

or not the suggested procedure was appropriate for quantitatively determining Pizotifen in pharmaceutical formulations.

1.4 Literature Review

One study that looked at how pharmaceutical companies use quantitative nuclear magnetic resonance spectroscopy was *Holzgrabe et al. (2005)*. According to the authors, qNMR has many applications beyond drug identification, including assay determination, impurity quantification, evaluation of degradation products, and investigation of residual solvents. According to their analysis, one major benefit of NMR is that it provides quantitative data that are directly related to the molecular structure and proton response because it is a main ratio measurement method. In pharmaceutical quality assessment, the study demonstrated that qNMR is a significant alternative and complementary tool to traditional chromatographic methods. In order to quantitatively determine pharmaceutical substances like Pizotifen, this work lays the groundwork analytically for utilizing ¹H-qNMR.

Using analytical techniques such as ¹H NMR spectroscopy, *Al Omari, El-Barghouthi, and Zughul (2008)* studied the interactions of Pizotifen and ketotifen with various cyclodextrins. They found that proton NMR could detect Pizotifen's molecular environment alterations by looking for shifts in chemical shifts caused by drug-cyclodextrin interactions. The interaction between β -Cyclodextrin and Pizotifen was especially significant. ¹H NMR proved effective for molecular level Pizotifen research, even though the main focus of the work was inclusion complexation and not quantitative test validation. Consequently, the study gives strong evidence in favor of using NMR spectroscopy as an analytical method to characterize Pizotifen. The source chapter also mentions this study as significant NMR work on Pizotifen.

In their 2021 publication, *Jaki, Bzhelyansky, and Pauli* traced the history of qNMR in pharmaceutical quality control and discussed the groundbreaking work of George M. Hanna at the US Food and Drug Administration. Their research demonstrated that qNMR might offer an orthogonal alternative to chromatographic quantification and has been effectively used for medicinal dosage forms for decades. Significantly, the writers delved into Hanna's initial quantitative examination of dicyclomine pharmaceutical goods, utilizing maleic acid as an internal standard. The qNMR findings varied from the matching USP titrimetric values by a margin of less than 1%. This review shows that the metrological and historical basis of internal-standard qNMR for pharmaceutical assays is solid. This data provide credence to the idea that the Pizotifen qNMR approach might benefit from using maleic acid as an internal reference.

To validate quantitative methods, *Sahu et al. (2021)* examined new advancements in nuclear magnetic resonance (NMR). Proper selection of an internal standard, solvent, quantitative resonance, acquisition settings, and validation conditions are some of the essential aspects of qNMR that the authors covered. In their review, they stressed the importance of well-resolved signals and well-controlled experimental conditions for accurate qNMR. In addition, the writers covered topics related to quantitative pharmaceutical applications and analytical validation. The suggested approach is comparable to theirs in that it employs a defined internal standard, deuterated solvent, chosen quantitative proton signal, and systematic validation of analytical performance; all of which are important to the current Pizotifen study.

In their review of the pharmaceutical qNMR literature, *Jaki et al. (2021)* emphasized how quantitative NMR is becoming more important for characterizing reference materials, chemicals, medicines, and natural products. Unlike many traditional quantitative methods, qNMR relies on nuclear response to determine concentrations rather than detector-response parameters that are particular to each compounds, as highlighted in the review. With a soluble analyte and an NMR-active nucleus that produces a well-resolved resonance, qNMR can yield precise quantitative data. When designing pharmaceutical assays, this quality is invaluable since it allows for simultaneous identification and quantification using a single spectral window. There is substantial theoretical support for using ¹H-qNMR on Pizotifen pills due to these methodological benefits.

Review articles published after Bharti and Roy's more general qNMR work solidified qNMR's position as a powerful analytical tool for assessing the composition and purity of organic compounds. Under suitable quantitative acquisition settings, the number of nuclei contributing to a resonance is directly proportional to the strength of the integrated NMR signal. This is the fundamental premise. By utilizing signal-integral ratios and proton stoichiometry, this enables the quantification of an analyte in relation to an internal standard. This method offers structural confirmation and can lessen reliance on analyte-specific calibration standards, making it ideal for use in pharmaceutical applications. These concepts are directly applicable to the determination of Pizotifen, as the current approach compares the methyl-proton response of Pizotifen with the internal-standard signal of maleic acid.

More recent studies on NMR-based quantitative analysis of completed medical products have added to the growing body of data that, with proper control over acquisition and processing parameters, qNMR can give satisfactory accuracy and repeatability. Under the tested low-field NMR circumstances, a comprehensive investigation including 33 completed pharmaceutical compounds found recovery rates with deuterated solvents ranging from about 97% to 103%. Researchers stressed that accuracy is very sensitive to factors such signal-to-noise ratio, solvent choice, spectral processing, and location of quantitative signals. Findings support an essential methodological principle: optimization and validation of acquisition and processing circumstances depend on pharmaceutical qNMR performance. This holds true even though the instrumental settings differ from the present Pizotifen investigation.

2. Materials and Methods

Chemicals and Reagents

Material	Source / specification
Pizotifen reference standard	V.V MED, Hyderabad, India
Tablet formulation	Migralin; 0.725 mg Pizotifen per tablet
Internal standard	Maleic acid, purity $\geq 99\%$, Sigma-Aldrich
NMR solvent	DMSO- d_6
Comparative HPLC reagents	Methanol, acetonitrile, orthophosphoric acid, ammonium dihydrogen phosphate

Instrumentation and NMR Conditions: We used a 5 mm broadband inverse probe and a Bruker Avance III HD 400 MHz NMR spectrometer to get the spectra. We integrated and acquired spectra using Bruker TopSpin 3.5.

Parameter	Setting
Spectrometer	Bruker Avance III HD, 400 MHz
Probe	5 mm broadband inverse (BBI)
Solvent	DMSO- d_6
Internal-standard signal	Maleic acid, δ 6.25 ppm, 2H, singlet
Target Pizotifen signal	Methyl protons, $\sim \delta$ 2.2 ppm
Number of scans	16
Relaxation delay	30 s
Acquisition time	3.5 s
Temperature	298 K

Specificity: We used spectra from a blank excipient matrix, Migralin tablet extract, and the Pizotifen standard solution to check for specificity. We looked for signals that overlapped in the analytical regions at $\delta \sim 2.2$ ppm for Pizotifen and δ 6.25 ppm for maleic acid. According to the source, there are no overlapping peaks in these areas caused by tablet excipients.

Linearity: We used the chosen qNMR parameters to examine six different Pizotifen doses ranging from 5 to 30 ppm. The depicted value represents the integral area of the Pizotifen methyl signal vs concentration. According to the source, R^2 is 0.998.

Precision and Accuracy: Measures of accuracy included repeatability within a single day and accuracy between days. In a replicate analysis of a 15 ppm Pizotifen solution, the source

reported an intraday RSD of 1.40% and an interday RSD of 1.07%. For accuracy testing, we used standard addition to 25%, 50%, and 75% spike levels in pre-analyzed tablet extracts.

Robustness, Stability, LOD and LOQ: Scanning rates (16–64), temperatures (296–300 K), and relaxation delays (28–32 s) were all varied to examine robustness. Chemical shifts and integral response were used to monitor solution stability for 48 hours at 25°C. There was a reported signal-to-noise ratio of about 3:1 for LOD and 10:1 for LOQ.

3. Results

Specificity and Signal Selection: There was a report of a separate singlet that was suitable for quantitative integration, which was the Pizotifen methyl resonance at around δ 2.2 ppm. At δ 6.25 ppm, maleic acid gave rise to a distinct singlet that corresponded to two identical protons. Both quantitative regions did not show any discernible excipient interference, lending credence to the uniqueness of the chosen signal combination.

Linearity: The integral response increased approximately proportionally across 5–30 ppm. The numerical data from the source are retained in Table 1.

Table 1. Linearity data for Pizotifen by ^1H -qNMR

Concentration (ppm)	Mean integral area
5.0	30,333
10.0	61,991
15.0	88,759
20.0	121,043
25.0	148,511
30.0	186,164

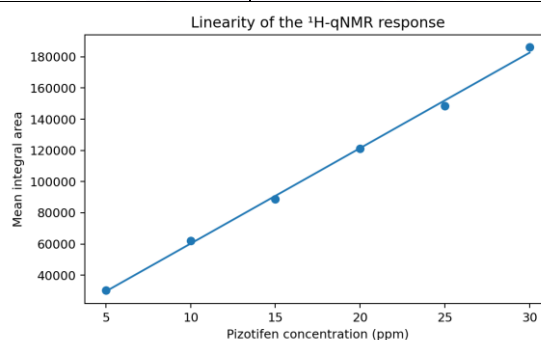


Figure 1. Linearity of Pizotifen concentration versus mean integral area. Regression recalculated directly from the tabulated source values: $y = 6119.99x - 966.40$; $R^2 \approx 0.9980$

Precision: The RSD readings for the intraday precision were 1.40% and for the interday precision were 1.07%. Both results fell short of the previously established cutoff of $\leq 2.0\%$, suggesting that the suggested ^1H -qNMR technique has acceptable repeatability and intermediate precision.

Table 2. Precision results for the ^1H -qNMR method

Precision type	%RSD
Intraday	1.40
Interday	1.07

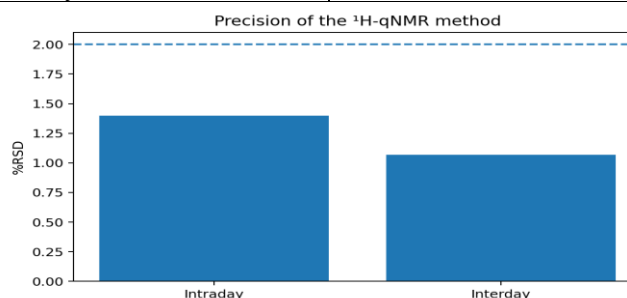


Figure 2. Intraday and interday precision. Dashed line indicates the 2% criterion used

Accuracy / Recovery: We used conventional addition at three different levels to evaluate recovery. While RSD dropped from 0.53% at the 25% spike level to 0.10% at the 75% level, mean recoveries stayed near 100%.

Table 3. Recovery data for Pizotifen by qNMR

Spike level	Recovery range (%)	Mean recovery (%)	%RSD
25%	99.6–100.4	100.02	0.53
50%	99.8–100.1	99.93	0.26
75%	99.9–100.1	100.06	0.10

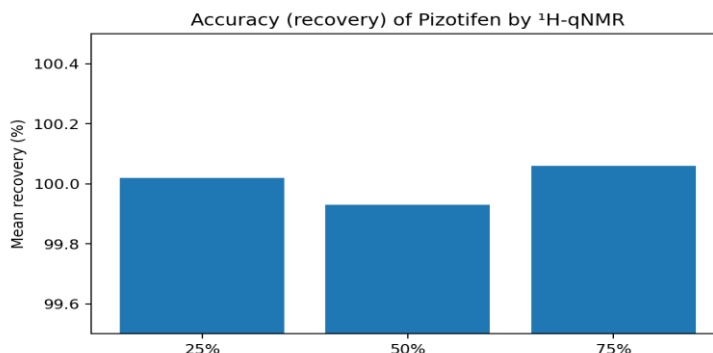


Figure 3. Mean recovery at the three spike levels

Robustness: The suggested ¹H-qNMR method's robustness was tested by intentionally introducing tiny changes to the acquisition parameters. This approach was found to be quite stable throughout the ranges that were examined, since the greatest deviation under all conditions was less than 0.6% (Table 4).

Table 4. Robustness conditions and reported maximum deviation

Parameter	Tested range	Maximum deviation
Number of scans	16–64 scans	<0.6%
Temperature	296–300 K	<0.6%
Relaxation delay	28–32 s	<0.6%

Solution Stability: We kept the Pizotifen standard and tablet extract solutions at room temperature (25°C) for 48 hours. There has been no discernible alteration in the integral responses or chemical shifts of the maleic-acid and Pizotifen signals, according to the source. Over the course of the research, recovery stayed over 98%, indicating that the solution was stable under the given conditions for the short term.

Sensitivity

Table 5. Reported sensitivity parameters

Parameter	Reported value	Criterion / interpretation
LOD	20 ng/mL	S/N approximately 3:1
LOQ	65 ng/mL	S/N approximately 10:1

The LOD and LOQ were found to be 20 ng/mL and 65 ng/mL, respectively, indicating high sensitivity of the proposed ¹H-qNMR method.

3.8 Summary of Validation Performance

Validation parameter	Result
Specificity	No reported interference at ~δ 2.2 or 6.25 ppm
Linearity	5–30 ppm; R ² = 0.998
Intraday precision	1.40% RSD
Interday precision	1.07% RSD
Accuracy	Mean recovery 99.93–100.06%
Robustness	Maximum deviation <0.6%
Solution stability	>98% recovery through 48 h
LOD / LOQ	20 / 65 ng/mL

4. Discussion

4.1 Analytical Performance

With well-resolved ^1H -qNMR signals from Pizotifen and maleic acid, selective integration was possible, free from interference from tablet excipients. A correlation coefficient of $R^2 = 0.998$ demonstrated a significant link between Pizotifen concentration and NMR integral response, and the method exhibited high linearity over the concentration range of 5-30 ppm.

4.2 Precision and Accuracy

With intraday RSD values of 1.40% and interday RSD values of 1.07%, respectively, both falling below the acceptability limit of 2%, the approach showed satisfactory precision. Mean recoveries of 100.02%, 99.93%, and 100.06% at the 25%, 50%, and 75% spiking levels, respectively, demonstrated satisfactory accuracy. The suggested approach for Pizotifen quantification appears to have good repeatability and accuracy based on these results.

4.3 Robustness and Solution Stability

Deliberately changing the number of scans, temperature, and relaxation delay by a small amount resulted in differences of less than 0.6%, proving that the approach is robust. There were no discernible changes in the chemical shifts or integral responses of the standard and sample solutions during the 48 hours at 25°C.

4.4 Sensitivity

The LOD and LOQ of the proposed ^1H -qNMR method were reported as **20 ng/mL** and **65 ng/mL**, respectively. These values were determined using the signal-to-noise approach, corresponding approximately to **S/N ratios of 3:1 for LOD and 10:1 for LOQ**. For complete experimental documentation, representative low-concentration ^1H NMR spectra, measured signal-to-noise values, and the calculations used for determining LOD and LOQ should be provided as supporting analytical evidence.

4.5 Methodological Comparison with HPLC

The proposed ^1H -qNMR method offers a different analytical approach from conventional HPLC by enabling quantitative determination through proton integral ratios without requiring chromatographic separation. It can also provide structural information and quantitative response within the same spectrum. However, the present study did not include a complete paired experimental comparison of Pizotifen assay results obtained by ^1H -qNMR and HPLC. Therefore, the comparison presented in Table 6 is limited to **general methodological features**, and no claim of analytical superiority or experimental equivalence between the two methods is made. A direct comparative study using identical pharmaceutical samples is recommended for further evaluation.

Table 6. Methodological Comparison of ^1H -qNMR and HPLC for Pizotifen Analysis

Aspect	^1H -qNMR	HPLC (general comparison in source)
Analytical basis	Proton integral ratio and internal standard	Detector response and reference calibration
Separation column	Not required	Required
Structural information	Direct chemical-shift information	Indirect; based mainly on retention/detector
Organic mobile phase	Not required for NMR acquisition	Commonly required
Sample recovery	Potentially recoverable	Sample typically consumed
Multi-component analysis	Possible with resolved signals	Possible after chromatographic resolution

4.6 Strengths of the Proposed Method

Direct integration of a distinctive Pizotifen signal, validation across various conventional analytical-performance metrics, an internally established standard, and simple sample analysis are the main strengths. Although the linearity experiment can still be used to show

proportionate response, the method also gets rid of the necessity to build a detector-response calibration for regular internal-standard quantification.

5. Discussion

Over the concentration range of 5–30 ppm, the technique demonstrated good linearity ($R^2 = 0.998$), with accuracy values around 1.5% RSD and recoveries near 100%. Under the assessed conditions, these results show satisfactory analytical performance. For accurate quantification, the choice of well-resolved signals was crucial. Selective integration was made possible by the obvious separation of the maleic acid singlet at about δ 6.25 ppm from the Pizotifen methyl signal at about δ 2.2 ppm. Adequate spectral resolution is necessary for reliable determination since qNMR enables structural identification and quantitative analysis from the same spectrum without chromatographic separation.

At each of the three spiking levels, the approach showed good accuracy. The RSD values ranged from 0.10% to 0.53%, while the mean recoveries were 100.02%, 99.93%, and 100.06%. The RSD values for intraday and interday were 1.40% and 1.07%, respectively, showing intermediate precision and excellent repeatability. The robustness investigation demonstrated that intentional changes in temperature, relaxation delay, and scan count produced differences less than 0.6%, suggesting that small adjustments to acquisition conditions had no discernible impact on quantitative performance. Additionally, the standard and sample solutions maintained sufficient solution stability throughout routine analysis for 48 hours at 25°C. According to the signal-to-noise method, the LOD and LOQ were 20 ng/mL and 65 ng/mL, respectively. Overall, the validation results show that the devised ^1H -qNMR process has the potential to be a straightforward, accurate, precise, and reliable analytical method for quantifying pizotifen in pharmaceutical formulations.

6. Conclusion

According to reports, matrix interference was absent from the chosen Pizotifen signal at about δ 2.2 ppm. The approach demonstrated mean recoveries of 99.93–100.06%, intraday and interday precision of 1.40% and 1.07% RSD, respectively, and linear response spanning 5–30 ppm ($R^2 \approx 0.998$). The generated solutions were reported to be stable for 48 hours, and intentional modifications to the acquisition conditions resulted in variations less than 0.6%. These findings validate the suggested qNMR method for quantitative pizotifen analysis under the assessed circumstances. The original experimental spectra, raw sensitivity data, and comprehensive bibliographic information should all be included in the final journal submission. Overall, the results demonstrate satisfactory analytical performance of the proposed ^1H -qNMR method under the investigated conditions. Further studies involving direct experimental comparison with a validated HPLC method and detailed experimental confirmation of LOD and LOQ would strengthen its applicability for routine pharmaceutical quality-control analysis.

7. Suggestions of the Study

Based on the findings of the present study, the developed ^1H -qNMR method may be further evaluated for routine quantitative analysis of Pizotifen in pharmaceutical formulations. Future studies may include a larger number of commercial tablet formulations from different manufacturers to establish wider applicability of the method. The method may also be compared with established analytical techniques such as HPLC using the same samples to assess agreement between the two approaches. Further investigation under forced-degradation conditions may help determine its applicability for stability-indicating analysis. Studies using different NMR instruments, acquisition conditions, and suitable internal standards may also be conducted to evaluate method reproducibility across laboratories. In addition, the reported LOD and LOQ may be further confirmed using low-concentration experimental spectra and replicate measurements. These investigations would provide additional evidence regarding the applicability of ^1H -qNMR for routine pharmaceutical quality-control analysis of Pizotifen.

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