auto-receptors increases histamine concentration and histaminergic activity in the brain **[5]**. By blocking the manufacture and release of histamine after binding to endogenous histamine, the H3 autoreceptors control histaminergic activity in the central nervous system (and, to a lesser degree, the peripheral nervous system). Pitolisant hydrocholride increases histaminergic activity in the brain by blocking natural histamine from binding to the H3 receptor and by causing an opposite reaction to that of endogenous histamine at the receptor (inverse agonism). Compared to more conventional CNS stimulants like amphetamine, euregoics are less likely to cause addiction and have fewer negative effects. Activating the histaminergic neurons in the brain by inhibiting the histamine 3 (H3) autoreceptor, Pitolisant hydrocholride is the first eugeroic medication. It has been demonstrated that pitolisant hydrocholride is a safe, efficient therapy for narcolepsy, whether or not cataplexy is present.

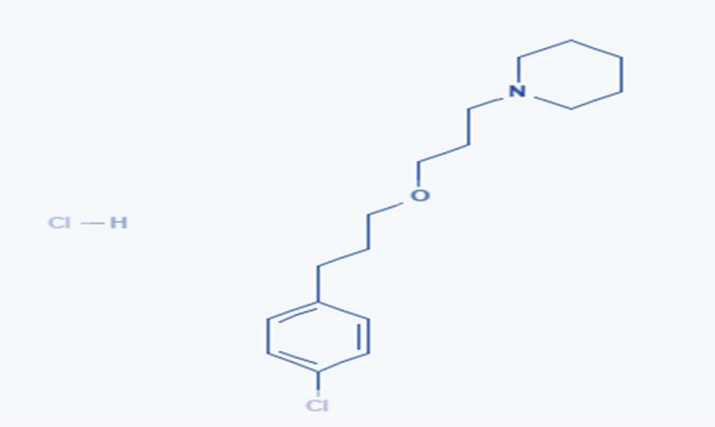

Figure 1: Chemical Structure of Pitolisant Hydrocholride

Pitolisant hydrochloride is chemically known as 1-[3-[3-(4-chlorophenyl)propoxy]propyl]piperidine;hydrochloride. It has a molecular formula $C_{17}H_{27}Cl_2NO$ and a molecular weight 332.3 g/mol. Pitolisant hydrocholride is white to beige-white powder and is soluble in methanol, water, DMSO and in EtOH.

The present work was aimed to develop and validate a simple, sensitive, precise, and specific UVSpectrophotometric method for estimation of pitolisant hydrocholride in its API.

Literature survey revealed that the drug has been estimated by LC-MS/MS **[6]**, & RP-HPLC **[7, 8]** methods. Moreover, the reported methods for pitolisant in bulk and pharmaceutical dosage form has been reported till now, but no UV spectrophotometric method for pitolisant hydrochloride is estimated, therefore the method development and validation was carried out through basic instrument (UV-Visible Spectrophotometric). Hydrocholride is the salt form of pitolisant which tends to overcome the challenges of the molecule solubilization in particular solvent.

Hence, the present work was aimed to develop and validate a simple, sensitive, precise, and specific UV Spectrophotometric method for estimation of pitolisant hydrochloride in its API

MATERIALS AND METHODS

Instrumentation

The absorbance and spectral measurements were done on a double-beam Labindia UV-Visible spectrophotometer with software UV Win. 1cm quartz cells were used for sample handling. A digital analytical balance was used for weighing.

Materials and Reagents

Pitolisant hydrochloride API was obtained from Bhisaj Pharmaceutical, Pune as a gift sample. Methanol was procured from the local market. All the chemicals and solvents used were of analytical grade.

Preparation of standard stock solution (100µg/ml)

Accurately weighed 10mg of pure Pitolisant hydrochloride API was taken in a clean, dry 100ml volumetric flask and dissolved in a few ml of Methanol, and the volume was made up to 100ml to obtain a concentration of 100µg/ml.

Selection of Analytical wavelength

Different aliquots (10, 15, 20, 25, 30) of working standard solution were transferred to a series of 10 ml volumetric flasks and then made up to 10ml with methanol to obtain a concentration range of 10-30 µg/ml. One of the solutions was scanned in UV range of 200-400 nm using water as a blank and the wavelength of maximum absorption was found to be 221 nm and it is selected for further analysis. The UV spectrum of different solutions was shown in **Figure 2**. and the UV spectrum of 20 µg/ml solution was shown in **Figure 4**.

