JOURNAL OF PHARMACEUTICAL ANALYSIS

VALIDATE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOCLOPERASTINE FENDIZOATE PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple, accurate, precise method was developed for the simultaneous estimation of the levocloperastine hydrochloride in suspension dosage form. Chromatogram was run through symmetry (250 × 4.6) mm; 5 micron, C18. Mobile phase containing buffer (pH 3.5) and acetonitrile in the ratio of 50:50 was pumped through column at flow rate of 1 ml/min. The mobile phase temperature was maintained at 30 °C, optimized wavelength for levocloperastine is 273 nm. Retention time of Levocloperastine was found to be 5.403 min, tailing factor and number of theoretical plate were 1.146 and 4455 respectively. Regression value of levocloperastine from linearity curve was found to be 0.997109 which suggest very strong linearity , expected in pharmaceutical and QC lab.
Index Terms: (250 × 4.6) mm; 5 micron, C18, Levocloperastine.

INTRODUCTION

RP-HPLC

Reversed-phase high performance liquid chromatography (RP-HPLC) separates molecules based on how non-polar or hydrophobic they are. The separation occurs due to hydrophobic interactions between the solute molecules present in the mobile phase and the hydrophobic ligands fixed onto the stationary phase (sorbent). The solute mixture is first introduced to the sorbent using aqueous buffer solutions, and Elution is carried out by gradually introducing the solute mixture dissolved in an organic solvent into the mobile phase. Elution can be carried out in an isocratic setting, where the organic solvent concentration stays constant, or using gradient elution, where the concentration of the organic solvent gradually increases over time. As a result, Solutes elute in sequence based on increasing molecular hydrophobicity. Initially, samples are applied in **aqueous buffer**, and separation is achieved by adding an **organic solvent [1]**.

In reversed-phase mode, sample components with greater hydrophobicity tend to be retained longer within the system. Several factors influence the separation and retention of solutes, including the following [2]:

a) The chemical characteristics of the stationary phase, such as the type of ligands attached to its surface and the density of their bonding, which reflects the degree of surface coverage.

b) The makeup of the mobile phase, particularly the types of bulk solvents used, as their combination determines the overall polarity. A solvent introduced to adjust this polarity is known as a 'modifier'.
c) Additives like buffers alter the pH of the mobile phase, thereby affecting the ionization state and polarity of the solutes.
d) The mobile phase flow speed through the column may have a profound effect o the separation process and resolution of analytes. A flow rate that is too high may cause band broadening and reduced resolution, while a flow rate that is too low can lead to excessively long analysis times.

To ensure the retention of the organic components in the mixture, the stationary phase packed in the columns is composed of hydrophobic groups affixed to the surface of porous silica gel particles. The surface of the particles is coated with chemically bonded hydrocarbon chains, such as C3, C4, C8, C18, and others. The length of the hydrocarbon chains on the stationary phase influences retention time-the longer the chains, the longer the sample components are retained [2].

Advantages of RP-HPLC

Reversed-phase HPLC provides several benefits compared to normal-phase HPLC, including the following advantages [3]:

- More cost-effective than other HPLC techniques.
- Reduced solvent toxicity, leading to a lower environmental impact.
- Requires smaller sample volumes while still delivering accurate results.
- Minimized solvent evaporation.
- The capability to selectively modify pH to enhance sample separation.
- Effective retention of most organic molecules by the stationary phase.
- Elimination of distorted or inconsistent analyte retention times.
  Enhanced precision in gradient separations.

Levocloperastine Fendizoate

Cough is a very common respiratory symptom observed in children and individuals of all ages. It can significantly impact a patient's quality of life, particularly when it disrupts sleep at night. In most cases, acute cough in patient is caused by a viral upper respiratory tract infection (URTI), such as the common cold. Cough is generally considered a protective reflex, and therefore, treatment is not always necessary. However, when caused by a viral upper respiratory tract infection (URTI), cough can become distressing, leading to the frequent use of empiric treatment with antitussive medications [4].

Currently available antitussive agents, when used at effective doses, often results in undesirable effects such as drowsiness, nausea, and constipation, which can limit their use. Moreover, recent evidence suggests that standard treatments like codeine may not be particularly effective in relieving cough related to upper respiratory infections. As a result, there is a clinical demand for improved and better-tolerated alternatives [19].

