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## A SIMPLE STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LEVOCETIRIZINE DIHYDROCHLORIDE, PHENYLEPHRINE HYDROCHLORIDE AND PARACETAMOL IN PHARMACEUTICALS

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Reverse phase,  
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Levocetirizine Dihydrochloride,  
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Paracetamol

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**ABSTRACT:** An accurate and precise HPLC method was developed and validated for simultaneous determination of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol in syrup formulations. The separation between Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol was achieved within 20 min using an L1 column of 100 × 4.6 mm, 3 μ dimension using gradient programme and detector wavelength 215 nm. The mobile phase solution A is a buffer solution of 0.015 Molar 1-octane sulphonic acid sodium salt adjusted pH 3.4 with orthophosphoric acid and mobile phase solution B is Acetonitrile. The Method was validated as per ICH guideline for parameters like Specificity, Precision, Accuracy, solution stability, filter compatibility, and robustness. Accuracy for Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol lies between 97.0 to 103.0%. The proposed method can be used for quality control assay of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol in pharmaceuticals.

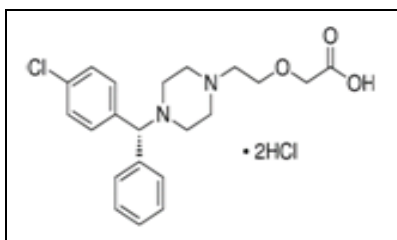
**INTRODUCTION:** In pharmaceutical industries, rapid analytical methods are required for simultaneous estimation of multiple components. There is a number of formulations available in which multiple active drugs are presents in single dosage forms. Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol syrup are widely used formulation in the pharmaceutical market.

Levocetirizine dihydrochloride is widely used as an antihistaminic drug. It is an active R-enantiomer of cetirizine, orally active, potent, and selective and long-acting H1-histamine receptor antagonist with no ant cholinergic activity. It is chemically (R)-[2-[4-[(4-chlorophenyl) phenyl methyl]-1-piperazinyl] ethoxy] acetic acid dihydrochloride <sup>1</sup>. It is a white to off white crystalline powder with a molecular weight of 461.82. It is used to relieve allergy symptoms such as watery eyes, runny nose, itching eyes/nose, and sneezing. It is freely soluble in water and soluble in methanol. According to the Biopharmaceutical Classification System (BCS), Levocetirizine dihydrochloride falls under the BCS Class III, highly soluble and poorly permeable drug.

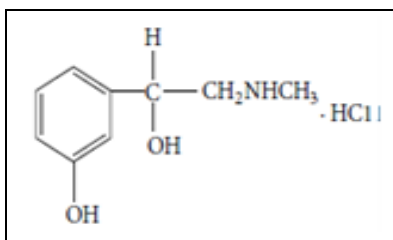
<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.10(5).2497-03</p> <hr/> <p>The article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a></p> <hr/> <p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.10(5).2497-03">http://dx.doi.org/10.13040/IJPSR.0975-8232.10(5).2497-03</a></p>
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Phenylephrine hydrochloride is an alpha-adrenergic (sympathomimetic) agent which stimulates alpha-adrenergic receptors, producing pronounced vasoconstriction. Chemically Phenylephrine hydrochloride (PHE), is (R)-2-methylamino-1-(3-hydroxyphenyl) ethanol hydrochloride. It is a white to almost white crystalline powder with a molecular weight of 203.7. Phenylephrine hydrochloride is official in IP, BP and USP<sup>2,3,4</sup>. It is freely soluble in water and alcohol. Paracetamol is a non-prescription combination analgesic for

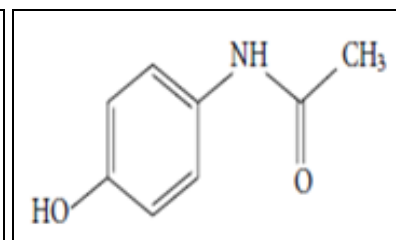
reducing fever and for relief for pain caused by heat, cancer, dental surgery, and arthritis. Chemically, Paracetamol is 4-Hydroxy acetanilide or p-hydroxyacetanilide. It is a white to almost white crystalline powder with a molecular weight of 151.2. It is sparingly soluble in water, freely soluble in Alcohol and very slightly soluble in Methylene Chloride<sup>5,6</sup>. Structure of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol shown in **Fig. 1, 2 and 3** respectively.