METHOD VALIDATION

Method validation as per ICH guidelines Q2(R1) [9]

1. Linearity

Different aliquots 10, 15, 20, 25, 30 ml of working standard were transferred to a series of 10 ml volumetric flasks and then made up to 10ml with methanol to obtain 10, 15, 20, 25, 30 µg/ml respectively. Then their absorbance was measured at 221nm. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The calibration plot was shown in **Figure 3**, And the optical characteristics and other parameters were shown in **Table 1**.

2. Precision

The precision of the method was demonstrated by intraday and inter-day studies. In the intra-day study, three different solutions of the same concentrations (20µg/ml) were prepared and analysed thrice a day (morning, afternoon, and evening).

In the inter-day variation study, the solutions of same concentration (20µg/ml) were prepared and analysed daily for three days, and the absorbance was recorded. The results of precision, intra-day and inter-day study were shown **Table 2 and 3**.

2.1 Repeatability

The method was determined by performing the 6 different solutions of the same concentrations(20µg/ml) were prepared and analysed. The results of repeatability study were shown in **Table 3**.

2.2 Intermediate Precision

Intermediate precision of the method was determined by performing the same method by using different analysts at similar operational and environmental conditions. The results were reported in the **Table 4**.

3. Robustness

Robustness is the ability of a method to remain unaffected by small deliberate variations in method parameters. It is determined by performing the analysis at slightly different wavelengths from the

selected wavelength of maximum absorption. The results were recorded in **Table 5**.

4. Limit of Detection (LOD)

Limit of Detection (LOD) of the method was found to be 0.037µg/ml which is calculated from the following

formula,

LOD=3.3 σ/S

Where,

σ=Standard deviation of the response of the analyte,

S=Slope of the linearity plot of the analyte.

LOD=2.894046 µg/ml

5. Limit of Quantitation (LOQ)

Quantitation limit is the concentration that can be quantitated reliably with a specified level of accuracy and

precision. Limit of Quantitation can be calculated from the following formula,

LOQ=10σ/S

Where,

σ=Standard deviation of the response,

S=Slope of the linearity plot of the analyte.

LOQ=8.769836 µg/ml

6. Stability

Pitolisant Hydrochloride API solution was checked for stability and was found to be stable at 2^0C -8^0C for 24 Hours.

RESULTS AND DISCUSSION

Table 1: Optical Characteristics and Other Parameters

Parameters	Results
Absorption Maxima	221nm
Beer's-Lamber's Range(µg/ml)	10-30 (µg/ml)
Regression Equation (y)	0.0305x-0.0273
Slope (m)	0.0305
Intercept (c)	0.0273
Correlation Co-efficient	0.9988
Limit of detection (µg/ml)	2.894046
Limit of quantification (µg/ml)	8.769836