Levocloperastine is a novel cough suppressants (antitussive) medication mainly used to relieve dry, non-productive cough. It is commonly formulated as a syrup or suspension and is available in countries such as India, Germany, Canada and Italy. Levocloperastine acts by inhibiting the cough receptor in the brai n, helping to reduce the urge to cough. It is commonly recommended

for the short-term relief of dry cough, non-productive cough. However, it does not effectively treat chronic coughs associated with conditions such as smoking, asthma, emphysema. It acts through a dual mechanism, affecting both the central cough center in the brainstem and peripheral receptors within the tracheobronchial tree. Preclinical research has shown that, levocloperastine provides antitussive effects similar to those of codeine. In both acute and repeated-dose toxicity studies, levocloperastine was well tolerated in rodents and dogs, with no notable significant gastrointestinal or cardiovascular side effects. Clinical trials have shown that it has a prompt effect and provides more significant decrease in cough severity and occurrence compared to DL-cloperastine, codeine, and levodropropizine. Its antitussive effects were evident from the day one of therapy across all age groups. In kids, it improved sleep and reduced irritability, while in mature individual, it was efficacious against ACE inhibitor-induced cough. Levocloperastine was generally exhibit good patient compliance, with fewer central side effects than comparator drugs, making it a promising alternative with better tolerability and quicker relief [5].

It is already discussed that levocloperastine is an antitussive agent but It also possesses antihistaminic properties, due to its ethylamine structure shared with H1 receptor antagonists, and exhibits papaverine-like activity comparable to codeine, but without the narcotic effects associated with codeine. Thanks to its dual mechanism of action, it is highly effective in treating cough linked to various acute and chronic conditions affecting all age groups. Medication research have demonstrated that the compound works directly on the central cough pathway without suppressing the respiratory function and does not adversely affect the cardiovascular system [6].

Levocloperastine can be formulated in various forms, including solids (e.g., tablets, capsules), liquids (solutions or suspensions), suppositories, and injectables. Solid oral forms are typically intended to be swallowed whole, which can be difficult for children, the elderly, and patients with swallowing difficulties. Therefore, a liquid dosage form, particularly an oral suspension of levocloperastine fendizoate, is preferred for easier administration in such populations. Pharmaceutical suspensions are two-phase systems in which water-insoluble drug particles are dispersed in a liquid medium. They differ from emulsions and syrups containing dissolved drugs. An ideal suspension should be physically and chemically stable, have suitable viscosity, be easily redispersed by shaking, simple to manufacture, and acceptable for patient use [6].

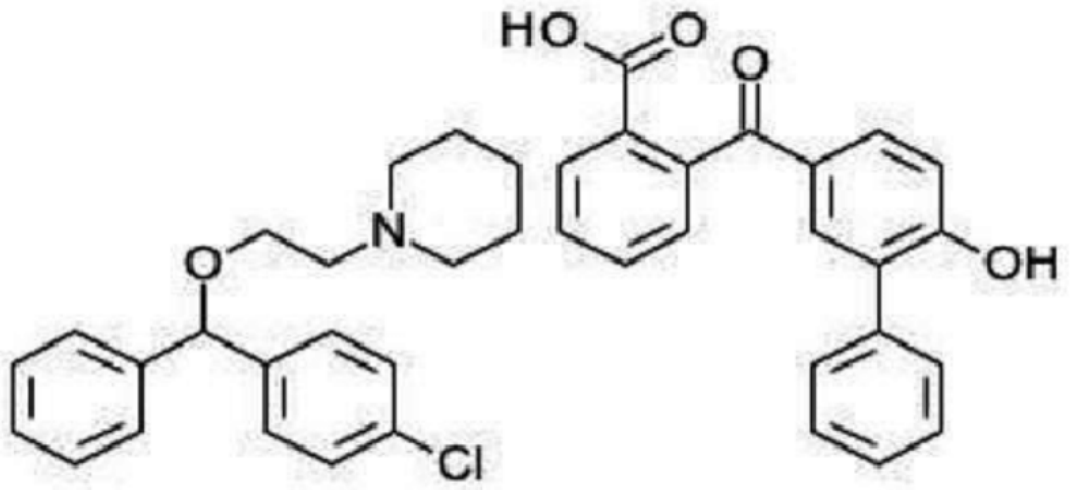

Fig: Levocloperastine Fendizoate

MATERIALS AND METHODS

Equipment

HPLC (Shimadzu), Digital weighing balance (Shimadzu), UV double beam (Shimadzu), pH meter, Ultrasonication, suction pump, (250 × 4.6) mm; 5 micron, C18 column.

