

## Research Article



## Development and Validation of Stability-Indicating RP-HPLC Method for the Analysis of Levocetirizine Dihydrochloride and Fexofenadine Hydrochloride in the Presence of Parabens in Liquid Dosage Forms

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### ABSTRACT

The study describes the development and validation of stability-indicating RP-HPLC method for the analysis of Levocetirizine 2HCl and its preservatives Methylparaben and Propylparaben in the Levocetirizine 2HCl oral solutions and for the analysis of Fexofenadine HCl and its preservatives Methylparaben and Propylparaben in the Fexofenadine HCl oral suspensions. Effective chromatographic separation was achieved using C18 column (150 mm, 4.6mm i.d., 5µm) with mobile phase consisting of phosphate buffer pH 6.0 and Acetonitrile (68:32) at a flow rate of 1.0 mL/min. The column temperature was maintained at 35 °C and the detection wavelength was 230 nm. Quantification was based on measuring the peak areas. Analytical performance of the proposed HPLC procedure was statistically validated with respect to system suitability, linearity, ranges, precision, accuracy, specificity, robustness, detection and quantification limits. The linearity ranges for Levocetirizine 2HCl, Fexofenadine HCl, Methylparaben and Propylparaben were 5–40, 6–48, 10-80 and 2–16 µg/mL respectively with correlation coefficients >0.9995. The four analytes were subjected to stress conditions of acidic and alkaline hydrolysis, oxidation, photolysis and thermal degradation. The proposed method proved to be stability-indicating by resolution of the analytes from their forced-degradation products. The method is very practical for routine quality control of Levocetirizine 2HCl and Fexofenadine HCl oral preparations as well as in stability studies.

**Keywords:** Levocetirizine, Fexofenadine, Methylparaben, Propylparaben, stability-indicating, RP-HPLC method, Forced degradation.

### INTRODUCTION

Levocetirizine (as Levocetirizine dihydrochloride), 2-[2-[4-[(R)-(4-chlorophenyl)-phenyl-methyl]piperazin-1yl]ethoxy] acetic acid dihydrochloride (Figure 1), is a second generation H1 antihistamines marketed for the treatment of perennial and seasonal allergic rhinitis and chronic idiopathic urticaria. It is the most active enantiomer of cetirizine and has a favorable pharmacokinetic profile.

Levocetirizine is rapidly and extensively absorbed, minimally metabolized and has a volume of distribution (Vd) which is lower than other compounds from the same group.<sup>1-2</sup>

Literature shows that Levocetirizine can be estimated by different analytical methods including HPLC<sup>3</sup>, HPTLC<sup>4-5</sup>, Chiral HPLC<sup>6</sup>, Spectrophotometric<sup>7-8</sup>, and Ratio derivative method.<sup>9</sup>

Fexofenadine hydrochloride, (2-[4-[(1RS)-1-hydroxy-4-[4-(hydroxydiphenylmethyl)piperidin-1-yl]butyl]phenyl]-2-methylpropanoic acid hydrochloride) (Figure 1), is an antihistamine pharmaceutical drug used in the treatment of hay fever, allergy symptoms, and urticaria.<sup>10</sup>

It was developed as a successor of, and alternative to terfenadine, an antihistamine that caused QT interval prolongation, potentially leading to cardiac arrhythmia. Fexofenadine, like other second- and third-generation antihistamines, does not readily cross the blood-brain barrier, and so causes less drowsiness than first-

generation histamine-receptor antagonists. It works by being an antagonist to the H1 receptor.<sup>11</sup>

The review of literature shows that several methods are available for the determination of Fexofenadine hydrochloride in dosage forms including spectrophotometry<sup>12-13</sup>, spectrofluorometry<sup>14</sup>, and RP-HPLC.<sup>15</sup>

Liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredients. Such preparations are protected by the addition of preservatives that prevent the alteration and degradation of the product formulation.

