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PITOLISANT HCl: A REVIEW ON DRUG CHARACTERISTICS AND COMPARATIVE HPLC METHOD DEVELOPMENT AND VALIDATION

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ABSTRACT

Pitolisant, a selective histamine H3 receptor antagonist/inverse agonist, is an innovative therapeutic agent for treating narcolepsy and related disorders of excessive daytime sleepiness. The review focuses on the development and validation of high-performance liquid chromatography methods and analysis of pitolisant in pharmaceutical formulations and bulk, critical aspects of HPLC method development, including the selection of mobile phase, stationary phase, flow rate and detection parameters, are examined to achieve optimal separation and sensitivity. This work aims to provide a comprehensive resource for researchers and analysts to ensure the quality control and regulatory compliance of pitolisant, contributing to its therapeutic efficacy and safety in clinical applications.

KEYWORDS: Pitolisant, HPLC, Method development, validation.

INTRODUCTION

Pitolisant is marketed in the European Union by Bio ProJet Pharma. It was approved for medical use in the European Union in March 2016 by the European Medicines Agency (EMA).

The U.S. Food and Drug Administration (FDA) approved pitolisant for excessive daytime sleepiness in participants with narcolepsy based primarily on evidence from two trials (Trial 1/NCT01067222, Trial 2/NCT01638403). An additional trial (Trial 3/NCT01800045), in which participants with a different type of narcolepsy were exposed to the same dose of pitolisant, was used to add data for evaluation of side effects. The trials were conducted in Europe and South America.

The two primary trials enrolled adults with narcolepsy and excessive daytime sleepiness. Participants received pitolisant, placebo, or an approved drug for narcolepsy for eight weeks. For participants receiving pitolisant, the dose could be increased during the first three weeks but had to remain the same for the next five weeks. Neither the participants nor the healthcare providers knew which treatment was being given during the trial.

The benefit of pitolisant was evaluated by comparing changes in daytime sleepiness during the trial between pitolisant- and placebo-treated participants. To measure the daytime sleepiness, the investigators used a scale called the Epworth Sleepiness Scale (ESS). The ESS asks participants to rate the likelihood that they would fall asleep while doing eight daily activities (such as sitting and reading or watching television). Participants rate each item from zero (would never doze) to three (high chance of dozing).

Pitolisant was approved by the FDA in August 2019. It was granted orphan drug designation for the treatment of narcolepsy, fast track designation for the treatment of excessive daytime sleepiness and cataplexy in people with narcolepsy, and breakthrough therapy designation for the treatment of cataplexy in people with narcolepsy.

DRUG PROFILE[15]

PARAMETER	DETAILS
Generic Name	Pitolisant Hydrochloride
Brand Name	Wakix®
Drug Class	Histamine H3 receptor inverse agonist/antagonist
Chemical Name	1-{3-[3-(4-Chlorophenyl)propoxy]propyl} piperidine hydrochloride
Molecular Formula	C17H26ClNO · HCl
Molecular Weight	295.85g/mol(base),332.31g/mol (hydrochloride salt)
Mechanism of Action	Inverse agonist of H3 receptor; enhances histamine, dopamine, ACh, NE(Fig-2)
Therapeutic Use	Narcolepsy; being explored for ADHD, Parkinson's
Dosage Forms	Tablets: 4.45 mg, 17.8 mg (equivalent to 5 mg and 20 mg Pitolisant base)
Route of Administration	Oral
Absorption	Oral bioavailability ~90%; Tmax: 3 hrs
Distribution	Vd ~7.2 L/kg
Protein Binding	~91–96%
Metabolism	Hepatic via CYP3A4, CYP2D6, and CYP1A2
Elimination	Primarily renal; t½ ~10–20 hrs
Adverse Effects	Insomnia, headache, nausea, anxiety, irritability
Contraindications	Hypersensitivity; severe hepatic impairment
Pregnancy Category	Category C
Storage Conditions	Store at 20°C–25°C (68°F–77°F)
Stability Considerations	Stable under standard conditions; sensitive to moisture

STRUCTURE[19]

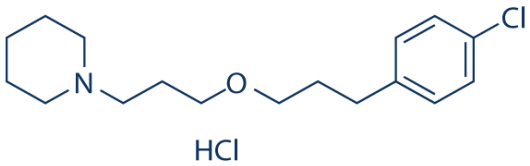

Figure 1: Structure of pitolisant HCl.

Mechanism of action

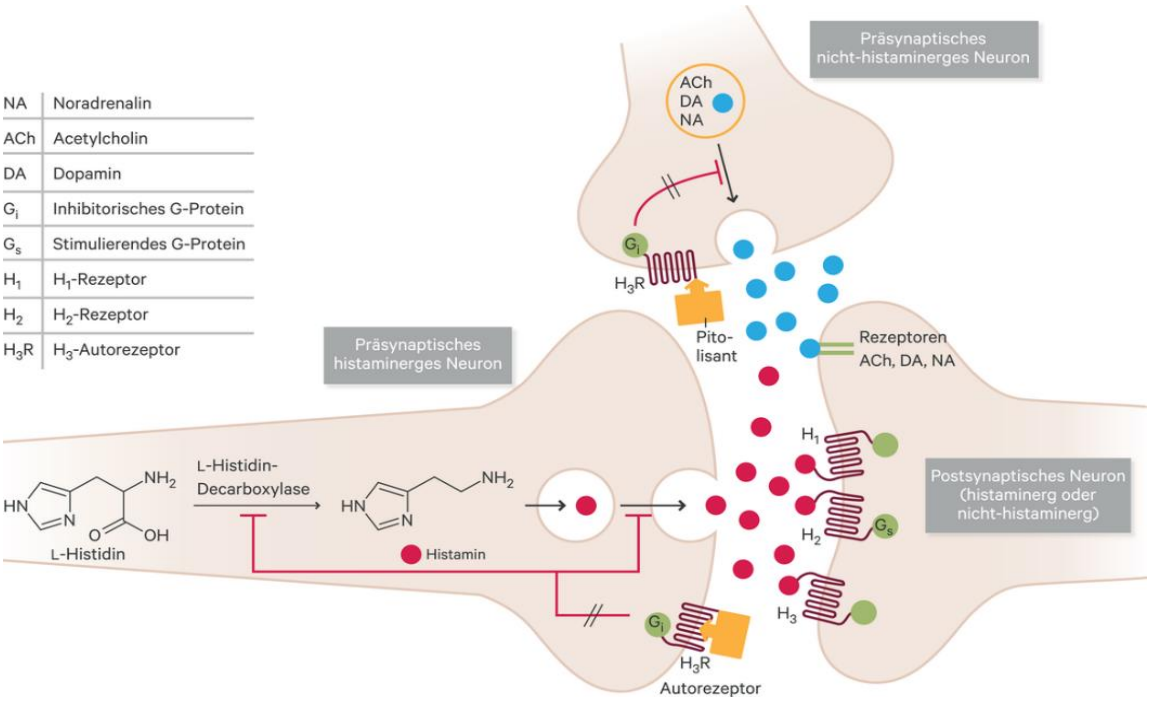

Figure 2: MOA of Pitolisant HCl.

PATENT[17]

Discovery and Origin

Pitolisant Hydrochloride was discovered by a team of scientists at Bioprojet, a French pharmaceutical company. The lead inventor was Professor Jean-Charles Schwartz, who worked extensively on histaminergic neurotransmission. The drug was designed based on the concept of targeting the histamine H3 receptor to treat disorders such as narcolepsy, ADHD, and cognitive dysfunctions.

First Patent Filing

- Patent Number: WO2001033418A1(WIPO)
- Title: Piperidine derivatives as H3 receptor ligands
- Filed by: Bioproject (France)
- Filing date: November 9, 2001
- Publication Date: May 17, 2001

The patent describes a family of piperidine-based compounds including Pitolisant. It claims their use as central nervous system stimulants and provides data on synthesis, pharmacology, and potential applications for neurological and psychiatric disorders.

REFERENCE

WO2001033418A1. Piperidine derivatives as H3 receptor ligands. Bioprojet. Link: https://patents.google.com/patent/WO2001033418A1/en

Innovation Behind Pitolisant

Pitolisant is the first-in-class drug acting as an H3 receptor inverse agonist. Unlike stimulant drugs, it promotes wakefulness by enhancing the brain's natural histaminergic tone. It does not directly stimulate dopamine like modafinil or amphetamines, giving it a low potential for abuse. Its unique mechanism makes it effective in narcolepsy and potentially useful in Parkinson's disease, ADHD, and other cognitive disorders.

Development Timeline

Year	Milestone
1999	Bioproject files initial patents on Pitolisant
Early 2000s	Preclinical studies show strong wake-promoting activity
2007–2012	Phase I–III clinical trials conducted in Europe for narcolepsy
2016	Approved by EMA (European Medicines Agency) for narcolepsy
2019	Approved by FDA (U.S.) under brand name Wakix®
2020	Commercial launch in the United States

REGULATORY AFFAIRS[18]

- Regulatory class: Prescription only medicine (Rx)
- Therapeutic class: CNS Stimulant (Non-amphetamine), H3 receptor inverse agonist
- Controlled substance: Not classified as a controlled substance in US or EU due to low abuse potential.

KEY APPROVALS

Agency	Country/Region	Approval Year
EMA	European union	2016
FDA	United states	2019
PMDA	Japan	Not yet approved

ORPHAN DRUG STATUS

- Unites states (FDA): Granted orphan drug designation for the treatment of narcolepsy, allowing marketing exclusivity for 7 years post-approval.
- European Union (EMA): Also granted orphan drug status under EU Regulation (EC) No.141/2000.

ANALYTICAL METHOD DEVELOPMENT[5]

Method development- It refer to the process of designing and optimizing an analytical technique to effectively quantify, identify, or characterize a substance of interest. In pharmaceutical analysis, this typically involves selecting appropriate instrumentation, reagents, conditions (e.g. Mobile phase, temperature, and flow rate) and sample analysis.

1. **Selection of mobile phase**
   - The mobile phase should be chosen to achieve good resolution of pitolisant from other components
   - A typical mobile phase might include methanol and phosphate buffer (pH 4.5–6.0), but this depends on the chemical properties of Pitolisant.
   - Gradient or isocratic elution can be tested based on the peak resolution.

2. **Column selection**
   - Common columns used for Pitolisant analysis are C18 reverse-phase columns.
   - Choose a column with an appropriate particle size (e.g., 3–5 µm) for good separation efficiency.

3. **Detection wavelength**
   - Pitolisant absorbs UV light, so a UV detector is commonly used.
   - A typical detection wavelength is 221 nm based on the absorption maxima of Pitolisant.

4. **Flow rate**
   - Typical flow rates range from 0.8 to 1.5 mL/min.
   - This can be adjusted based on column dimensions and desired resolution.

5. **Injection volume**
   - Injection volumes of around 10–20 µL are commonly used.

6. Temperature

- The column temperature may be controlled at 30–40°C to ensure reproducibility and reduce peak tailing.

ANALYTICAL METHOD VALIDATION[6]

It is a process of assessing the reliability and performance of an analytical method. This ensures that the method meets predefined criteria for its intended purpose.

1. Specificity

- Specificity is typically used to describe the ultimate state, measuring unequivocally a desired analyte.
- Ensure the method can differentiate Pitolisant from other compounds (e.g., excipients, impurities).
- Test for interference from commonly used solvents or additives.

2. Linearity

- Prepare standard solutions of Pitolisant at different concentrations (e.g., 10, 50, 100, 200 µg/mL).
- Plot a calibration curve and calculate the correlation coefficient (r^2). It should be >0.99 for good linearity.

3. Accuracy

- Perform recovery studies by spiking known quantities of Pitolisant into the sample matrix and comparing the measured concentration to the expected value.
- Typically, recoveries should be within 98–102%.

4. Precision

- Repeatability (Intra-day precision): Inject the same sample multiple times (at least 6 times) on the same day and calculate the % RSD (Relative Standard Deviation).
- Intermediate precision (Inter-day precision): Perform the same test on different days to check for consistency.

5. Limit of detection & limit of quantification

- Determine the lowest detectable and quantifiable concentration of Pitolisant in the sample
- LOD is a lowest concentration of an analyte that can be detected but not necessarily quantified under the stated experimental conditions. It ensures the method's sensitivity.
- LOQ is a lowest concentration of an analyte that can be quantitively measured with acceptable precision, accuracy, and reliability.

6. Robustness

- The robustness of an analytical procedure is a measure of its capacity to meet the expected performance criteria during normal use. Robustness is tested by deliberate variations of analytical procedure parameters**.**
- Test small variations in method parameters (e.g., mobile phase composition, flow rate, temperature) to ensure consistent results.

7. System suitability

- System suitability tests are developed and used to verify that the measurement system and the analytical operations associated with the analytical procedure are fit for the intended purpose and increase the detectability of unacceptable performance.
- Verify the HPLC system's performance before each run by checking parameters like theoretical plates, resolution, tailing factor, and capacity factor.

8. Stability

- Stability Testing of New Drug Substances and Products" to propose a retest period or shelf life in a registration application. This guideline describes when and how extrapolation can be considered when proposing a retest period for a drug substance or a shelf life for a drug product that extends beyond the period covered by "available data from the stability study under the long-term storage condition**".**
- Ensure that the sample and standard solutions of Pitolisant remain stable under storage conditions (e.g., refrigerated or at room temperature).

9. Selectivity

- Selectivity is a relative term to describe the extent to which particular analytes in mixtures or matrices can be measured without interferences from other components with similar behaviour.
- Ensure no co-eluting peaks with Pitolisant at the retention time under the given conditions.

Table 1: Trade name and respective companies of Pitolisant.

Brand Name	Dosage Forms	Company
Wakix	Tablet	Bioprojet (France)
Ozawade	Tablet	Bioprojet (EU)

Table 2: Analytical methods described in literature for determination of Pitolisant in pharmaceutical dosage forms and raw material. [7-12]

S.no	Title	Chromatographic conditions	Result
1.	Stability indicating estimation of Pitolisant in pharmaceutical formulations using RP-HPLC: Method development and validation	**Column-** YMC pack ODSA, 150 x 4.6 x 3µ **Mobile phase-** 0.2% Formic acid: ACN (40:60). **Flow rate-** 1.0 ml/min **Detector**-PDA. **Wavelength**-220nm **Rt-** 2.216 min	**Specificity**-No interference **Linearity-** 0.997 **Accuracy**-100.24% **Precision**-RSD-0.72 **LOD**-0.06 µg/ml **LOQ-** 0.21µg/ml
2.	Analytical method development, validation of Pitolisant drug by using RP-HPLC Method.	**Column-** C18 column (250 mm x 4.6mm, 5 µm **)** **Mobile phase-** Methanol and 0.1% water with OPA (45:55 v/v) **Flow rate-** 0.8 ml/min **Detector**-UV **Wavelength**-266nm **Rt-** 4.627 min	**Specificity**-No interference **Linearity-** 0.9994 **Accuracy-** RSD 80% (0.40), 100% (0.03), 120% (0.23). **Precision**-5µg-0.88%,15µg-0.54, 25µg-0.20 **LOD**-0.1004 **LOQ**-0.3043.
3	Stability indicating RP-HPLC method for determination of Pitolisant in bulk and pharmaceutical dosage form	**Column-**X-Bridgephenyl 150mm x 4.6mm, 3.5µm **Mobile phase-** 0.1% OPA: ACN (30:70) **Flow rate-** 1.0 ml/min **Detector-** PDA **Wavelength-**210nm **Rt-** 4.358 min	**Specificity**-No interference **Linearity-** 0.997 **Accuracy**-100.24% **Precision-** RSD-0.72% **LOD**-0.18µg/ml **LOQ**-1.8µg/ml
4	Quantification of Pitolisant: A RP-HPLC study	**Column-Inertsil ODS** 250mm x 4.6mm, 3.5µm **Mobile phase-** ACN: 0.1% formic acid (90:10) **Flow rate-** 1.0 ml/min **Detector-** PDA **Wavelength-**268nm **Rt-** 3.680 min	**Specificity**-No interference **Linearity-** 0.998 **Accuracy**-100.4% **Precision-** RSD-0.79% **LOD**-0.3 µg/ml **LOQ-** 1.0 µg/ml
5	Analytical method development and validation of Pitolisant hydrochloride in synthetic mixture: A Novel treatment for narcolepsy	**Column-C18**250mmx4.6mm, 3.5µm **Mobile phase**-MP A- 10mm Ammonium acetate buffer MP B- ACN **Flow rate-** 1.0 ml/min **Detector-** PDA **Wavelength**-268nm **Rt-** 4.4 min	**Specificity**-No interference **Linearity-** 0.9999 **Accuracy**-95- 99.3% **Precision**-0.5% **LOD**-7-8µg/ml **LOQ**-22.5µg/ml
6	LC-MS/MS method for the determination of Pitolisant: application to rat pharmacokinetic and brain penetration studies.	**Column-**X-Bridgephenyl 2.1 x 50mm, 3.5µm **Mobile phase-** Ammonium formate with 0.2% formic acid **Rt-** 2.5min **Detector-**	**Specificity**-No interference **Linearity-** 0.9999 **Accuracy-** 99% **Precision-** 1.6-10.3% **LOD**-0.03-0.05ng/ml

CONCLUSION

Pitolisant Hydrochloride is a clinically approved drug for narcolepsy, recognized by both EMA and FDA for its therapeutic potential. This review aimed to compare the previously reported RP-HPLC methods for the estimation of Pitolisant Hydrochloride in bulk and tablet dosage forms. Each method was critically evaluated based on key validation parameters such as retention time, linearity, accuracy, precision, robustness, and system suitability, all aligned with ICH guidelines.

The comparative analysis confirmed that all methods exhibited acceptable performance, with strong linearity, reproducibility, and sensitivity. The validated parameters consistently met the required analytical standards, making these HPLC methods suitable for routine quality control applications. This review highlights the analytical reliability of the existing methods and serves as a reference for future method optimization and regulatory support.

AUTHORS CONTRIBUTIONS: All the authors have contributed equally.

CONFLICT OF INTERESTS: Declared none.

REFERENCES

1. Fifield F W and Kealey D. Principles and practice of analytical chemistry. 5 th Edition, Wiley-Blackwell, 2005; 256-286.
2. Douglas A Skoog. Principles of Instrumental Analysis. 5 th Edition, Thomson Business Information India pvt Ltd, New Delhi., 2006; 1-18.
3. Beckett A H and Stenlake J B. Practical Pharmaceutical Chemistry. 4 the edition, CBS Publishers and Distributors, New Delhi., 2007; 278-312.
4. Beckette A H and Stenlake J B. Practical pharmaceutical chemistry, part B. 3rd edition, CBS Publishers and Distributors, New Delhi., 2001; 275–282.
5. ICH Harmonized Tripartitie Guideline. ICH Q2 (R1) Text and Methodology, November 2007.
6. International Conference on Harmonization guidance for Industry. Q2A Text on validation of Analytical methods. Switzerlandl, 1994: IFPMIA, 1, 4.
7. P. Venkateswara Rao* *et al..,* "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.
8. Dr. Kanjarla Narasimha *et al..,* "Stability-Indicating Estimation of Pitolisant in Pharmaceutical Formulations Using RP-HPLC: Method Development and Validation". African Journal of Biomedical research, Sep. 2024; 27(3s): 352-361.
9. Dipak R. Borase *et al..,* **"**Analytical method development, validation of pitolisant drug by using RP-HPLC Method". International Journal of Pharmaceutical Sciences, 2024; 2: 2784-2793.
10. Keerthivasan B *et al..,* "Analytical Method Development Validation of pitolisant in synthetic mixture: A Novel Treatment for Narcolepsy by Using RP-HPLC Method", International Journal of Biology, Pharmacy amd Allied Sciences, 2025; 4(2): 960-968.
11. Rajya Lakshmi N *et al.,* **"**Quantification of Pitolisant:RP-HPLC Study". YMER, 2022; 21: 896-907.
12. Ramakrishna Nirogia *et al.,* "LC-MS/MS method for the determination of pitolisant: application to rat pharmacokinetic and brain penetration", Biomedical Chromatography, 2013; 27: 1431-1437.
13. European medicines agency-Committee for Medicinal Products for Human Use.
14. https://www.accessdata.fda.gov/
15. https://www.drugbank.ca/drugs/DB05272
16. https://pubchem.ncbi.nlm.nih.gov/
17. https://patents.google.com/patent/WO2001033418A1/en
18. https://www.accessdata.fda.gov/
19. https://www.ema.europa.eu/en/medicines/human/EPAR/wakix.