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Research Article

Stability-Indicating RP-HPLC Method Development and Validation for Simultaneous estimation of Cinnarizine and Piracetam bulk and in Capsule dosage form

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Abstract

This work presents the development and validation of a solid Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for concurrently quantifying Cinnarizine and Piracetam in pharmaceutical formulations. The technique used an Agilent 1200 series high-performance liquid chromatography (HPLC) machine equipped with a C18 column. The mobile phase composition consisted of Methanol and Orthophosphoric Acid.

Correlation coefficients of 0.9992 for Cinnarizine and 0.9998 for Piracetam indicate the calibration curves exhibit a robust linear relationship. The present investigation determined the Limits of Detection (LOD) for Cinnarizine to be 0.1575 µg/ml, other than for Piracetam it was found to be 1.9926 µg/ml.

The Limits of Quantification (LOQ) for both compounds were found to be 0.4774 µg/ml and 6.0384 µg/ml, respectively. In interday precision tests, the approach exhibited a high level of accuracy, as evidenced by % Relative Standard Deviation (RSD) values of 0.3601 for Cinnarizine and 0.7080 for Piracetam. Assay results for Phescetam capsules revealed 100.53% purity for Cinnarizine and 100.19% for Piracetam, with % RSD values within acceptable limits, indicating high accuracy and precision. The method proved robust and rugged, showing consistent results under varying analytical conditions and across different analysts.

Stability studies confirmed the method's suitability for routine quality control, with both drugs demonstrating stability under acidic, basic, oxidative, and photolytic conditions.

Keywords: RP-HPLC, Cinnarizine, Piracetam, Calibration Curves, Validation, Stability studies**.**

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INTRODUCTION

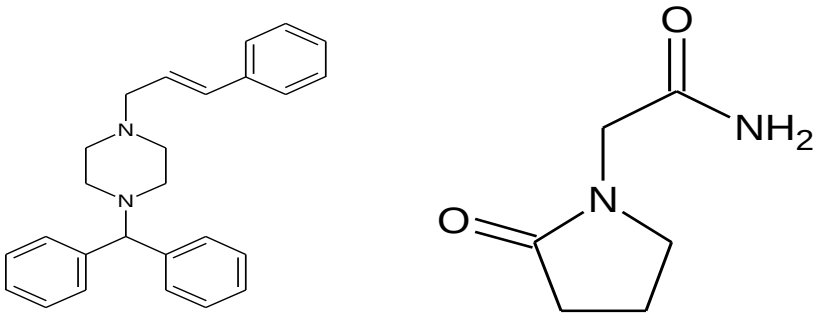
Cinnarizine is chemically known as 1-[(2-chlorophenyl) (phenyl)methyl]-4-(3-phenylpropyl) piperazine) and Piracetam is also known as 2-oxo-1-pyrrolidine acetamide) are two pharmacologically distinct agents commonly used in combination to treat neurological and vestibular disorders [1,2]. Cinnarizine, a selective calcium channel blocker, is known for its ability to inhibit calcium ion influx into cells, which reduces the excitability of the vestibular system and alleviates symptoms of vertigo and motion sickness [3,4]. Piracetam, a nootropic compound, enhances cognitive function by modulating neurotransmission, particularly by increasing the fluidity of cell membranes and improving the efficiency of synaptic transmission [5]. The structure of cinnarizine and piracetam is shown in Figure 1.

The combined therapeutic use of Cinnarizine and Piracetam aims to leverage their synergistic effects to manage both cognitive and vestibular dysfunctions, particularly in conditions like vertigo with associated cognitive impairments.

Several analytical methods have been reported for the individual quantification of Cinnarizine and Piracetam. For instance, chromatographic techniques such as High-Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography (UPLC) have been utilized for the determination of these drugs in various matrices [6-8]. Studies have demonstrated the successful application of HPLC for the estimation of Cinnarizine in combination with other drugs, highlighting its efficacy in separation and quantification. Similarly, Piracetam has been analyzed using different chromatographic methods, often focusing on its stability and interactions with other substances [9-11].

Previous studies have explored the use of various mobile phases, columns, and detection techniques to optimize the separation and quantification of these compounds [12,13]. Despite these advancements, there remains a need for a stability-indicating method that can address the complexities associated with their combined formulation and provide reliable results under different stress conditions.