Reagents and Chemicals

Pharmaceutical pure sample of Levocloperastine Fendizoate, potassium dihydrogen phosphate, orthophosphoric acid, Acetonitrile HPLC grade, Dimethyl sulphoxide (DMSO), methanol were obtained from NHC Pvt. Ltd. Nepal.

Chromatographic condition

Experiments, method development and validation were performed using HPLC (Shimadzu), Data collection was done using Lab solution software. Mobile phase consisting of acetonitrile and phosphate buffer (pH 3.5) at ratio (50:50 V/V) was used in an isocratic mode. The flow rate was maintained at rate 1 ml/min and the injection volume was 20 µl. The UV detection was performed at 273 nm and the separation was achieved at temperature 30 °C.

EXPERIMENTAL

Preparation of standard solution

Approximately 35.5 mg of levocloperastine fendizoate an accurate amount of the working standard was transferred into a 100 ml volumetric flask. 10 ml of DMSO were added, and the mixture was shaken and sonicated for 10 minutes to dissolve the substance. After cooling to room temperature, the volume was made up to 100 ml with methanol. The solution was then mixed for 10 minutes and filtered.

Preparation of sample solution

An accurately measured amount of the suspension, containing approximately 20 mg of levocloperastine hydrochloride, was transferred to a 100 mL volumetric flask. To this, 10 mL of DMSO was added, and the mixture was shaken and sonicated for 10 minutes to ensure complete dissolution. After allowing the solution to cool to room temperature, the volume was brought up to 100 mL with methanol. The resulting solution was mixed for 10 minutes and then filtered.

METHOD VALIDATION

According to ICH guidelines, this method remains been unvalidated. To validate the method, following parameters were assessed [7].

System suitability

The separation parameters were configured, the mobile phase was pumped through the column at a controlled flow rate of 1.0 mL/min to allow complete saturation. Once the column was fully equilibrated, six individual injections of the levocloperastine fendizoate reference standard were injected. Ensure that, upon injecting the standard solution, the tailing

factor stays below 2.0, the theoretical plates exceed 2000, and the relative standard deviation (RSD) for repeated injections remains within 2.0%.

Linearity

An analytical methods linearity is its capacity to yield results proportional to analyte concentration over a defined range.

Linearity of levocloperastine fendizoate shall be study by taking minimum of five concentration of a standard in the range of the drug substance from 80% - 120% content [8].

Precision

The precision study of an analytical method refers to the degree of repeatability of results obtained from a series of experiment conducted in a single session by one operator using the same reagents and instruments [9].

Accuracy (% recovery)

Accuracy of an analytical method refers to how closely the test results match the true value. It is often expressed as the percentage recovery of a known amount of analyse added during the assay.

The method's accuracy is assessed by spiking known amounts of analyte at levels both below and above 100% of the normal analyte concentration. The accuracy of the assay method of levocloperastine fendizoate shall be determined by adding the known amount of levocloperastine fendizoate in spiked [10].

Specificity

Specificity testing conducted to unambiguously confirm the presences of the analyte amid potential co-existing component impurities, interference with excipients, degradation of products and matrix components. Different sample of standard and test has been prepared and injected [11].

LOD and LOQ

The detection limit refers to the smallest concentration of an analyte in a solution which is detectable but not accurately quantified. It is typically determined using the signal-to-noise ratio, commonly around 3:1. According to ICH guidelines, a signal-to-noise ratio of 10:1 is recommended. These data points are generated from the calibration curve [12].

Robustness

As per ICH standards, slight deliberate changes in mobile phase concentration were applied like ratio of buffer and ACN changed from 50:50 to 55:45, like study was also performed by change in volume injection, at two different wavelength [13].

Assay calculation for levocloperastine

Assay calculation for levocloperastine was carried out by injecting 20 µl volume of standard and sample solution of levocloperastine in six and triplicate respectively. The assay mean, standard deviation and percentage RSD of sample peak area were computed and reported.

RESULTS AND DISCUSSION

System suitability

This study focuses on the development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the routine analysis of levocloperastine in pharmaceutical formulations. The RP-HPLC method was initially employed to optimize chromatographic conditions for the quantification of levocloperastine in the selected dosage form. The most suitable combination identified was a binary mixture of acetonitrile (ACN) and phosphate buffer (pH 3.5) (50:50 V/V), as the mobile phase. The resulting chromatographic peaks were sharp and symmetrical, with no evidence of tailing factor. The developed chromatographic method was validated in accordance with ICH guidelines. Levocloperastine exhibited a retention time of 5.403 minutes. Figure 1 presents the optimized chromatogram, clearly demonstrating the separation of levocloperastine at the specified retention time.

System suitability of the HPLC method was evaluated using six injections of levocloperastine at 100% concentration. The results showed a tailing factor of less than 2 and a theoretical plate count exceeding 2000, indicating acceptable system performance.

Table 1: Result of system suitability parameters

Sample Name	RT	Area	TF	NTP
Std. 1	6.499	10062154	0.944	5539
Std. 2	6.503	10382172	0.943	5559
Std. 3	6.516	10384081	0.944	5566
Std. 4	6.518	10298923	0.945	5618
Std. 5	6.520	10536516	0.945	5618
Std. 6	6.511	10583646	0.944	5668
Blank	0.000	0	--	--
Average	6.511	10374582	0.944	5594
%RSD	0.130	1.795	0.079	0.855

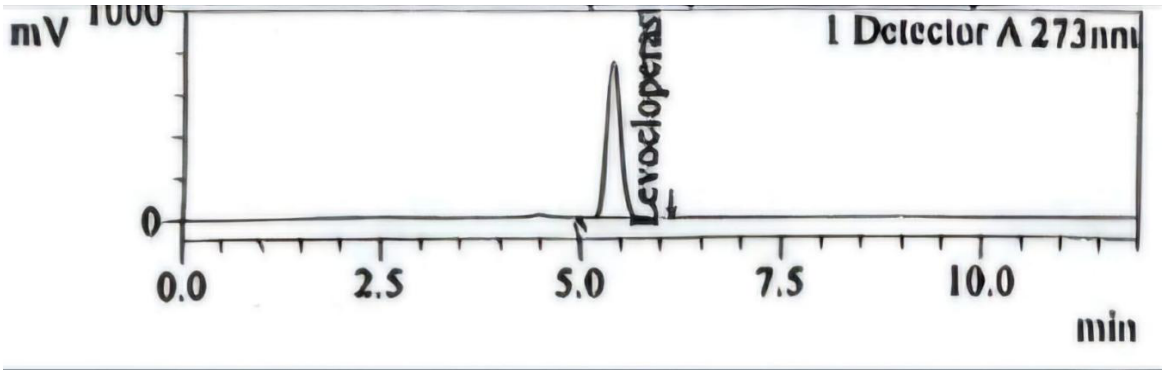

Table 1: Optimized chromatogram of levocloperastine

Linearity

The linearity was observed from concentration 80% to 120% of levocloperastine. The correlation coefficient was found 0.997109 which indicate good linearity at specified concentration range. The calibration curve for levocloperastine is shown in figure 2.

Table 2: Results of linearity

Sr. No.	Concentration (%)	Area	%RSD
1.	80	6917753	0.239
2.	90	7799318	0.009
3.	100	8870615	0.168
4.	110	9549943	0.086
5.	120	10775837	0.081

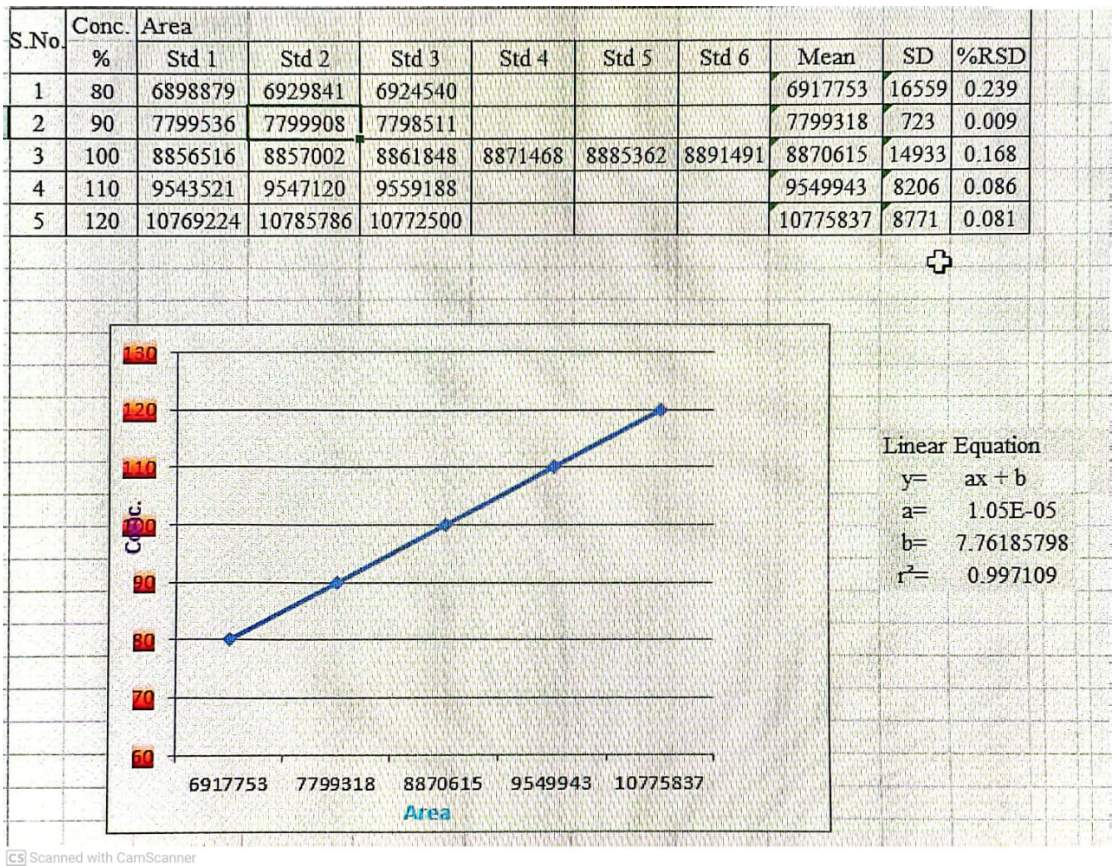

Figure 2: Plotting of calibration curve

Precision

A thorough study was conducted to assess intraday and interday variability. The corresponding response was evaluated on same day and at different days. Results are observed in percentage relative standard deviation (%RSD), all results are lies within acceptance limits (%RSD < 2).

Table 3A: Result of interday precision

Sr. No.	Area	%RSD
1.	8797728	0.887

Table 3B: Result of intraday precision

Sr. No.	Area	%RSD
1.	10998469	1.542

Accuracy

Recovery studies were performed by spiking pre-analyzed samples with a known quantity of standard medication to evaluate the accuracy of the proposed method. The standard addition technique was applied at three concentration levels—80%, 100%, and 120% of the original sample-in triplicate, to confirm the reliability and precision of the analytical procedure. The percentage RSD was found to be below 2%, demonstrating that the method's accuracy is consistent and closely aligns with the labeled claim.

Table 4: Result of recovery study (Accuracy)

Sr. No.	Concentration	Test		Standard	
		Area	%RSD	Area	%RSD
1.	80%	7657207	0.413	-	-
2.	100%	9140157	0.756	8870615	0.168
3.	120%	11250011	0.339	-	-

Specificity

The specificity of the method was assessed by analyzing the chromatograms obtained from the blank injection, sample injection and standard injection shown in figure respectively. No interference from excipients with the target analyte was observed, confirming the method's specificity. Thus, the method was deemed suitable for the analysis of the commercial pharmaceutical formulation.

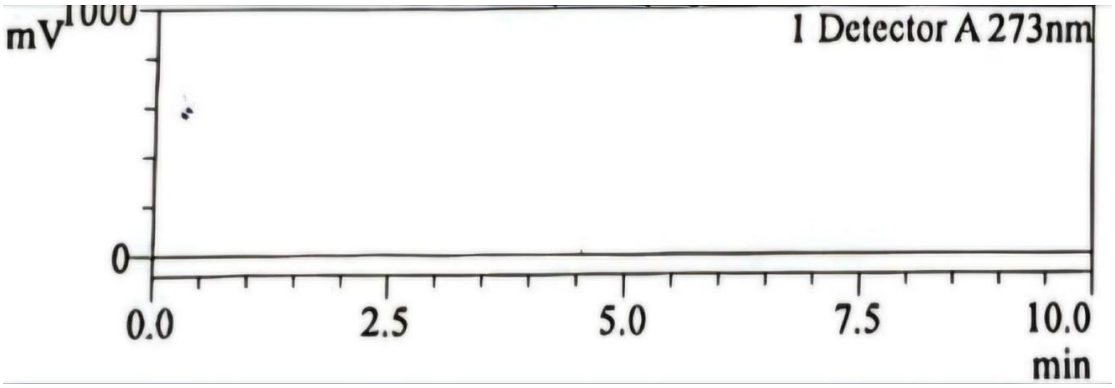

Figure 3A: Chromatogram of blank injection

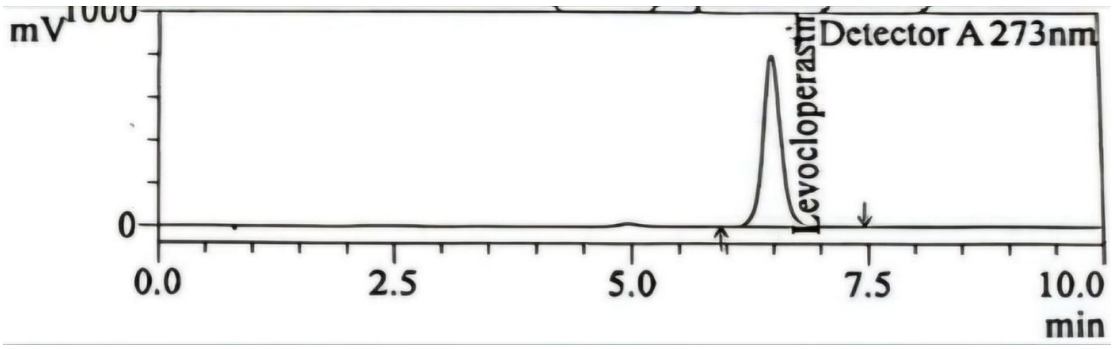

Figure 3B: Chromatogram of sample injection

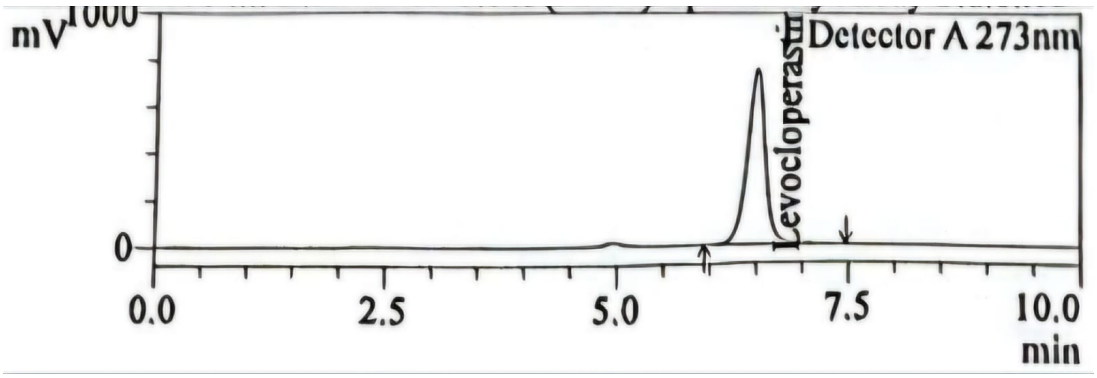

Figure 3C: Chromatogram of standard injection

LOD & LOQ

The minimum concentration level at which the analyte can be reliable and detected LOD & quantified LOQ were found 0.496 to 1.504 µg/ml respectively.

Robustness

The robustness of the method was evaluated by introducing deliberate variations in experimental conditions, including changes to the mobile phase composition, detection wavelength, and injection volume. Specifically, the mobile phase ratio (phosphate buffer (pH 3.5) + acetonitrile, 55:45 %v/v), wavelengths (271 nm and 275 nm), and injection volumes (14 µL and 18 µL) were altered. For each modified condition, a 100% concentration solution was prepared and injected in triplicate. The percentage RSD for all conditions remained below 2%, confirming the robustness of the method.

Table 5: Result of Robustness study

Sr. No.	Condition	Variation	Levocloperastine	
			Area	%RSD
1.	Change in mobile phase ratio (50:50)	55:45	9129289	0.438
2.	Change in wavelength (273±2nm)	271	9379340	0.551
		275	9917698	0.091
3.	Change in injection volume	IV16	8087139	0.050
		IV18	8777357	0.031

Conclusion

Levocloperastine dosage forms were analyzed using a specifically developed and validated RP-HPLC method. This method proved to be cost-effective due to its low solvent consumption (1 mL/min) and short run time (7 minutes), making it an environmentally friendly chromatographic technique suitable for high-throughput analysis. Additionally, the method demonstrated a broad linearity range and utilized a readily available mobile phase, contributing to its efficiency and overall superiority.

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