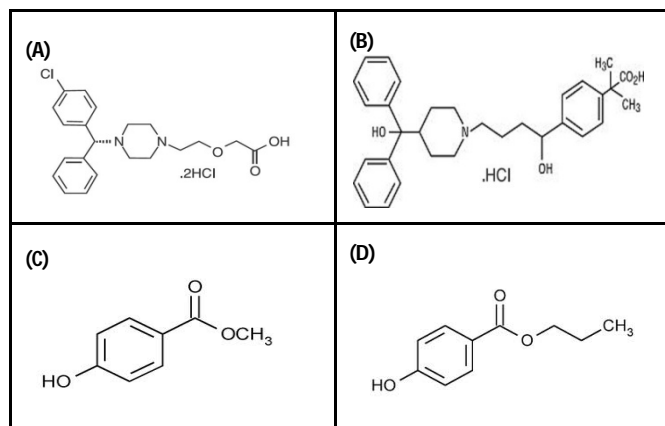
Methylparaben and Propylparaben (Figure 1) are used as either single or in combinations in drug products as antimicrobial preservatives to prevent alteration of product preparations. Methylparaben is the methyl ester of p-hydroxybenzoic acid and propylparaben is the propyl ester of p-hydroxybenzoic acid.

Based on the guidelines of International Conference on Harmonization (ICH), the requirement of establishment of a stability-indicating assay method has become more clearly mandatory. The guidelines require conducting of forced decomposition studies under a variety of conditions, like pH, light, oxidation, heat and others followed by separation of drug from degradation products.<sup>16</sup> Because of the requirement of separation of multiple components during the analysis of stability samples, HPLC has gained popularity in stability studies



due to its high-resolution capacity, sensitivity and specificity.

This study focused on the chromatographic behavior of Levocetirizine 2HCl, Fexofenadine HCl, Methylparaben and Propylparaben and their degradation products in order to attain the required method that separates all four analytes as well as their perspective degradation products in oral liquid dosage forms.



**Figure 1:** Chemical structures of (A) Levocetirizine dihydrochloride, (B) Fexofenadine hydrochloride, (C) Methylparaben and (D) Propylparaben.

## MATERIALS AND METHODS

### Chemicals and solutions

Levocetirizine dihydrochloride was obtained from Auctus Pharma. Fexofenadine hydrochloride was obtained from Ranbaxy Laboratories. Methylparaben was obtained from Salicylates and Chemicals. Propylparaben was obtained from Clariant Production. Acetonitrile and Methanol used were of HPLC grade. All other reagents used in this study were of AR grade. Purified water was used for making the solutions.

### Chromatographic conditions

Separations were performed with a HPLC (LA Chrom ELITE, VWR Hitachi, Germany, equipped with L-2130 pump, L-2200 auto sampler, L-2300 column oven, and UV photo diode array detector L-2455). The out-put signal was monitored and processed using EZ Chrom ELITE software.

The chromatographic column used was Thermo Hypersil C18 column (150 mm, 4.6mm i.d., 5 $\mu$ m). The mobile phase comprised of mixture of Potassium dihydrogen phosphate buffer (pH 6.0; 0.01 M) and Acetonitrile (68:32, v/v). The pH of the Potassium dihydrogen phosphate solution was adjusted to 6.0 with potassium hydroxide solution (10% w/v).

The mobile phase was filtered through 0.45 micron membrane filter, degassed in ultrasonic bath and pumped from the respective solvent reservoir to the column at a flow rate of 1 mL/min. The column temperature was maintained at 35°C and the detection wavelength was 230 nm. The injection volume was 50  $\mu$ L. The column was

equilibrated for 60 min prior to the injection of the drug solution.

### Preparation of standard stock solutions

Levocetirizine 2HCl (500  $\mu$ g/mL) stock solution was prepared in the purified water. Fexofenadine HCl (600  $\mu$ g/mL), Methylparaben (1000  $\mu$ g/mL) and Propylparaben (200  $\mu$ g/mL) stock solutions were prepared in HPLC-grade methanol.

### Preparation of the standard solution for the analysis of Levocetirizine 2HCl oral solution

1 mL of the Levocetirizine 2HCl stock solution, 1 mL of the Methylparaben stock solution and 1 mL of the Propylparaben stock solution were transferred in a 25 mL volumetric flask and diluted with the mobile phase to 25 mL. The concentrations obtained were (20  $\mu$ g/mL), (40  $\mu$ g/mL) and (8  $\mu$ g/mL) for Levocetirizine 2HCl, Methylparaben and Propylparaben respectively. The standard solution was filtered using a 0.45  $\mu$ m nylon syringe filter.