This work aims to develop and validate a stability-indicating reversed-phase high-performance liquid chromatography (RP HPLC) method for simultaneously quantifying Cinnarizine and Piracetam in both bulk and capsule forms. We will thoroughly validate the proposed approach by ICH standards to guarantee its correctness, precision, specificity, and robustness [14]. By addressing the limitations of existing methods, this research seeks to provide a comprehensive analytical tool for quality control and stability assessment of Cinnarizine and Piracetam formulations, ultimately contributing to improved therapeutic outcomes and patient safety.



Cinnarizine **Piracetam**

Figure 1: Structure for cinnarizine and piracetam

Experimental

Chemical and Reagents

The drug samples used in this study were Cinnarizine and Piracetam, which were obtained by The Madras Pharmaceutical Limited, Chennai. The formulation employed was Phescetam capsules, each containing 25 mg of Cinnarizine and 400 mg of Piracetam, procured from a local pharmacy. The solvents used included Carbinol (Methanol), Water HPLC analytical grade from Qualigens; Acetonitrile and Orthophosphoric Acid, analytical grade from Merck. These chemicals and solvents were selected based on their purity and suitability for the analytical procedures involved.

HPLC Instrumentation and Conditions

An HPLC analysis was performed using an Agilent 1200 series HPLC system fitted with a C18 column measuring 150 × 4.5 mm and containing particles of 5 µm in size. In the first chromatographic setup, an isocratic separation technique was employed using a mobile phase consisting of Methanol and Orthophosphoric Acid (pH 2.0) in a volumetric ratio of 45:55%. At a temperature of 30°C, the flow rate was selected as 1.0 ml/min and the detecting wavelength was set at 229 nm. For optimization, the mobile phase composition was adjusted to Methanol and Orthophosphoric Acid (pH 2.5) in a 40:60 % v/v ratio. The sample injection volume was 20 µl. The HPLC system was coupled with a Shimadzu UV-1900 ultra-visible spectrophotometer, and other instruments used included a Labman ultra-sonicator, DLab water bath, Hanna pH meter, and RADWAG analytical balance to support the preparation and analysis of samples.

Method Validation

Preparation of Standard Stock Solution:

Accurately weigh 25 mg of Cinnarizine and 400 mg of Piracetam, and dissolve in 100 ml of mobile phase to prepare stock solutions. The initial stock solutions contain 250 µg/ml for Cinnarizine and 4000 µg/ml for Piracetam. Using these, create working solutions with final concentrations of 7.5 µg/m for Cinnarizine and 120 µg/m for Piracetam.

Preparation of Calibration Graph (Linearity):

Aliquots of stock solutions (0.1 to 6.0 ml) are transferred into 10 ml volumetric flasks and made up with a mobile phase. The concentration ranges are 2.5-15.0 µg/ml for Cinnarizine and 40-240 µg/ml for Piracetam. Inject 20 µl of these solutions, record

chromatograms at 229 nm, and plot peak areas against concentrations to construct a calibration curve.

LOD and LOQ:
The determination of the LOD and LOQ is based on the calibration curve approach, which involves calculating the average slope and intercept.

Quantification of Formulation:
Weigh 10 capsules of Phescetam, determine the average weight, and accurately weigh the equivalent of 25 mg of Cinnarizine and 400 mg of Piracetam. The solution should be dissolved in the mobile phase, subjected to sonication for 10 minutes, filtered using Whatman filter paper No. 41 with a filter size of 0.45 microns, and then purified to a final of Cinnarizine (7.5 µg/ml) and Piracetam (120 µg/ml).

Precision:
Perform interday and intraday precision tests by analyzing the formulation six times with the same concentrations. The percentage Relative Standard Deviation (RSD) was calculated

Robustness:
The adaptability of the chromatography is assessed by altering the mobile phase composition (± 2 ml), flow rate (± 0.2 ml/min), and wavelength (± 2 nm). Inject 20 µl solutions and record chromatograms, checking system suitability parameters.

Ruggedness:
Assess ruggedness by analyzing samples with different analysts to check the reproducibility of results.

Forced Degradation Studies

Acid Hydrolysis:
Develop solutions of Cinnarizine for 7.5 µg/ml and Piracetam for 120 µg/ml. Dispense 1 ml of 0.1 N hydrochloric acid, and allow it to stand at ambient temperature for two hours. and thereafter dilute with the mobile phase.

Base Hydrolysis:
Develop solutions of 7.5 µg/ml of Cinnarizine and 120 µg/ml of Piracetam. Dispense 1 ml of a 0.1 N NaOH solution, allow it to stand at ambient temperature for two hours, and thereafter dilute it with the mobile phase.