**FIG. 1: LEVOCETIRIZINE DIHYDROCHLORIDE**



**FIG. 2: PHENYLEPHRINE HYDROCHLORIDE**



**FIG. 3: PARACETAMOL**

Literature survey revealed that there are so many methods of analysis were reported for individual analysis of Levocetirizine Dihydrochloride, Phenylephrine hydrochloride, and Paracetamol, as well as with combination with other drugs<sup>7-19</sup>, but no method is reported for Simultaneous determination of Levocetirizine Dihydrochloride, Phenylephrine hydrochloride and Paracetamol in combination formulations. A proposed analytical method has been developed and validated as per ICH guideline<sup>20</sup>. Hence, the proposed method is advantageous over the reported method and can be conveniently adopted for routine quality analysis in Pharmaceutical industries.

## MATERIALS AND METHODS:

**Chemicals and Reagents:** Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol syrup samples with Label claim 2.5 mg, 10 mg, and 250 mg respectively were procured from the market. Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol standards are taken from LGC Promochem India Ltd. 1-Octane sulphonic acid sodium salt (AR Grade-spectrochem), Milli Q grade water, orthophosphoric acid (GR grade-Merck), triethylamine (GR Grade-spectrochem), and acetonitrile (HPLC grade-Merck) were used. Water's 2695 Alliance HPLC system with Autosampler with UV and PDA detector was used.

## Chromatographic Conditions for Developed

**Method:** 0.015 Molar 1-octane sulphonic acid sodium salt adjusted pH 3.4 with Orthophosphoric acid is used as a buffer solution (Mobile phase A) with Acetonitrile (Mobile solution B).

Column: L1 100 × 4.6 mm, 3 μ, Column oven temperature: 25 °C, Wavelength: 215 nm, Injection volume: 20 μL, Flow rate: 1.3 mL/minute, Retention Times: Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol eluted at about 22 min, 13 min, and 5 min respectively.

## Gradient Programme:

Time (in min)	Mobile Phase A	Mobile Phase B
0	100	0
16	70	30
20	60	40
30	60	40
32	20	80
35	100	0
40	100	0

**Diluent:** Water and Acetonitrile in the ratio 80:20 v/v.

**Preparation of Standard Solutions:** Mixed Standard solution in diluent was prepared to contain Levocetirizine dihydrochloride (25 μg/mL), Phenylephrine hydrochloride (100 μg/mL) and Paracetamol (50 μg/mL) with sonication for 5 min to dissolve.

**Preparation of Sample Solution:**

**Sample Stock Solution:** Weighed Sample equivalent to 5 mg of Levocetirizine dihydrochloride into 200 volumetric flasks. Added a sufficient amount of diluents about 50% of the volumetric flask and sonicated for about 25 minutes with intermittent shaking. Cooled and dilute up to volume with diluent. Filtered through 0.45 $\mu$  PVDF membrane filter with discarding 5 mL of filtrate (Stock solution).

**Sample Solution for Levocetirizine Dihydrochloride and Phenylephrine Hydrochloride:** The above Sample Stock solution is used as such for analysis of Levocetirizine dihydrochloride and Phenylephrine hydrochloride having concentration about 25  $\mu$ g/mL and 100  $\mu$ g/mL respectively.

**Sample Solution for Paracetamol:** Diluted above Sample Stock solution to achieve the concentration of the solution which contains 50  $\mu$ g/mL of Paracetamol.

**RESULTS AND DISCUSSION:**

**Method Development:** For systematic method development for determination of Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol, developmental parameters were studied which includes but not limited to Solubility, Selection of diluents, UV Detection Wavelength, Selection of working pH range for Mobile phase, Choice of Buffer, Buffer Concentration, Selection of Columns and final method optimization.

To select proper diluents for standard and sample solution, it's very important to know the solubility or miscibility of compound/s to be used for method development. Therefore solubility of compound/s to be used in method development was checked in some HPLC compatible solvents like Methanol, Acetonitrile, Tetrahydrofuran, Isopropyl alcohol, water well as a mixture of solvents and water. By solubility study and some initial trials water and Acetonitrile in the ratio of 80:20 v/v was selected as a diluent for this method development. All the three compounds are showing a comparatively better response at 215 nm UV wavelength; therefore 215 nm wavelength was selected for this method.