### Preparation of the standard solution for the analysis of Fexofenadine HCl oral suspension

1 mL of the Fexofenadine HCl stock solution, 1 mL of the Methylparaben stock solution and 1 mL of the Propylparaben stock solution were transferred in a 25 mL volumetric flask and diluted with the mobile phase to 25 mL. The concentrations obtained were (24  $\mu$ g/mL), (40  $\mu$ g/mL) and (8  $\mu$ g/mL) for Fexofenadine HCl, Methylparaben and Propylparaben respectively. The standard solution was filtered using a 0.45  $\mu$ m nylon syringe filter.

### Preparation of the sample solution for the analysis of Levocetirizine 2HCl oral solution

The commercially available Levocetirizine 2HCl oral solution contains 0.5 mg/mL Levocetirizine 2HCl. From this solution, 1 mL was carefully transferred in a 25 mL volumetric flask containing about 10 mL of the mobile phase. The solution was mixed and diluted to volume with the same diluent. The sample solution was filtered using a 0.45  $\mu$ m nylon syringe filter. The concentration obtained was (20  $\mu$ g/mL) of Levocetirizine 2HCl.

### Preparation of the sample solution for the analysis of Fexofenadine HCl oral suspension

The commercially available Fexofenadine HCl oral suspension contains 6 mg/mL Fexofenadine HCl. From this well shaken suspension, 5 mL was carefully transferred in a 50 mL volumetric flask containing about 20 mL of HPLC-grade methanol, sonicated for about 10 minutes, diluted to volume with the same diluents and filtered. From this filtrate, 1 mL was carefully transferred in a 25 mL volumetric flask containing about 10 mL of the mobile phase. Then the solution was mixed and diluted to volume with the mobile phase. The sample solution was filtered using a 0.45  $\mu$ m nylon syringe filter. The

concentration obtained was (24 µg/mL) of Fexofenadine HCl.

### Method validation

Validation was done with respect to various parameters, as required under ICH guidelines.<sup>17,18,19</sup> Analytical performance of the proposed HPLC procedure was statistically validated with respect to system suitability, linearity, ranges, precision, accuracy, specificity, robustness, detection and quantification limits.

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. System suitability tests are an integral part of method development and are performed to evaluate the behavior of the chromatographic system. Plate number (N), tailing factor (T), resolution (R) and RSD were evaluated for five replicate injections of the standard solutions.

The linearity of the method was confirmed using standard solutions at different concentrations of analytes within the ranges of 5–40, 6–48, 10–80 and 2–16 µg/mL for Levocetirizine 2HCl, Fexofenadine HCl, Methylparaben and Propylparaben respectively. 50 µL of each solution was injected into the HPLC system and the peak area of the chromatogram obtained was noted.

The intra-day and inter-day precision of the assay method was evaluated at three concentration levels (25%, 100%, and 200%; n=3) for each analyte and the RSD% of three obtained assay values on three different days was calculated.

Accuracy was expressed as percentage recovery [(amount found / amount applied) × 100]. For the determination of recovery (%), pre-analyzed samples were spiked with 50%, 100% and 150% of each analyte labeled concentration. For each level, three determinations were performed.

In addition, the specificity was evaluated by analyzing solutions containing the excipients employed for the preparation of Levocetirizine 2HCl oral solutions and Fexofenadine HCl oral suspensions.

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included mobile phase composition, mobile phase pH, flow rate, column temperature, wavelength, and injection volume. Robustness of the method was studied using six replicates at a concentration level of 100% of each analyte.

Ratios of 3:1 and 10:1 signal-to-noise were considered acceptable for estimation of the detection limit and quantification limit, respectively.

### Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.

### Forced degradation studies for the analysis of Levocetirizine 2HCl oral solution

**Stock solution (x):** 10 mL of the Levocetirizine 2HCl stock solution, 10 mL of the Methylparaben stock solution and 10 mL of the Propylparaben stock solution were transferred in a 100 mL volumetric flask and diluted with the mobile phase to 100mL.

**Acidic hydrolysis:** 2 mL of 2 M HCl was added to 10 mL of stock solution (x) and was kept at 70 °C for about 3 hours in water bath. Then the solution was allowed to attend ambient temperature and was neutralized by 2 mL of 2 M NaOH and volume was made up to 25 mL with the mobile phase.

**Alkaline hydrolysis:** 2 mL of 2 M NaOH was added to 10 mL of stock solution (x) and was kept at 70 °C for about 3 hours in water bath. Then the solution was allowed to attend ambient temperature and was neutralized by 2 mL of 2 M HCl and volume was made up to 25 mL with the mobile phase.

**Oxidation:** 3 mL of 3 % H<sub>2</sub>O<sub>2</sub> was added to 10 mL of stock solution (x) and was kept at 70 °C for about 3 hours in water bath. Then the solution was allowed to attend ambient temperature and volume was made up to 25 mL with the mobile phase.

**Thermal Degradation:** 10 mL of stock solution (x) was kept at 80 °C for 3 hours in water bath. Then the solution was allowed to attend ambient temperature and volume was made up to 25 mL with the mobile phase.

**Photolytic degradation:** 10 mL of stock solution (x) was subjected to UV irradiation at 370 nm for 4 hours. After the specified time, the solution was allowed to attend ambient temperature and volume was made up to 25 mL with the mobile phase.

### Forced degradation studies for the analysis of Fexofenadine HCl oral suspension

**Stock solution (y):** 10 mL of the Fexofenadine HCl stock solution, 10 mL of the Methylparaben stock solution and 10 mL of the Propylparaben stock solution were transferred in a 100 mL volumetric flask and diluted with the mobile phase to 100 mL.

**Acidic hydrolysis:** 2 mL of 2 M HCl was added to 10 mL of stock solution (y) and was kept at 70 °C for about 3 hours in water bath. Then the solution was allowed to attend ambient temperature and was neutralized by 2 mL of 2 M NaOH and volume was made up to 25 mL with the mobile phase.

**Alkaline hydrolysis:** 2 mL of 2 M NaOH was added to 10 mL of stock solution (y) and was kept at 70 °C for about 3 hours in water bath. Then the solution was allowed to attend ambient temperature and was neutralized by 2 mL of 2 M HCl and volume was made up to 25 mL with the mobile phase.

**Oxidation:** 1 mL of 3 % H<sub>2</sub>O<sub>2</sub> was added to 10 mL of stock solution (y) and was kept at 70 °C for about 3 hours in

water bath. Then the solution was allowed to attend ambient temperature and volume was made up to 25 mL with the mobile phase.

**Thermal Degradation:** 10 mL of stock solution (y) was kept at 80°C for 3 hours in water bath. Then the solution was allowed to attend ambient temperature and volume was made up to 25 mL with the mobile phase.

**Photolytic degradation:** 10 mL of stock solution (y) was subjected to UV irradiation at 370 nm for 4 hours. After the specified time, the solution was allowed to attend ambient temperature and volume was made up to 25 mL with the mobile phase.

All treated solutions were filtered with a 0.45 µm nylon syringe filter and injected in stabilized chromatographic conditions.

## RESULTS AND DISCUSSION

### Method validation

The correlation coefficients ( $R^2 > 0.9995$ ) of the calibration plots indicate good linearity. The developed method was found to be precise as the RSD% values for intra-day and inter-day were found to be less than 2%. Recovery varied from (99.13% to 101.21%) of the analytes at each added concentration, indicating that the method was accurate.

The drugs peaks were well separated in the presence of the excipients (Figure 2) reflecting specificity of the method.

Robustness was examined by evaluating the influence of small variations in different conditions such as mobile phase composition ( $\pm 2\%$ ), mobile phase pH ( $\pm 0.2$ ), flow

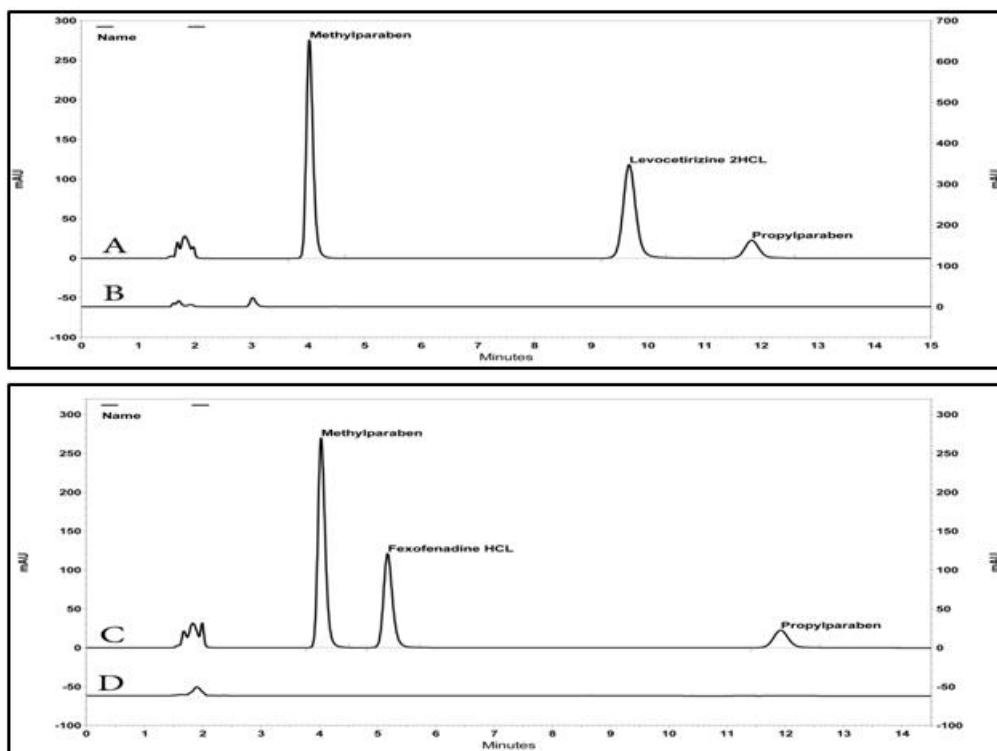
rate ( $\pm 0.1$  mL/min), column temperature ( $\pm 5^\circ\text{C}$ ), wavelength ( $\pm 2$  nm), and injection volume ( $\pm 5$  µL). These variations did not have any significant effect on the measured responses or the chromatographic resolution. RSD% for the measured peaks areas using these variations did not exceed 1%. The summary of validation parameters of proposed method is shown in (Table 1). Calibration curves of the analytes are shown in (Figure 3).

### Forced degradation results:

The stress degradation studies showed that the most significant degradation happened under alkaline hydrolysis and oxidative stress conditions. The decomposition products were resolved from the intact drugs under all stress conditions of acidic and alkaline hydrolysis, oxidation, photolysis and thermal conditions. The peak purity spectrum of each analyte was recorded using PDA detector. Peak purity results were greater than 0.990. That indicates that the peaks are homogeneous in all stress conditions tested and thus establishing the specificity and confirming the stability indicating power of the assay method. Typical chromatograms obtained following the assay of stressed samples are shown in (Figures 4 and 5) and the summary of forced degradation studies is shown in (Table 2).

### Analysis of commercial formulations

The validated method was applied for the analysis of local formulations of Levocetirizine 2HCl oral solution and Fexofenadine HCl oral suspension that contain Methylparaben and Propylparaben as preservatives. Results were summarized in (Table 3).



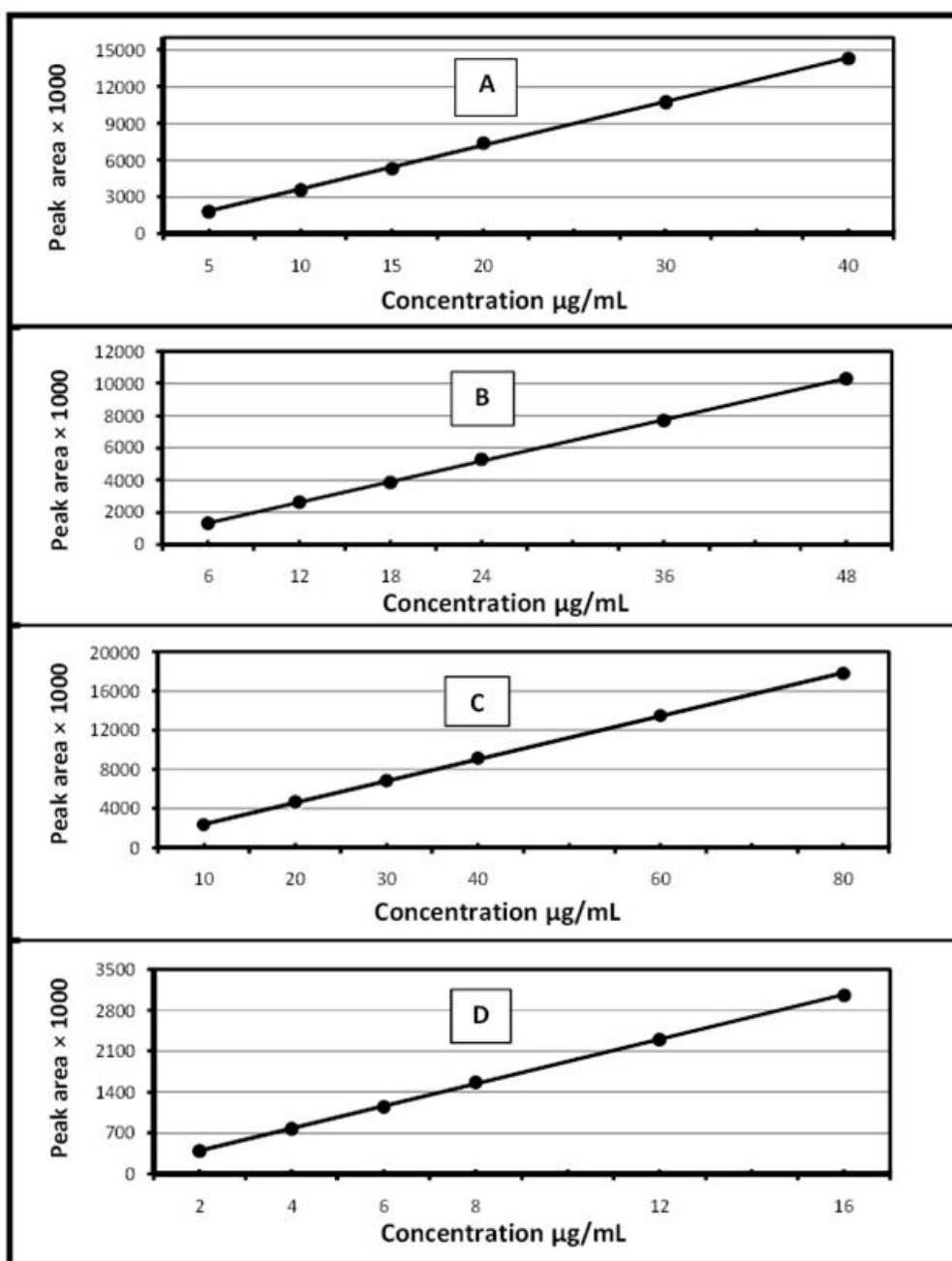
**Figure 2:** Representative chromatograms of (A) Standard solution for the analysis of Levocetirizine 2HCl oral solution, (B) Excipients solution for the analysis of Levocetirizine 2HCl oral solution, (C) Standard solution for the analysis of Fexofenadine HCl oral suspension and (D) Excipients solution for the analysis of Fexofenadine HCl oral suspension.



**Table 1:** Summary of validation parameters

Parameter		Methylparaben	Fexofenadine HCl	Levocetirizine 2HCl	Propylparaben
<b>System Suitability</b>	RSD% of Area	0.167	0.140	0.445	0.584
	RSD% of R.T	0.345	0.746	0.605	0.695
	Tailing factor (T)	1.20	1.14	1.04	1.01
	Resolution (R)	-	5.95	20.98	3.98 <sup>A</sup> -18.89 <sup>B</sup>
	Theoretical plate (N)	6132	6832	10476	11638
<b>Accuracy</b>	mean recovery %	100.75	99.13	101.08	101.21
<b>Precision</b>	intra-day, RSD %	0.98	0.55	1.20	1.09
	inter-day, RSD %	1.22	0.71	1.15	0.94
<b>Linearity</b>	Range	10-80 µg/mL	6–48 µg/mL	5–40 µg/mL	2–16 µg/mL
	Equation	$y = 2208x + 204$	$y = 1282x + 65$	$y = 1785x + 52$	$y = 381x + 16$
	R <sup>2</sup>	0.99986	0.99970	0.99951	R <sup>2</sup> = 0.99978
<b>Sensitivity</b>	Detection limit (ng/mL)	6.0	9.0	6.8	12.0
	Quantification limit (ng/mL)	20.0	30.0	22.7	40.0

<sup>A</sup>Resolution value in the analysis of Levocetirizine 2HCl oral solution; <sup>B</sup>Resolution value in the analysis of Fexofenadine HCl oral suspension.



**Figure 3:** Calibration curves of (A) Levocetirizine dihydrochloride, (B) Fexofenadine hydrochloride, (C) Methylparaben and (D) Propylparaben.

**Table 2:** Summary of forced degradation studies

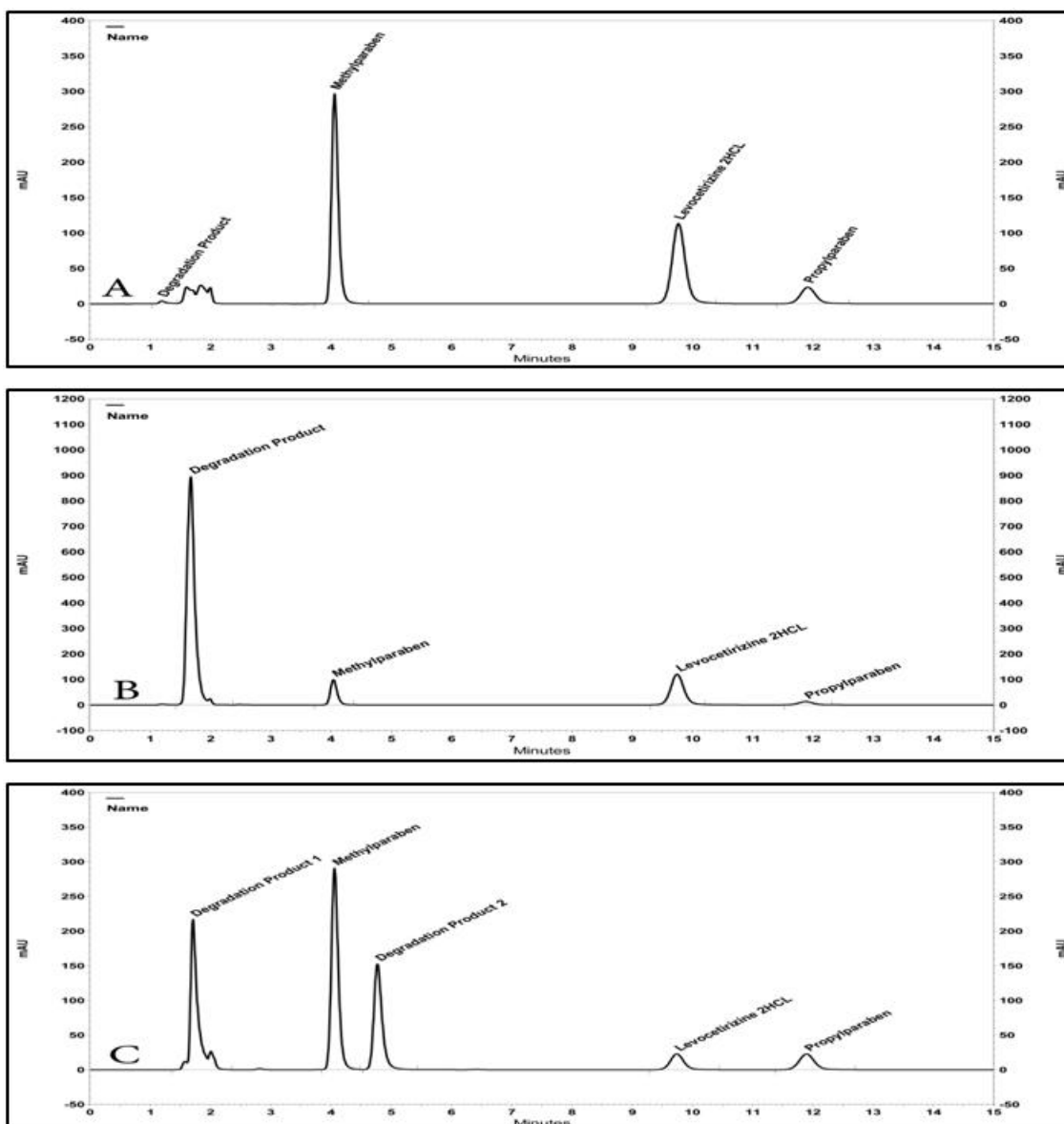
Stress conditions	<sup>A</sup> Recovery (%)			
	Levocetirizine 2HCl	Fexofenadine HCl	Methylparaben	Propylparaben
Acidic hydrolysis	94.12	98.50	100.51	100.95
Alkaline hydrolysis	99.55	100.29	33.59	55.89
Oxidative degradation	19.18	45.17	99.87	99.44
Thermal degradation	99.02	99.83	99.25	99.29
Photolytic degradation	99.21	99.45	100.07	99.87

<sup>A</sup>Mean of three replicates.

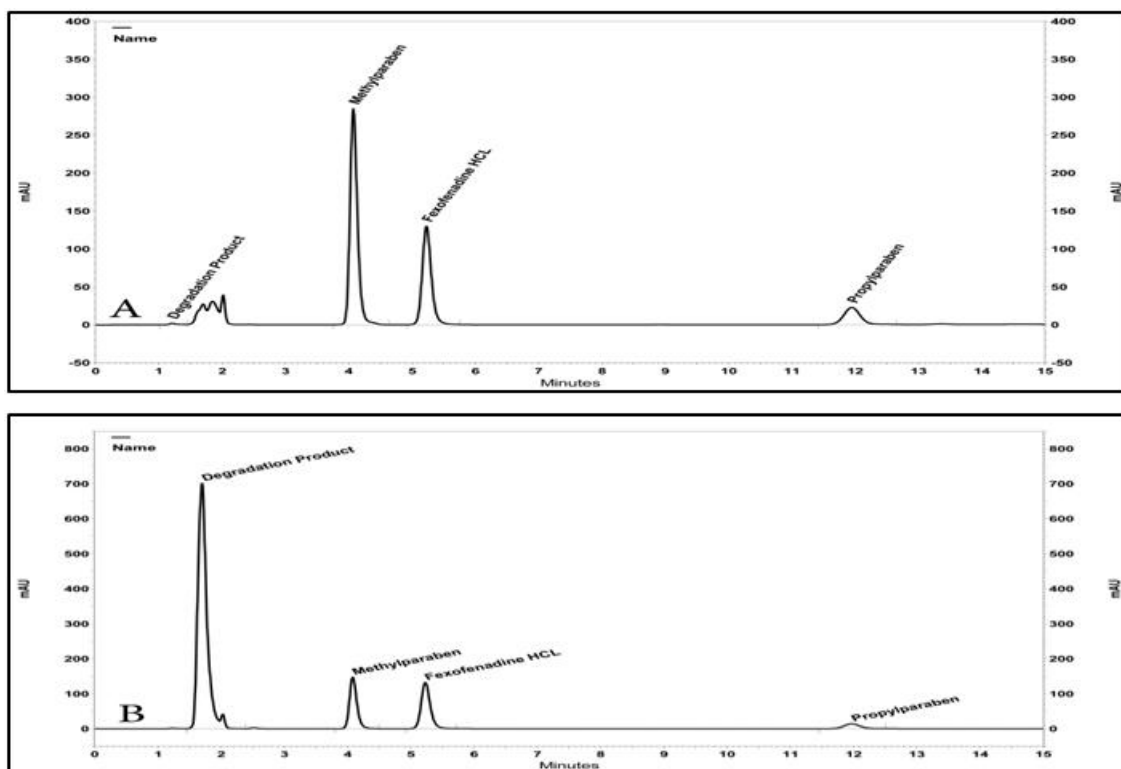
**Table 3:** Analysis of commercial formulations

Levocetirizine 2HCl oral solution			Fexofenadine HCl oral suspension		
Recovery (%) <sup>A</sup>			Recovery (%) <sup>A</sup>		
Levocetirizine 2HCl	Methylparaben	Propylparaben	Fexofenadine HCl	Methylparaben	Propylparaben
101.42	99.36	98.81	102.80	101.39	98.19

<sup>A</sup>Mean of three replicates.



**Figure 4:** Representative chromatograms of (A) Levocetirizine 2HCl oral solution sample degraded in acidic conditions, (B) Levocetirizine 2HCl oral solution sample degraded in alkaline conditions and (C) Levocetirizine 2HCl oral solution sample degraded in oxidative stress conditions.



**Figure 5:** Representative chromatograms of (A) Fexofenadine HCl oral suspension sample degraded in acidic conditions and (B) Fexofenadine HCl oral suspension sample degraded in alkaline conditions.

## CONCLUSION

This study describes a simple, cost effective stability-indicating assay method that showed good accuracy, precision, sensitivity, and robustness making it a valid choice for quality control laboratories to use in the assessment of Levocetirizine 2HCl and Fexofenadine HCl in the presence of parabens in typical liquid oral dosage forms.

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