Oxidative Degradation:
Treat sample solutions with 1% hydrogen peroxide at room temperature for 2 hours. Inject 20 µl samples and record chromatograms.

Photolytic Degradation:
Expose solid APIs to UV light for 24 hours, prepare solutions, and inject 20 µl. Record chromatograms.

Results and Discussion
No stability indicating the method is available in the official compendia using RP-HPLC for analyzing Cinnarizine and Piracetam in dosage forms till now. The present proposed method was compared with the reported methods in the literature and shown in Table 1. From the comparison Table 1 between the developed method for UV and RP-HPLC method the LOD and LOQ values were less when compared with the UV method. DL and QL values were very low when compared to the UV method so it indicated the sensitiveness of the method. Hence the method was more sensitive when compared with the UV method. Additionally, the linearity ranges of the
UV method were more when compared to the RP-HPLC method. So, the RP-HPLC method can be applied for regular quality control analysis, and the least amount of drug can be required.

Recovery Studies:
Conduct recovery studies by adding known concentrations (50%, 100%, 150%) of Cinnarizine and Piracetam standards to pre-analyzed formulations. The % RSD was calculated.

Table 1: Comparison of the performance characteristics of the present method with the published methods

Parameters	Drugs	Developed Method	Reported Method [15]
Retention Time	Cinnarizine	2.548	8.103
	Piracetam	3.241	3.888
LOD (µg/ml)	Cinnarizine	0.011	1.04
	Piracetam	0.018	16.0
LOQ (µg/ml)	Cinnarizine	0.034	3.4
	Piracetam	0.055	48
Linearity (µg/ml)	Cinnarizine	2-12	10-80
	Piracetam	40-240	160-960

Linearity
The linearity of the RP-HPLC technique was assessed by the preparation of working stock solutions containing Cinnarizine and Piracetam. The samples were diluted by the addition of a mobile phase consisting of methanol and orthophosphoric acid (pH 2.0) at a volumetric ratio of 45:55%. Cinnarizine was tested within concentration ranges of 2.5-12.5 µg/ml, whereas Piracetam was tested within concentration ranges of 40-240 µg/ml. Analyzed using chromatography at a wavelength of 229 nm, the results demonstrated linearity within the specified limits. The correlation coefficients were 0.9992 for Cinnarizine and 0.9998 for Piracetam, indicating strong linear relationships. The regression equations derived were y = 109857X + 5114.2 for Cinnarizine and y = 115991X + 71054 for Piracetam. Figure 2 shows as Chromatograms illustrating the linearity of Cinnarizine and Piracetam. Figure 3 displays linearity curves for Cinnarizine and Piracetam. Table 2 shows as Linearity for Cinnarizine and Piracetam by the RP-HPLC method.

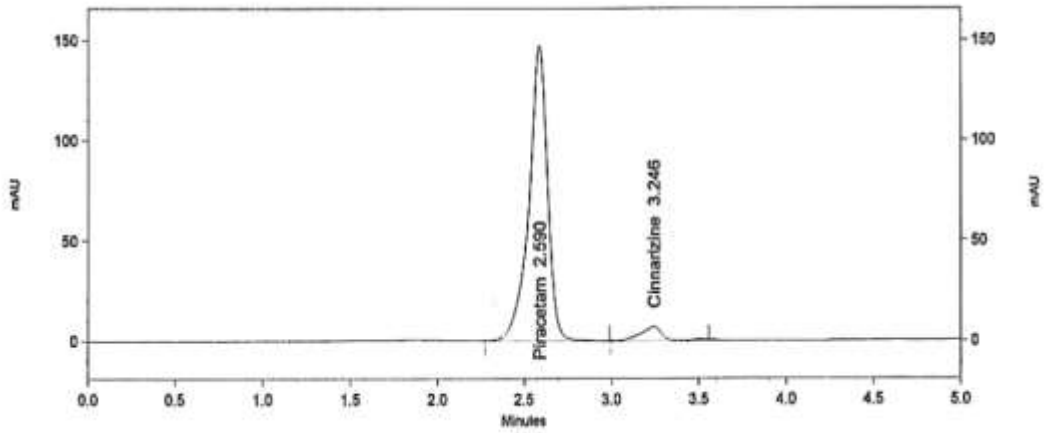


Figure 2: Chromatograms illustrating the linearity of Cinnarizine and Piracetam.