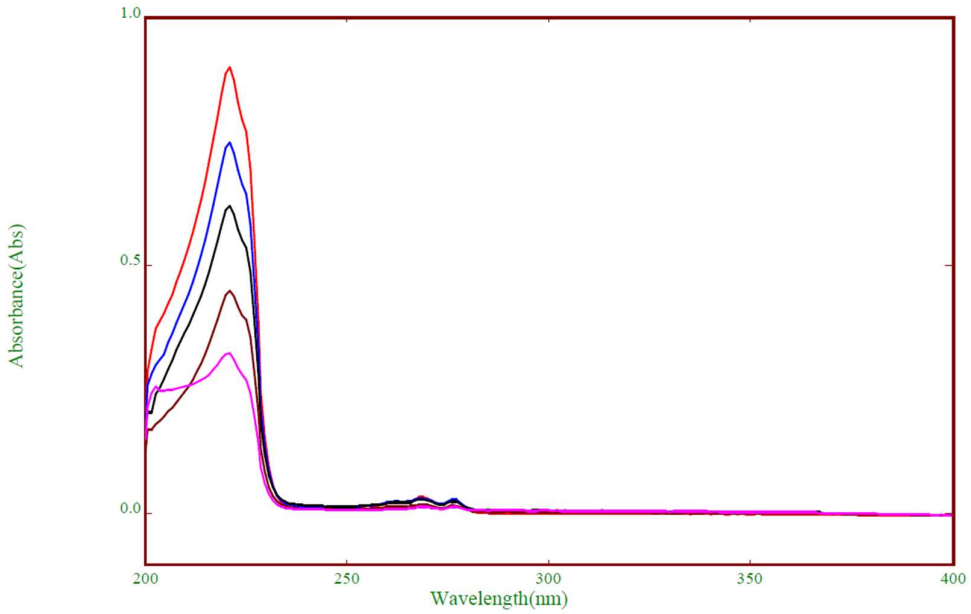

Figure 2: UV spectrum of 10-30 µg/ml pitolisant hydrocholride solution

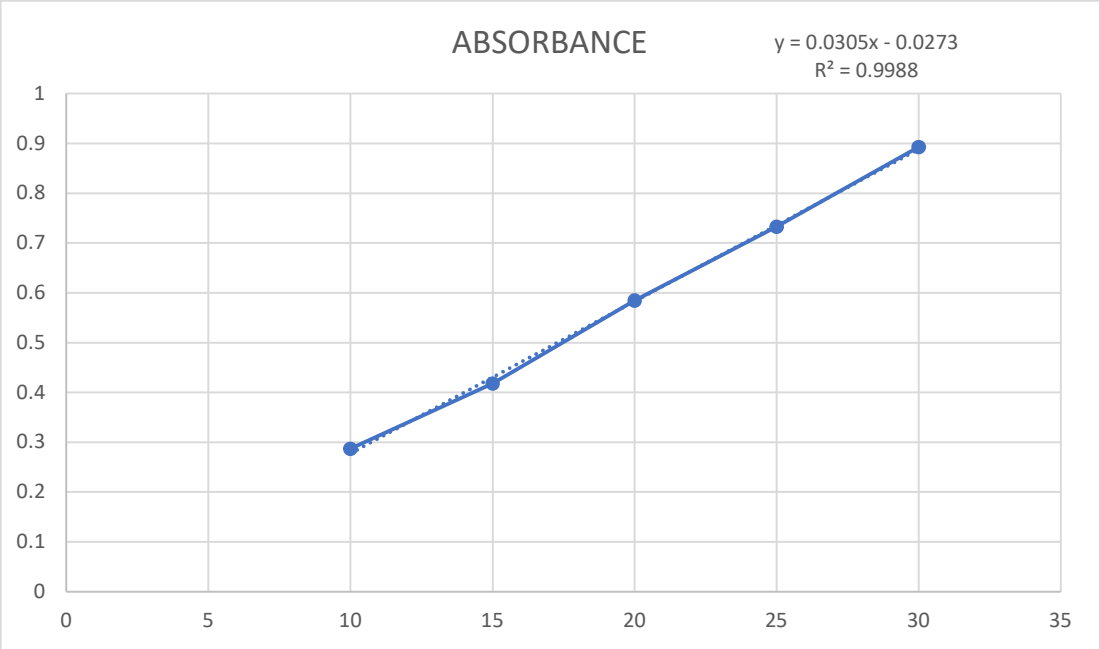

Figure 3: Calibration Curve of Pitolisant Hydrochloride

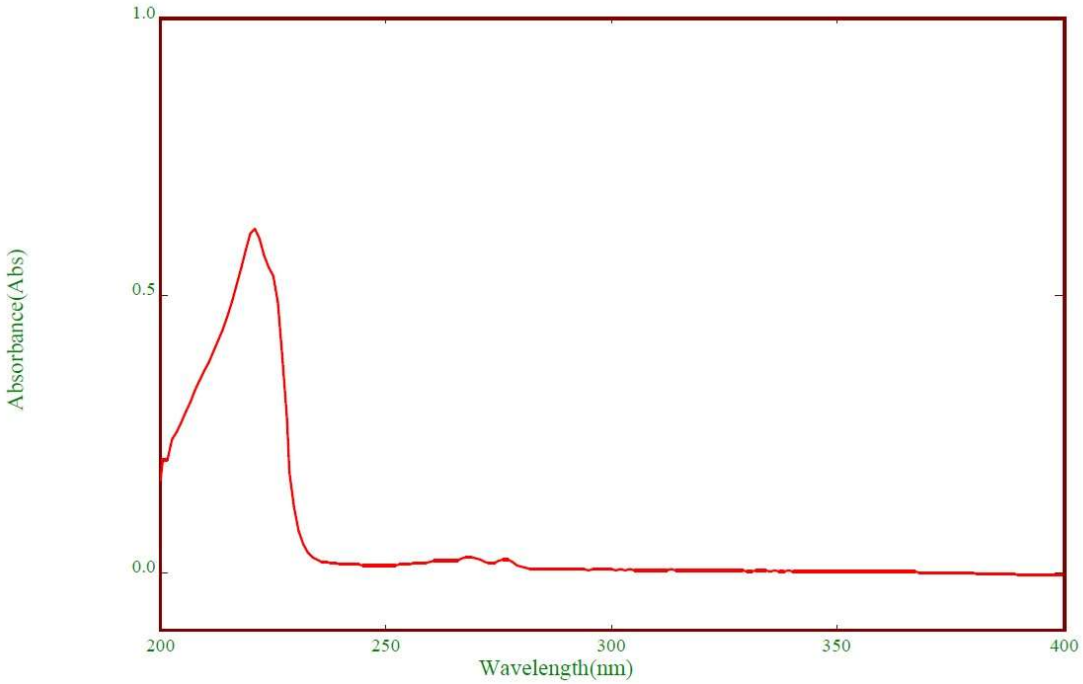

Figure 4: UV spectrum of 20 µg/ml Pitolisant Hydrocholride Solution

Table 2: Intra-day and Inter-day precision study results

Concentration (20µg/ml)	Intra-day Study			Inter-day Study		
	Morning	Afternoon	Evening	Day 1	Day 2	Day 3
Avg. Abs.	0.5631	0.563667	0.555267	0.586367	0.5695	0.586367
SD	0.008022	0.009691	0.00807	0.007516	0.009714	0.007516
%RSD	1.424697	1.71935	1.453338	1.281827	1.705691	1.281827

Table 3: Repeatability Study Results

Concentration	Absorbance
20	0.5695
20	0.5595
20	0.5658
20	0.5425
20	0.553
20	0.5499
Average	0.5567
Standard Deviation	0.010159
%RSD	1.82492

Table 4: Intermediate Precision Study Results

Concentration (20 µg/ml)	Analyst 1	Analyst 2
Mean Abs.	0.5672	0.558467
SD	0.006265	0.009902
%RSD	1.104545	1.773012

Table 5: Robustness Study Results

Concentration (20 µg/ml)	At 220	At 222
Mean Abs.	0.578333	0.568533
SD	0.002219	0.010276
%RSD	0.383664	1.807519

The UV spectrum of 20 µg/ml Pitolisant Hydrocholride solution was shown in **Figure 2**. From this spectrum, 221nm was selected as the wavelength of maximum absorption (λ max). The linearity of the method was found to be within the range of 10-30 µg/ml with a correlation coefficient of 0.9988. The linearity plot was shown in **Figure 3**. Precision of the method was determined by repeatability, intra-day and inter-day studies and the results were shown in **Table 2 and Table 3**. The LOD and LOQ of the method were calculated as 2.894046 and 8.769836µg/ml respectively. Intermediate Precision of the method was estimated by performing the same analysis by two different analysts and the results were found to be within the limits and were shown in **Table 4**. Robustness was estimated by performing analysis at slightly different wavelengths from the actual wavelength of maximum absorption and the results were reported in **Table 5**. Stability was estimated and drug was found to be stable at 2^0 C - 8^0 C.