Initially, trials were conducted by using the USP method of Levocetirizine Dihydrochloride, pH of

mobile phase and after comparative study, it was observed that pH 3.45 is suitable for separation of these compounds. Therefore, 0.015M 1-octane sulphonic acid sodium salt adjusted pH 3.45 with Orthophosphoric acid is used as a buffer solution (Mobile phase A) with Acetonitrile (Mobile solution B) as it gives better baseline when compared with methanol.

Column selection is the major part in the reverse phase chromatography for method development; column acts as a stationary phase. In reverse phase chromatography, a very wide range of columns can be used as a stationary phase depending on column chemistry. These columns include C-18, C-8, Cyano, phenyl, amide, etc.

By the literature survey, the number of runs was taken on L1, 15 cm and 10 cm columns of different make having 4.6 mm diameter and 5  $\mu$ m, 3  $\mu$ m particle size. The Inertsil ODS 100  $\times$  4.6 mm, 3.0  $\mu$ m particle size column was selected for method development.

Finally, Method was optimized by making required changes in mobile phase gradient composition, flow rate, column oven temperature, standard and sample concentration. After several trials and gradient optimization, final parameters selected which gives better separation between Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol with good peak shapes and resolution between the three peaks.

Representative chromatograms of standard solution and sample solution are shown in **Fig. 4, 5a** and **5b** respectively.

To validate the method, parameters like specificity, Linearity, Precision, Accuracy, Robustness, Solution stability, Filter compatibility, and System suitability were performed as per ICH guideline.

**System Suitability:** The six replicate injections of Standard solution containing Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol were injected. The system suitability of analysis was confirmed by calculating Mean of area response, standard Deviation, and % Relative standard deviation (% RSD).

System suitability data is shown in **Table 1**.

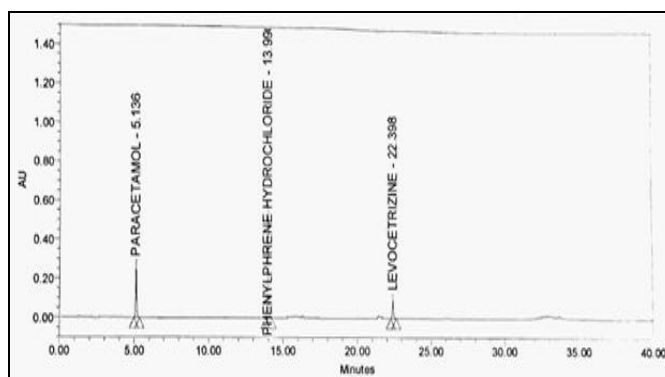


FIG. 4: CHROMATOGRAM – STANDARD SOLUTION

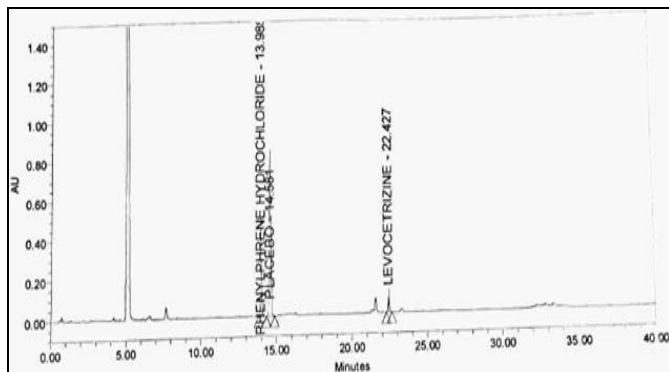


FIG. 5A: CHROMATOGRAM – SAMPLE SOLUTION FOR LEVOCETIRIZINE DIHYDROCHLORIDE AND PHENYLEPHRINE HYDROCHLORIDE

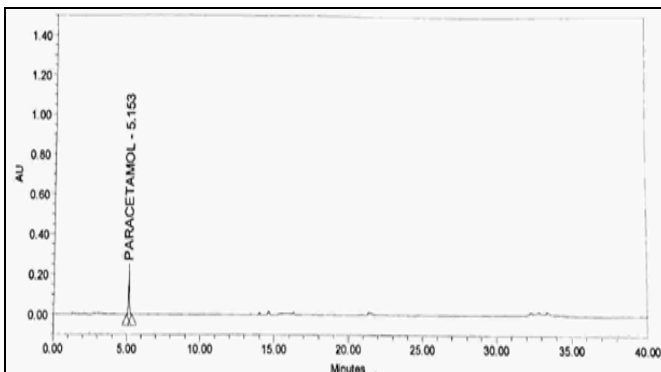


FIG. 5B: CHROMATOGRAM – SAMPLE SOLUTION FOR PARACETAMOL

TABLE 1: SYSTEM SUITABILITY DATA

S. no.	Standard Replicates	Area Response		
		Levocetirizine dihydrochloride	Phenylephrinehydrochloride	Paracetamol
1	Standard-1	489115	2700433	1275059
2	Standard-2	486614	2699533	1275785
3	Standard-3	486102	2694742	1272776
4	Standard-4	486718	2693780	1272190
5	Standard-5	488034	2699645	1275110
6	Standard-6	488471	2697383	1275180
	Mean	487509	2697586	1274350
	Standard deviation	1198.56	2783.92	1481.43
	% RSD	0.2	0.1	0.1

**Specificity:** Interference from diluents and placebo was checked at the retention time of Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol peaks. There is no any interference observed at the retention time of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol from the either of diluents and placebo. For identification purpose, the Retention time of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol peaks in sample solution matches the retention time of peaks in the standard solution. This result shows that the method is specific enough for the determination of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol without any interference.

**Forced Degradation Study:** Forced degradation study was performed under acid hydrolysis, base hydrolysis, and peroxide degradation conditions. The degradation sample solutions of Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol were prepared (having concentration about 25 µg/mL, 100 µg/mL and 50 µg/mL respectively). For acid hydrolysis samples are treated using 10 mL of 0.1N HCl, heated on a water bath at 80 °C for 30 min, cooled and neutralized the solution with 0.1N NaOH. For base hydrolysis samples are treated using 10 mL of 0.1N NaOH, heated on a water bath at 80 °C for 30 min, cooled and neutralized the solution with 0.1N HCl. In peroxide degradation of sample, added 30% H<sub>2</sub>O<sub>2</sub> solution and heated on a water bath at 80 °C for 30

min. The peak purity of all the three components Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol are passes. From this study, it was demonstrated that the developed analytical HPLC method can separate the degradant from analyte peaks.

**Linearity:** Linearity was performed by preparing solutions of different concentrations in the range of

50% to 150% level for Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol obtained from graph was 1.00, 1.00, and 0.999 respectively. The Linearity plot contains concentration in ppm on x-axis and peak area response obtained on the y-axis. The linearity graph and correlation coefficient are as below. On x-axis concentration in µg/mL and y-axis peak response.

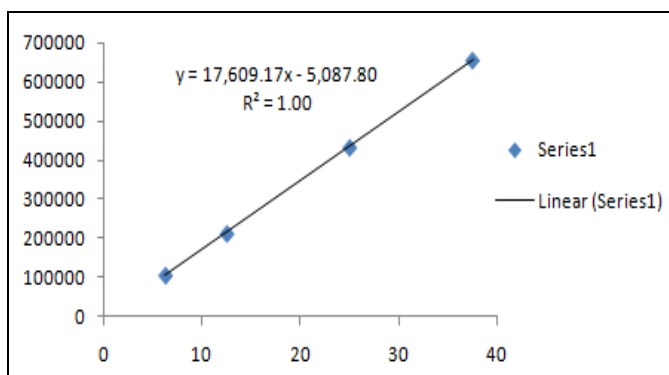


FIG. 6: LINEARITY GRAPH- LEVOCETIRIZINE DIHYDROCHLORIDE

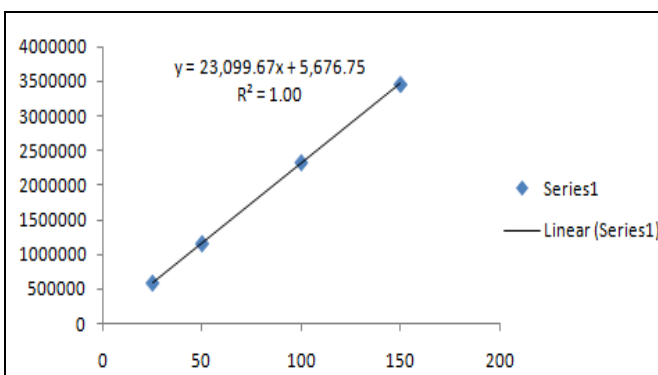


FIG. 7: LINEARITY GRAPH-PHENYLEPHRINE HYDROCHLORIDE

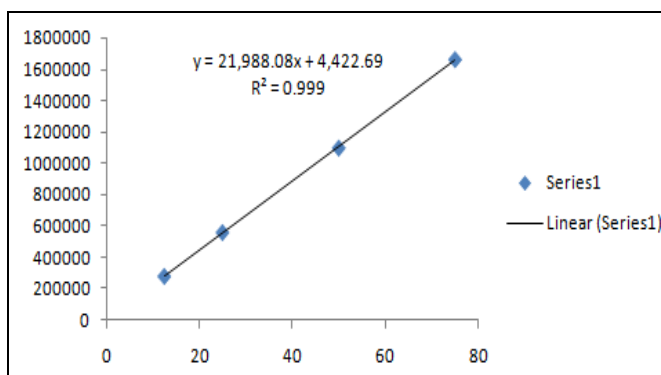


FIG. 8: LINEARITY GRAPH- PARACETAMOL

**Precision/Repeatability:** For Precision prepared six different samples of the same concentration and calculated for the content of Levocetirizine dihydrochloride, Phenylephrine hydrochloride, and Paracetamol. Relative Standard Deviation (RSD) and the content of all components show method is repeatable.

**Accuracy (Recovery):** Accuracy of the method was performed by recovery study by spiking standards into placebo and calculated the added amount and recovered the amount. Recovery values for all three components were between 95 to 105%. Recovery results are shown in **Table 2**.

TABLE 2: RECOVERY DATA

Recovery Level (%)	% Recovery		
	Levocetirizine dihydrochloride	Phenylephrine hydrochloride	Paracetamol
50%	101.5	100.4	102.8
100%	102.7	100.5	101.7
150%	104.1	100.8	101.8

**Robustness:** Robustness study confirmed that the method is sensitive towards pH. The slight change in pH from pH 3.45, the impurity-G and impurity-C of Levocetirizine dihydrochloride were getting

merged with Levocetirizine dihydrochloride main peak. The pH of the mobile phase should be adjusted to pH of 3.45 ± 0.05.

**Filter Compatibility:** Filter compatibility of the method was confirmed by comparing the centrifuged sample with filtered samples with discarding 5mL of filtrate. The observed difference between the centrifuged sample and filtered samples through Millipore PVDF, Whatman PVDF

having 0.45  $\mu\text{m}$  membrane filter was not more than 2.0%. It was observed that Millipore PVDF and Whatman PVDF filter paper does not adsorb the compound of interest during filtration of sample solutions. Observations of filter study are reported in **Table 3**.

**TABLE 3: FILTER STUDY OBSERVATIONS**

Filter used	Levocetirizine dihydrochloride		Phenylephrine hydrochloride		Paracetamol	
	% Assay	% Diff.	% Assay	% Diff.	% Assay	% Diff.
Centrifuge	101.9	-	99.2	-	101.2	-
Millipore PVDF	101.0	0.8	99.3	0.1	101.5	0.3
Nylon	102.2	0.3	95.8	3.4	100.7	0.5
Whatman PVDF	103.0	1.1	100.2	1.0	101.4	0.2
Acrodisc	102.7	0.8	99.7	0.5	99.2	2.0

**Solution Stability:** Solution Stability was performed by comparing the results of the freshly prepared sample solution and stored sample solution at room temperature for 24 h. It was observed that the solution was stable for 24 h.

**CONCLUSION:** The present paper describes the simple and accurate method for simultaneous determination of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol content from pharmaceutical samples using reverse phase HPLC. There is no method reported for this combination of products. The proposed method is very simple, accurate and precise. Hence, this validated method can be adopted for routine analysis of Levocetirizine dihydrochloride, Phenylephrine hydrochloride and Paracetamol content from pharmaceuticals.

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**CONFLICT OF INTEREST:** Nil

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