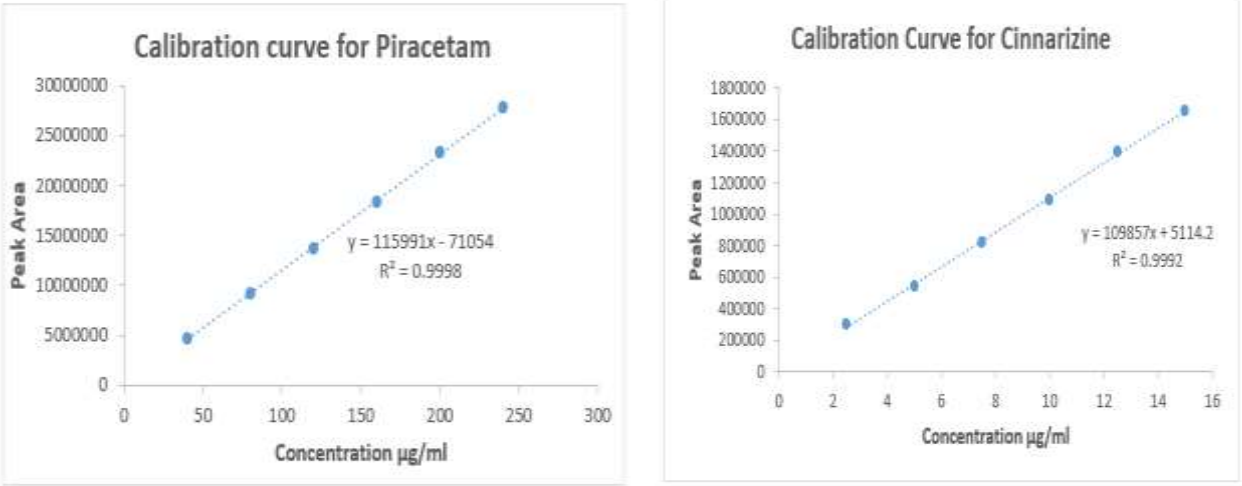


Figure 3: Linearity curves for Cinnarizine and Piracetam

Table 2: Linearity for Cinnarizine and Piracetam by RP-HPLC method

Parameters	**Cinnarizine**	**Piracetam**
Range (µg/ml)	2.5-12.5	40-240
Correlation Coefficient (r)	0.9992	0.9998
Regression equation (Y=mx+c)	y= 109857X+ 5114.2	y= 115991X +71054
Slope(m)	109857	115991
Intercept(c)	5114.2	71054
LOD (µg/ml)	0.1575	1.9926
LOQ (µg/ml)	0.4774	6.0384

Detection and Quantitation Limits

The method's sensitivity was assessed through the limits of detection (LOD) and limits of quantitation (LOQ). The LOD values were established as 0.1575 µg/ml for Cinnarizine and 1.9926 µg/ml for Piracetam. The LOQ values for Cinnarizine and Piracetam were 0.4774 µg/ml and 6.0384 µg/ml, respectively (table 1), indicating the method's capability to identify and measure trace amounts of the analytes.

Assay

The assay of the Phescetam capsule formulation showed that the percentage purity was 100.53% for Cinnarizine and 100.19% for Piracetam. The % RSD values were 0.3476 for Cinnarizine and 0.0320 for Piracetam, both within acceptable limits. The obtained results validate the application of the method for the precise quantification of the active components in the formulation. The corresponding findings are displayed in Table 3.

Table 3: Assay of Phescetam capsule formulation

Drug Name	Labeled amount(mg)	Amount found(mg)	%Purity	Average (%)	SD	%RSD
Cinnarizine	25 25 25	25.11 25.06 25.23	100.44 100.24 100.92	100.53	0.3494	0.3476
Piracetam	400 400 400	400.15 400.19 399.95	100.03 100.04 99.92	100.19	0.0321	0.0320

Precision

Precision in an analytical procedure is the degree of agreement among a collection of measurements obtained from multiple samples of a homogeneous sample, all taken under well-defined conditions. The precision of the approach was confirmed by conducting six repeated analyses of the formulation. The calibration curve yielded a nominal concentration of 7.5 µg/ml when 120 µg/ml solutions of Cinnarizine and Piracetam were chosen. The relative standard deviation (RSD) for precision was determined to be 0.3601 and 0.7080 for Cinnarizine and Piracetam, respectively. This study found that the percentage of the RSD for the region of six replicate injections was under the specified level. Table 4 presents the measured statistical precision values.

Table 4: Interday Precision for Cinnarizine and Piracetam

Drug Name	Injection No	Peak area	Average	SD	(%) RSD
Cinnarizine	**1** **2** **3** **4** **5** **6**	819811 812640 814215 814522 814522 811265	814655.66	2933.60	0.3601
Piracetam	**1** **2** **3** **4** **5** **6**	13711554 13621415 13569312 13452204 13703021 13643092	13616766.33	96407.36	0.7080

Recovery studies (Accuracy)

The accuracy study was established by recovery experiments. For the previously examined formulation, a predetermined amount of a solution containing Cinnarizine and Piracetam raw materials was added at varying concentrations of 50%, 100%, and 150%. A quantification of the substance found was performed. The Cinnarizine recovery percentage was determined to be 100.68%, while the Piracetam recovery percentage was discovered to be 100.04%. The calculated RSD values for Cinnarizine and Piracetam were 0.2027 and 0.0263 respectively. The percentage true standard deviation (RSD) value was found to be less than 2%. The minimal absolute standard deviation (RSD) value indicates that the excipients utilized in the formulation did not create any interference. Consequently, the accuracy of the method was confirmed. The data is shown in Table 5.

Table 5: Accuracy for Cinnarizine and Piracetam by RP-HPLC method

Drug Name	Concentration (%)	Amount added (µg/ml)	Amount found ((µg/ml)	Mean	Mean recovery	SD	%RSD
Cinnarizine	50 100 150	5 7.5 10	5.04 7.53 10.08	100.86 100.40 100.80	100.68	0.2041	0.2027
Piracetam	50 100 150	60 120 180	60.02 120.09 180.04	100.03 100.08 100.02	100.04	0.0262	0.0263

Robustness

This study assessed the method's resilience by implementing minor modifications in the flow rates and the chemical makeup of the mobile phase. The system fit results consistently conformed to acceptable limits, indicating the method's robustness and reliability across the different settings presented in Table 6.

Table 6: Robustness for Cinnarizine and Piracetam by RP-HPLC method

Peak Name	Parameter	Conditions	Theoretical plate	Asymmetric factor
Piracetam	Mobile phase	35ml	2434	0.83
		40ml	3135	0.83
		45ml	2232	0.84
	Wavelength	227nm	2434	0.83
		229nm	3135	0.83
		231nm	2232	0.84
	Flow rate	0.8ml/min	2492	0.85
		1.0ml/min	3135	0.83
		1.2ml/min	2233	0.85
Cinnarizine	Mobile phase	35ml	3448	0.78
		40ml	3820	0.79
		45ml	2536	0.79
	Wavelength	227nm	3448	0.78
		229nm	3820	0.79
		231nm	2536	0.79
	Flow rate	0.8ml/min	3559	0.79
		1.0ml/min	3820	0.79
		1.2ml/min	2632	0.76

Ruggedness

Ruggedness was assessed by testing the reproducibility of the results across different conditions and analysts. The % RSD values for Cinnarizine were 1.6987 and 1.7012 for Analyst I, and 0.1816 and 1.1328 for Analyst II. For Piracetam, the % RSD values were 0.5589 and 0.5999 for Analyst I, and 0.2845 and 1.0316 for Analyst II, confirming the method's reproducibility and ruggedness. The results are shown in table 7.

Table 7: Ruggedness for Cinnarizine and Piracetam by RP-HPLC method

Drug Name	Different conditions	Mean%	SD	%RSD
Cinnarizine	Analyst-I	99.96	1.7012	1.7018
	Analyst-II	101.43	1.1491	1.1328
Piracetam	Analyst-I	100.14	0.6004	0.5999
	Analyst-II	99.74	1.0289	1.0316

Degradation Studies

The Stability of the analytes was confirmed by stability studies. The degradation studies were carried out by using 0.1N HCl, 0.1N NaOH, 0.1%H2O2 and photolytic. Based on the results the percentage degradation was found to be for 0.1N HCl – 7.55 and 6.28 %, 0.1N NaOH-8.75 and 3.57%, 0.1% H_2O_2 – 5.47 and 6.48 %, Photolytic degradation-4.27 and 3.80% for Cinnarizine and Piracetam respectively. The degradation percentage was found to be for all stress conditions below 20% (ICH guidelines within the limit). Hence conclude that the analytes were stable under the above stress conditions. The report is shown in table 8.

Table 8: Degradation study of cinnarizine and piracetam

Degradation Condition	% Assay		% Degradation	
	Cinnarizine	Piracetam	Cinnarizine	Piracetam
0.1N HCl Acidic / 2hr	92.45	93.72	7.55	6.28
0.1N NaOH Basic / 2hr	91.27	96.43	8.75	3.57
1% H2O2 Peroxide/2hr	94.53	93.52	5.47	6.48
Photo/ UV light/ 24hr	95.73	96.20	4.27	3.80

Discussion

The linearity analysis showed that the RP-HPLC technique designed for the concurrent quantification of Cinnarizine and Piracetam exhibits linearity throughout the concentration ranges that were evaluated. The calibration curves exhibited superior linearity, as evidenced by correlation coefficients of 0.9992 for Cinnarizine and 0.9998 for Piracetam. These high correlation coefficients confirm that the method can accurately quantify both drugs within the specified ranges, which is crucial for ensuring consistent drug dosage in pharmaceutical formulations [16]. The high linearity observed suggests that the method is highly reliable for quantifying Cinnarizine and Piracetam in various formulations. The regression equations derived from the calibration curves can be used to predict concentrations in unknown samples, making this method suitable for routine quality control.

The LOD and LOQ for Cinnarizine were found to be 0.1575 µg/ml and 0.4774 µg/ml, respectively. For Piracetam, the LOD and LOQ were identified as 1.9926 µg/ml and 6.0384 µg/ml. These values indicate the method's sensitivity, allowing for the detection and quantitation of low concentrations of the analytes. The low LOD and LOQ values are indicative of the method's high sensitivity, which is essential when analyzing pharmaceutical samples that may contain trace amounts of these

drugs [17]. This sensitivity makes the method particularly useful in detecting impurities or degradation products in quality control settings. According to the assay results, the formulation of Phescetam capsules included 100.53% and 100.19% pure cinnarizine and piracetam, respectively. The % RSD values were well within acceptable limits, confirming the method's accuracy and precision in quantifying the active ingredients in the capsule formulation [18]. The close agreement between the observed and labelled amounts of the drugs in the Phescetam capsules confirms the method's accuracy. The low % RSD values further suggest that the method is both precise and reliable, making it suitable for routine pharmaceutical analysis. Interday and intraday precision tests yielded % RSD values of 0.3601 for Cinnarizine and 0.7080 for Piracetam, demonstrating the method's precision. These values are within the acceptable range, indicating consistent performance of the method across multiple runs. The recovery studies showed mean recoveries of 100.68% for Cinnarizine and 100.04% for Piracetam, with low % RSD values, confirming the method's accuracy. The method's excellent recovery rates and low % RSD values ensure precise quantification of medicines in the presence of excipients, crucial for maintaining the correct dose in pharmaceutical formulations. The results of robustness testing showed that minor changes in chromatographic conditions had no main impact on the system suitability parameters, therefore confirming the robustness of the approach. The robustness of the method ensures that it can withstand minor variations in analytical conditions without compromising accuracy or precision, making it suitable for use in different laboratory environments. The ruggedness study confirmed that the method produced consistent results across different analysts, with % RSD values within acceptable limits. The reproducibility of the results across different analysts demonstrates the method's ruggedness, making it reliable for use in various analytical settings.

The forced degradation studies indicated that both Cinnarizine and Piracetam were stable under acidic, basic, oxidative, and photolytic conditions, with degradation within acceptable limits. The stability of Cinnarizine and Piracetam under these stress conditions suggests that the method is suitable for stability-indicating analysis. This capability is crucial for ensuring the long-term stability and efficacy of pharmaceutical products.

Conclusion

The RP-HPLC method developed and validated in this work for the concurrent quantification of Cinnarizine and Piracetam in pharmaceutical formulations has demonstrated exceptional dependability and operational effectiveness. Due to its exceptional linearity, sensitivity, precision, and accuracy, the approach is well-suited for routine analysis in quality control laboratories. The method's capacity to detect and quantify even minute quantities of the analytes is highlighted by the low LOD and LOQ limits. This is especially beneficial in guaranteeing the purity and potency of pharmaceutical items. Additionally, the robustness and ruggedness of the method ensure its applicability across different laboratory settings and analysts without compromising the quality of results. Further confirmation of the stability-indicating nature of the approach was obtained by forced degradation experiments, therefore establishing its suitability for evaluating the stability of Cinnarizine and Piracetam under different stress situations. In summary, this approach offers a thorough and dependable procedure for ensuring the quality of formulations that include Cinnarizine and Piracetam.

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