CONCLUSION

The proposed UV- Spectrophotometric method for estimation of Pitolisant Hydrochloride in API and its validation was carried out as per ICH guidelines. By studying various parameters finally we conclude that the method is simple, precise, sensitive, economic, and specific and can be applied for the determination of Pitolisant Hydrocholride API. The method was found to be linear in the specified range. All the required validation parameters were estimated.

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REFERENCES

[1] American Academy of Sleep Medicine. International classification of sleep disorders. Diagnostic and coding manual. 2005:148-52.

[2] Scammell TE. Narcolepsy. New England Journal of Medicine. 2015 Dec 31;373(27):2654-62.

[3] https://aasm.org/resources/factsheets/narcolepsy.pdf

[4] FDA Approved Drug Products: WAKIX (pitolisant) tablets, for oral use (August 2019)

[5] Sarfraz N, Okuampa D, Hansen H, Alvarez M, Cornett EM, Kakazu J, Kaye AM, Kaye AD. pitolisant, a novel histamine-3 receptor competitive antagonist, and inverse agonist, in the treatment of excessive daytime sleepiness in adult patients with narcolepsy. Health Psychol Res. 2022 May 30;10(3):34222. doi: 10.52965/001c.34222. PMID: 35774905; PMCID: PMC9239364.

[6] Nirogi R, Ajjala DR, Kandikere V, Pantangi HR, Jonnala MR, Bhyrapuneni G, Muddana NR, Vurimindi H. LC-MS/MS method for the determination of pitolisant: application to rat pharmacokinetic and brain penetration studies. Biomedical Chromatography. 2013 Nov;27(11):1431-7.

[7] P. Venkateswara Rao*, J. Leela Rani, M. Roja Kumari, G. Kalyani, G. Sirisha and P. Naveen Reddy Stability indicating rp-hplc method for determination of pitolisant in bulk and pharmaceutical dosage form 2020, 10(1), 137-143 https://dx.doi.org/10.33289/IJRPC.10.1.2020.10(29)

[8] Sowjanya Gummadi & Rajya Lakshmi Nimmagadda Quantification of Pitolisant: A RP - HPLC study 2022

[9] https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf