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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PITOLISANT IN SYNTHETIC MIXTURE: A NOVEL TREATMENT FOR NARCOLEPSY

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ABSTRACT

This study presents the development and validation of a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of pitolisant, a novel treatment for narcolepsy. Narcolepsy is a chronic neurological condition that causes excessive daytime drowsiness and other severe symptoms. Pitolisant drug was purchased as a synthetic mixture and the separation was accomplished with C_{18} (250 mm 4.6 mm, 5µm spherical particles) column. The mobile phase contains mobile phase A- 10mm ammonium acetate buffer calibrated to pH 4 and mobile phase B- as acetonitrile in a gradient mode at a wavelength 268nm. The flow rate of the mobile phase and the sample volume injected were 1ml/min and 10L, respectively. Pitolisant had a retention time of 4.4±0.2 minutes. Pitolisant showed a linear association (R2=0.999) over concentrations ranging from 22.5µg to 360µg. Validation studies confirmed the method's reliability, accuracy, precision, linearity, and sensitivity, making it suitable for regular examination in quality control laboratories. The validated method will aid drug manufacturers, regulatory agencies, and researchers in assessing pitolisant's content in pharmaceutical formulations and pharmacokinetic studies, ultimately benefiting narcolepsy

patients. The devised approach is simple, precise, specific, accurate, and fast, making it appropriate for estimating pitolisant in bulk and pharmaceutical dose form. It was found that the current RP-HPLC technology is simple, quick, and accurate, and hence suitable for routine quality control analysis in the pharmaceutical business.

Keywords: pitolisant, Narcolepsy, Validation, HPLC

INTRODUCTION

Pitolisant is a first-in-class novel medicine that has been approved by the European Medicines Agency to treat type 1 or type 2 narcolepsy. Pitolisant is chemically1-[3-[3-(4-chlorophenyl)propoxy]propyl]piperidine **[1]**. The molecular formula of Pitolisant $C_{17}H_{26}ClNO$ has a molecular weight of 295.8 g/mole. It is a non-chiral molecule with no stereoisomerism that exists in the form of a white/almost white crystalline powder **[2]**. Pitolisant is insoluble in cyclohexane but extremely soluble in water, methylene chloride, and ethanol **[3]**. Pitolisant is available on the market under the trademark Wakix, Ozawade manufactured by Harmony and it is approved by the Food and Drug Administration (FDA). Pitolisant is a first-of-its-kind antagonist/inverse agonist of histamine H3 receptors (H3Rs) **[4]**. It stimulates histaminergic cell activity and other neurotransmitters in the brain (ie, dopamine, acetylcholine, noradrenaline). It is available as an oral tablet in various strengths. Narcolepsy is a persistent neurological condition affecting roughly one in every 2,000 people globally **[5-7]**. Excessive daytime sleepiness, a rapid loss of muscular tone (cataplexy), sleep paralysis, and hallucinations are its defining characteristics. Narcolepsy significantly impacts the quality of life and functional abilities of affected individuals, leading to impaired cognitive function, reduced productivity, and increased risk of accidents. While several therapeutic options are available for managing narcolepsy, the need for novel and more effective treatments remains. As with any medication, Pitolisant can have side effects and should only be taken under the supervision of a doctor. Some common side effects of Pitolisant include nausea, headaches, and anxiety **[8-10]**. However, it has shown to be a promising treatment option for people with narcolepsy who struggle with excessive daytime sleepiness and other associated symptoms. Clinical studies have shown that Pitolisant is effective at reducing excessive daytime sleepiness in people with narcolepsy **[11-12]**. A review of the literature on Pitolisant gave information regarding the method developed by various authors. Ramakrishna Nirogia *et al*. discuss the development and application of an LC-MS/MS method for the determination of

Pitolisant in rat plasma and brain samples. P. Venkateswara Rao *et al*. present a validated RP-HPLC method for quantifying Pitolisant in bulk and pharmaceutical dosage forms. Sowjanya Gummadi and Rajya Lakshmi Nimmagadda developed and validated an RP-HPLC method for quantifying Pitolisant, a drug used in narcolepsy treatment. This research paper aims to address this critical aspect by reporting the development and validation of high-performance liquid chromatography (HPLC) technology for the analysis of pitolisant in pharmaceutical formulations. The HPLC technique offers high sensitivity, precision, and accuracy, making it a preferred choice for drug analysis. The new technique will be verified according to globally accepted norms, guaranteeing that it is suitable for routine analysis in quality control laboratories **[13]**. And establishing a robust and validated analytical method, this research intends to contribute to the broader scientific and medical community's efforts to explore and utilize Pitolisant as a novel treatment for narcolepsy. The availability of such a method will not only facilitate the production and quality assessment of pharmaceutical formulations but also support pharmacokinetic studies, further advancing our understanding of pitolisant's therapeutic potential and ultimately benefiting patients suffering from narcolepsy **[14]**.

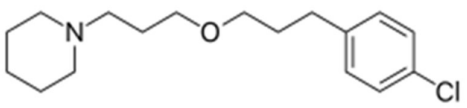

Figure 1: Pitolisant chemical structure

MATERIALS AND METHODS

Instrumentation:

HPLC was equipped with a high-pressure pump which maintained a gradient flow, and an auto sampler, A Shimadzu HPLC system with a Shimadzu PDA detector was utilized. A double beam UV-visible spectrophotometer (Shimadzu) model UV1800. The separation was made using the RP-HPLC C_{18} column (250 mm x 4.6 mm, 5µm spherical particles).

Chemicals and reagents:

Acetonitrile HPLC, methanol HPLC AR Grade, Ammonium acetate 99% AR Grade, Millipore Water HPLC Grade, Pitolisant 0.1% Acidic acid, Microcrystalline cellulose, talc, magnesium stearate, and colloidal anhydrous silica.

Chromatographic condition:

The HPLC system is fixed with a C_{18} column (250 mm × 4.6 mm). 10 µL volume was injected into the system. A Shimadzu HPLC system equipped with a Shimadzu PDA detector was used. The gradient flow via a symmetric C_{18} column (250 mm × 4.6 mm, 5µm spherical particles) was operated by the solvent delivery system with mobile phase A 10mm ammonium acetate buffer calibrated to pH 4 and acetonitrile as mobile phase B. The flow rate of the mobile phase

was 1 mL/min with a gradient flow of 30% buffer, and the run time was 8 minutes. Filtration via a 5 m Millipore membrane filter and sonication for 10 minutes were used to degas the mixture. At 25 °C the HPLC system was operated and detection was at 268 nm.

Preparation of Stock Solutions:

Standard Preparation:

10 mg of Pitolisant reference standard was weighed and transferred into a 10ml standard flask. A small amount of Millipore distilled water was added, mixed thoroughly, and shaken in vortex for 5 minutes. Then the volume of the standard flask was made with Millipore distilled water.

Working standard solution preparation:

180µl of stock solution was pipetted out using a micropipette and transferred to 1ml vial and 820µl of acetonitrile was added to make 180µg/ml concentration solution.

Synthetic mixture preparation:

0.2mg of a mixture of Microcrystalline cellulose, talc, magnesium stearate, and colloidal anhydrous silica was added to 17.8mg of API.

RESULTS:

Selection of analytical UV wavelength (λ_{max}):

The prepared stock solution was scanned to fix wavelength for analysis in ultraviolet spectroscopy over the range of 200-800nm from the resultant spectrum wavelength at 268nm was chosen as in this range maximum absorption of the drug occurs. So, this range is taken to analyze the sample **(Figure 2)**.

Pitolisant was diluted with 10µL to create a solution, which was then scanned between 200 and 400 nm. The drug reported a maximum absorption of 268 nm. This is why this was chosen as the wavelength for detection. Upon taking into account every system suitability parameter, with mobile phase A 10mm ammonium acetate buffer calibrated to pH 4 and mobile phase B as acetonitrile. The gradient flow of the 30% buffer was used with a 1 mL/min mobile phase flow rate. The run time of Pitolisant was found to be 8 minutes **(Figure 2)**.

ANALYTICAL METHOD VALIDATION:

1. System suitability:

The %RSD of Pitolisant was found to be 0.8%. From the system suitability studies, it is clear that the analytical procedures linked with the analytical technique and the measurement system are suitable for the desired analysis **(Table 1)**.

2. Linearity:

A linearity graph of the area at each level against the concentration (µg) of Pitolisant yielded a linearity graph, which was confirmed. Pitolisant's correlation coefficient was determined to be 0.9999. The approach is adequate for its intended concentration range and is linear over the whole concentration range of 22.5µg to

360µg for Pitolisant, according to the linear regression data **(Table 2) (Figure 4)**.

3. Specificity:

There was no peak visible on the placebo's chromatograms during the primary peak's retention period. It follows that the approach is determined to be specific **(Figure 5, 6)**.

4. Accuracy:

The percentage recovery of Pitolisant from the placebo at each of the levels is more than 90.0%. Therefore, the method is considered accurate and precise concerning measuring the Concentration of Pitolisant in placebo-spiked samples **(Table 3)**.

5. Repeatability:

The %RSD of the assay method was found to be less than 2% for Pitolisant. The analytical method meets the pre-established acceptance criteria and hence considered that the method is precise **(Table 4)**.

6. Intermediate precision:

The %RSD of the assay method was found to be less than 2% for Pitolisnt. The analytical method meets the pre-established acceptance criteria of intermediate precision **(Table 5)**.

7. Robustness:

After individually modifying the flow rate from the suggested technique, no appreciable variation in retention time was seen. All other system suitability parameter calculations were made following the accepted standards, and the data produced is equivalent to the real conditions. According to the above-mentioned findings, it can be said that the approach is unaffected by slight, intentional changes in flow rate **(Table 6)**.

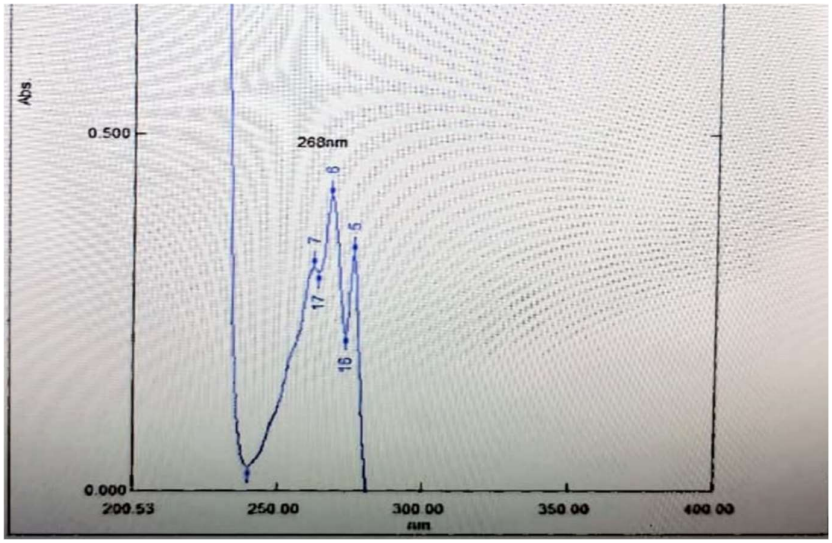

Figure 2: λ_{max} of pitolisant at 268nm

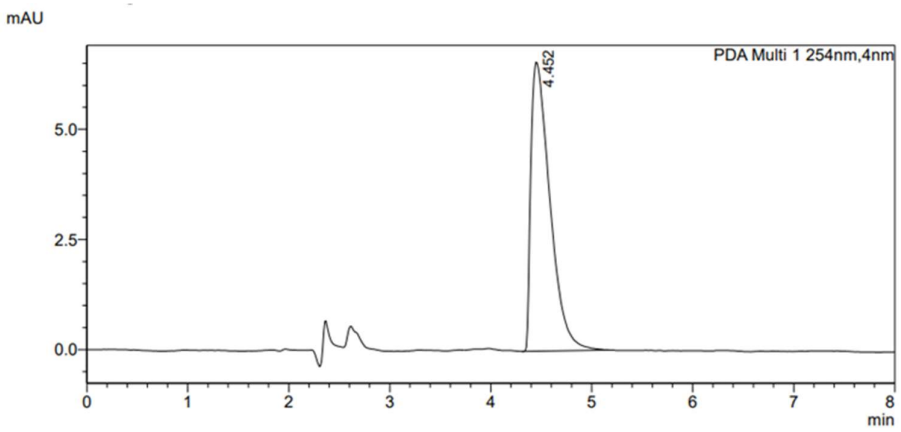

Figure 3: Chromatogram of Pitolisant

Table 1: System suitability

S. No.	PEAK AREA
1	95355
2	96460
3	94504
4	95142
5	96553
MEAN	95579.33333
%RSD	0.8

Table 2: Linearity of pitolisant raw material by HPLC

Concentration	Peak Area
22.5	10587
45	21195
90	41796
180	86272
360	174054
Correlation coefficient r2 Slope	R^2= 0.999

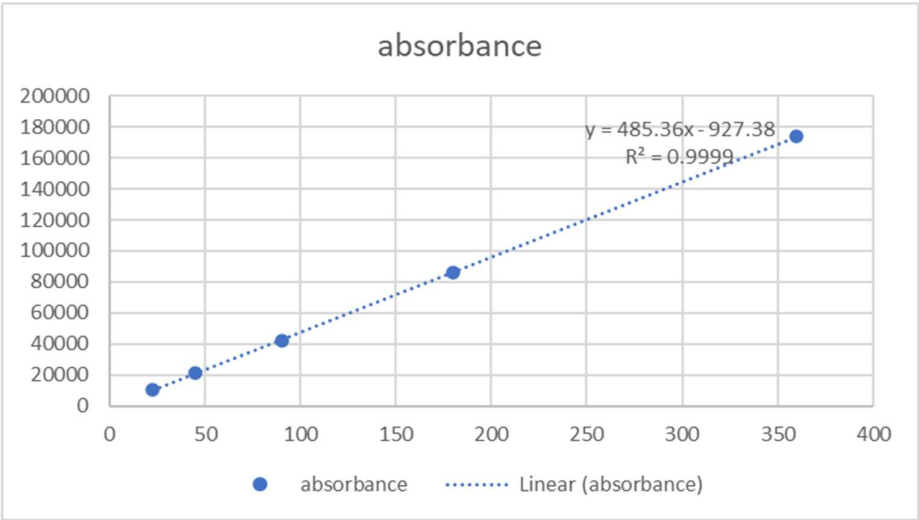

Figure 4: Linearity graph

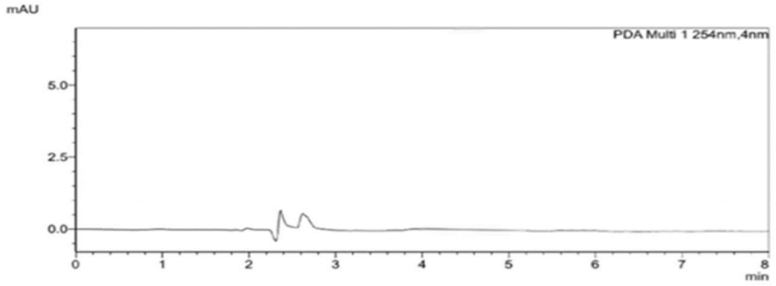

Figure 5: Chromatogram of blank

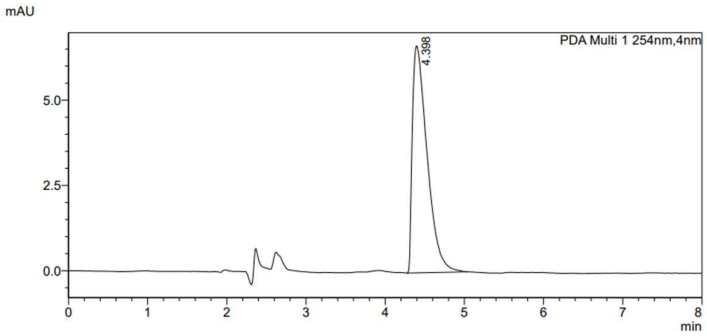

Figure 6: Chromatogram of Pitolisant synthetic mixture

Table: 3 Accuracy

Sample		Peak area	Amount added(mg)	Amount found(mg)	%recovery	Average % recovery	%RSD
80%	P1	65515	14.11	13.40	95.0	96.1	1.7
	P2	64854	14.08	13.80	98.0		
	P3	64578	14.19	13.54	95.4		
100%	P1	86188	17.66	17.45	98.8	97.3	0.3
	P2	79542	17.20	16.85	98		
	P3	81765	17.90	17.02	95.1		
120%	P1	110653	21.19	20.85	98.4	98.5	0.7
	P2	110045	21.22	20.77	97.9		
	P3	110547	21.12	20.98	99.3		
LIMIT					NLT90.00&NMT 110.00%		NMT 2.0

Table:4 Repeatability

SAMPLE	MEAN PEAK AREA	%ASSAY
preparation 1	86272	101.4
preparation 2	82471	97.0
preparation 3	84751	99.6
preparation 4	79845	93.9
preparation 5	80214	94.3
preparation 6	81556	95.9
mean		97.0
RSD%		0.5

Table: 5 Intermediate precision

SAMPLE	MEAN PEAK AREA	%ASSAY
preparation 1	92412	101.0
preparation 2	89451	97.8
preparation 3	85745	93.7
preparation 4	92788	101.4
preparation 5	88745	97.0
preparation 6	91325	99.8
mean		98.5
RSD%		0.5

Table:6 Robustness

Variation condition	System suitability	Results	
		%Assay 1	%Assay 2
As such method results.	Complies	98.0%	97.5%
Flow variation (-10%)	Complies	98.4%	97.6%
Flow variation (+10%)	Complies	97.7%	98.2%

CONCLUSION

A simple, sensitive, exact, and accurate RP-HPLC method was created for the current study to quantify Pitolisant in pharmaceutical and bulk medication dosage forms. Since diluted samples are used immediately without requiring any prior chemical derivatization or purification processes, this approach proved convenient. The development and validation of the analytical method for Pitolisant in this study mark a remarkable milestone in pharmaceutical analysis. Pitolisant, as a promising treatment for narcolepsy, holds immense potential, and the establishment of a dependable analytical method sets the stage for its extensive use and therapeutic efficacy. The approach was confirmed to be accurate, and the %RSD results were within 2. The RP-HPLC method produced encouraging findings, which are shown in the Tables. The solvent system used in this method was economical. This research represents a significant contribution to enhanced patient care, opening up new avenues for effectively managing narcolepsy.

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Conflict of interest

Each author declares that they have no competing interests with regard to this work.

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