Annals of Phytomedicine 12(1): 616-627, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.80

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Analysis of second-generation anti histamine fexofenadine soft gelatin capsules and its related compound by using RP-HPLC

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Article Info

Article history
Received 14 April 2023
Revised 29 May 2023
Accepted 30 May 2023
Published Online 30 June-2023

Keywords
Fexofenadine HCl
Keto fexofenadine
Capsules
Rapid high-performance liquid Chromatographic
Validation Parameters

Abstract

A simple, novel, sensitive, fast high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the quantitative determination of fexofenadine HCl and its related (compound A) in bulk and formulations. The drug was degraded by oxidation, acidity, basicity, neutrality, photolysis, and heat. In a total analytical run time, all degradation products were eluted. The chromatographic development was carried out on RP-HPLC. The column used as zorbax SB phenyl, L11, 250 mm x 4.6 mm x 5 µm column or equivalent with mobile phase consisting of acetonitrile 700 ml, 300 ml of buffer and 6 ml of triethylamine. The particles were seen at 220 nm and the flow rate was 1.5 ml/min. The retention time was found to be 8.415 min for fexofenadine and its related (compound A) is 13.961 min. In terms of linearity, accuracy, precision, and robustness, the technique was verified in accordance with the ICH Q2 R1 guidelines. With a regression value of 0.999, the calibration curve was discovered to be linear throughout a range of 45.2867-135.8601 µg/ml. The technique has demonstrated good specificity and sensitivity. The results of the research demonstrated the utility of the suggested RP-HPLC technique for the routine detection of fexofenadine in bulk medication and in its pharmaceutical formulation.

1. Introduction

On a chemical basis, fexofenadine hydrochloride is (±)4-[1-hydroxy-4-[4-(hydroxyl diphenylmethyl)-1-piperidinyl] butyl] bezeneacetic acid.-α,α-Dimethyl-hydrochloride.

This H1 antagonist is a selective, periphery-focused the second-generation durable H1 receptor antagonist (Nimje *et al.,* 2012). It is a terfenadine active enzyme and, like terfenadine, binds to H1 receptor areas on inflammatory cells in the digestive system, arteries, bronchi, and the breathing system to interact with histamine (Ravisankar *et al.,* 2014). Fexofenadine appears to have a low blood-brain barrier penetration rate, which has an adverse impact on its ability to sedative. The beneficial antihistaminic properties of fexofenadine, the active acid metabolite of terfenadine, remain while the parent molecule's detrimental arrhythmogenic actions are completely absent (Chandran *et al.,* 2007).

The FDA is currently investigating it as a potential treatment for seasonal allergic rhinitis. It is utilized to treat the seasonally rhinitis caused by allergies sensations (often referred to as "hay fever") in adults (Radhakrishna *et al.,* 2002). These runny nose, coughing, and red, itchy, or irritated eyes are signs of symptoms, as well as inflammation of the mouth's roof, throat, or nostrils (Maher *et al.,* 2011). It is a hydroxyl acid metabolite of the histamine H1 receptor antagonist terfenadine. Fexofenadine hydrochloride is a crystalline powder that ranges from white in colour to white (Tokumura *et al.,* 2016). In neither methanol nor ethanol, it is easily soluble. Not frequently soluble in water and chloroform, and insoluble in hexane. There are not many LC methods that have been documented for fexofenadine determination (Arayne *et al.,* 2011).

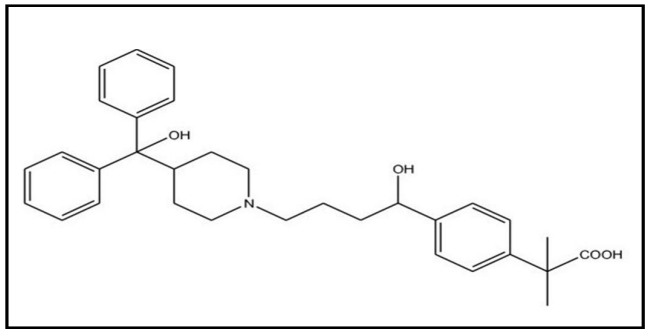

Figure 1: Chemical structure of fexofenadine.

Also characterized are two distinct LC methods (Araujo *et al.,* 2009). In the US pharmacopoeia previews of sites, one of which is used to separate and determine related (compound-B meta-isomer) using a pricey beta-silica column subjected to cyclodextrin USP L45, and the other to determine both fexofenadine and its related (compound A) using a phenyl bonded column USP L11 (Chikanbanjar *et al.,* 2020). This project's objective was to develop an analytical LC procedure that would be a rapid and accurate way to measure both fexofenadine and its impurities. Using fexofenadine as an internal standard, the contaminants were effectively, isolated from the drug in the proposed LC technique and eluted before the 18-min run time (Sanam *et al.,* 2018).

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The current research offers a validated RP-HPLC method that is straight forward, sensitive, economical, and proved to correspond to ICH Q2 R1 criteria for fexofenadine force degradation and evaluation of fexofenadine (Raghubabu *et al.,* 2014). There are several methods for estimating fexofenadine using different mixtures using HPLC that have been reported. It was also shown that the retention period for separation was extended (Pinto *et al.,* 2020). A few RP-HPLC techniques have also been created employing a gradient method with a prolonged run time (Pradeep *et al.,* 2022). Contradictorily; however, a number of the confirmed procedures were created by fusing polar and non-polar mobile phases (Sivakumar *et al.,* 2022). Certain validated procedures have been examined in an acidic buffer in light of the fact that fexofenadine has been identified as an acidic substance (Tandulwadkar *et al.,* 2012).

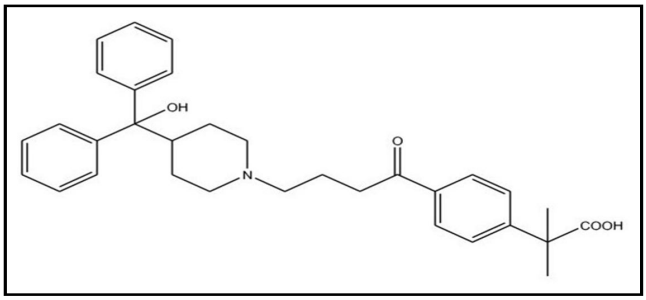

Figure 2: Chemical structure of keto fexofenadine.

The recommended verified approach is better, practical, accurate, and focused for quantifying fexofenadine in pharmaceuticals (Baira venkatesham *et al*., 2021). This method uses the isocratic method to alter pH up to 2.0 (the basic buffer of acetate), which might be helpful to reduce the usage of surplus excess chemicals (Hofmann *et al*., 2002). Additionally, to produce the ideal chromatogram, here, it was prevented to mix polar and non-polar ingredients. With a flow rate of 1.5 ml/min, we used a zorbax SB phenyl, L11, 250 mm x 4.6 mm x 5 µm column (or equivalent) to perfectly separate the compounds and degradation products (Kiran *et al*., 2019).

2. Materials and Methods

2.1 Chemicals and reagents

Monobasic sodiumphosphate-analytical research grade, sodium per chlorate-analytical research grade, orthophosphoric acid-analytical research grade, acetonitrile-analytical research grade, triethylamine-analytical research grade, working standard of Fexofenadine and fexofenadine related compound A (keto fexofenadine) from Soft Gel Healthcare PVT. Ltd. Using the filter of 0.45 µm nylon syringe filter 0.45 µm PVDF syringe filter.

2.2 Instruments and apparatus required

With a pH range of 1-14, the highest pressure is 44 MPa/440 bar (up to 5 ml/min). A standard inclusion is an injection volume of up to 5 ml. Usually, this takes between 4,000 and 8,000 h. The great majority of detectors used in (U) HPLC are techniques which concentrate on absorbing ultraviolet (UV) radiation and visible (Vis) the spectral wavelength ranges. These devices are frequently referred to as UV-Vis or UV/Vis. The UV region is where most research on organic analytical substances is conducted between 190 and 350 nm.

2.3 Instrumentation and chromatographic conditions

HPLC with UV/PDA detector-based examination was done on zorbax SB phenyl, L11, and 250 mm x 4.6 mm x 5 mm columns or equivalent reversed phase column. The mobile phase consisting of mix 700 ml of acetonitrile, 1300 ml of buffer and 6 ml of triethylamine. The flow rate of mobile phase was 1.5 ml/min. An examination carried out by at 220 nm. Injection volume 20 µl and the run time is 35 min. All analyses were performed under isocratic conditions at a temperature of 25°C. Lab solution data analysis software was used for data collection and analysis. The relevant retention intervals for the fexofenadine and its related compound A the peaks are about 8.415 min and 13.961 min, respectively.

2.4 Dilute orthophosphoric acid preparation

Transfer 10 ml of concentrated orthophosphoric acid into a 50 ml volumetric flask. Added 30 ml of water mixed well dilute to volume with water.

2.5 Buffer preparation

Weigh accurately 13.28 g of sodium phosphate monobasic dihydrate and 1.68 g of sodium per chlorate in a 2000 ml beaker, add 2000 ml of water, mix and sonicate for 10 min to dissolve. Then adjust the pH to 2.0 ± 0.05 with dilute orthophosphoric acid filter through 0.45 µ membrane.

2.6 Preparation of mobile phase ml

Mix 700 ml of acetonitrile, 1300 ml of buffer and 6 ml of triethylamine in a 2000 ml beaker, mix well, sonicate for 10 min.

2.7 Diluent

Mix acetonitrile and buffer in the ratio of 1:1 Filter and degas.

2.8 Preparation of standard

2.8.1 Solution A (0.45 mg/ml of fexofenadine hydrochloride)

In a 100 ml volumetric flask, correctly weigh 45.0 mg of fexofenadine hydrochloride WS/ RS, add 30 ml of mobile phase, and ultrasound for five min to dissolve. Then use mobile phase to dilute to volume.

2.8.2 Solution B (0.05 mg/ml of fexofenadine related compound A)

In a 50 ml volumetric flask, correctly weigh 2.5 mg of the fexofenadine related compound A WS/RS, add 20 ml of mobile phase, and ultrasound for 5 min to dissolve. Then use mobile phase to dilute to volume.

2.8.3 Standard solution (0.09 mg/ml of fexofenadine hydrochloride and 0.005 mg/ml of fexofanadine related compound A)

Transfer and pour with a pipette in a 50 ml volumetric flask, combine 10 ml of solution A and 5 ml of solution B. Add 20 ml of mobile phase, and then dilute to volume with mobile phase.

2.9 Preparation of sample

2.9.1 Sample stock solution (1.8 mg/ml of fexofenadine hydrochloride)

10 whole capsules should be added to a 1000 ml volumetric flask together with 300 ml of diluent, stirred by hand for 2 min, and then

dissolved using the sonicator for 15 min. Cool it down and add diluent to increase its volume.

2.9.2 Sample solution (0.05 mg/ml of fexofenadine hydrochloride)

Dilute 5.0 ml of sample stock solution to 100 ml with mobile phase.

2.10 Placebo preparation

2.10.1 Placebo stock solution

In a 1000 ml volumetric flask, add 300 ml of diluent, 10 whole placebo capsules, stir by hand for 2 min, and then sonicate for 15 min to dissolve. Dilute with diluent to volume once cooled to room temperature.

2.10.2 Placebo solution

To make 100 ml, add mobile phase to 5.0 ml of the placebo stock solution.

3. Results

3.1 Assay validation

The suggested RP-HPLC technique was validated for a number of parameters in accordance with the ICH Q2 R1 requirements.

3.2 Method validation

According to ICH Q2 R1 requirements, the HPLC process was verified in terms of precision, accuracy, and linearity. Final experimental settings were purposefully changed, and the findings were scrutinised to evaluate the robustness of the procedure.

3.3 Validation parameters

3.3.1 System suitability

Standard solution of fexofenadine hydrochloride is six duplicates were produced and injected into HPLC System. Estimated suitability of the system parameters as shown in Table 1.

3.3.2 Specificity

The method's specificity determines its capacity to quantify the substance occurs when a matrix's various components are present. Show the specificity by interference with the peak analyte concentration and maximum analyte concentration with a blank and a placebo. To establish specificity, the following concentrations were produced and loaded into an HPLC system. Blank placebo standard solution sample solution. Blank and placebo peak should not conflict with one other with main fexofenadine and keto fexofenadine. The principal peak's retention time in the sample solution should match that of the standard solution. The retention time for standard (fexofenadine and keto fexofenadine) and sample is 8.4 min, 13.9 min, and 8.4 min**.** The peak purity index for both fexofenadine and ketofexofenadine is 1.0 not less than 0.99. The chromotagrams as listed below.

Table 1: System suitability studies for fexofenadine and keto fexofenadine

Parameter	Fexofenadine	Keto fexofenadine
Retention time	8.3 min	13.9 min
Average area	2304435 mAU	94914 mAU
SD	1143	144
% RSD	0.0	0.1
Tailing factor	1.1	1.0
Theoretical plate	7701	8373
% recovery	99.6%	99.0%

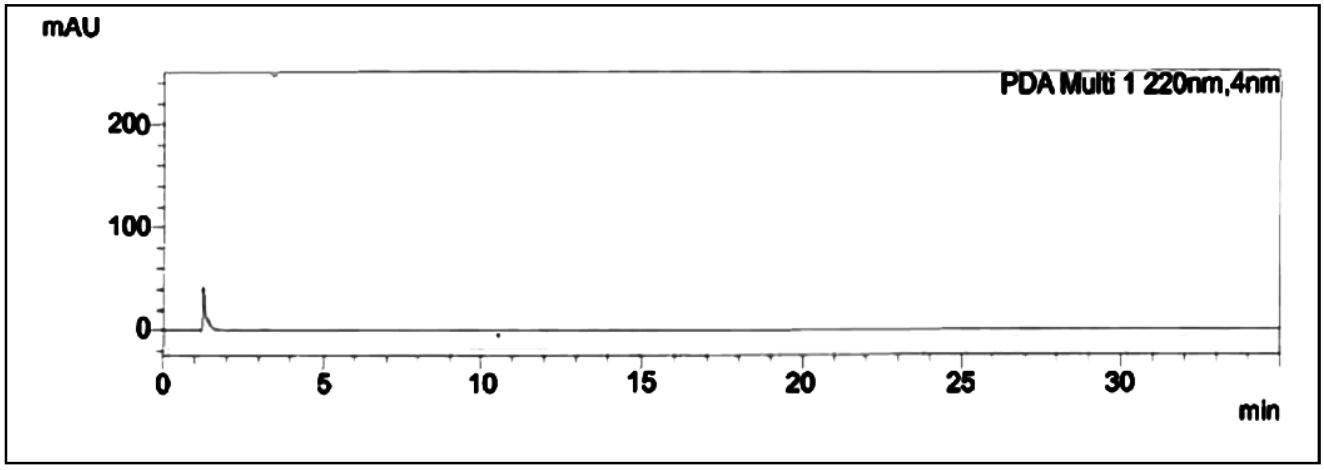

Figure 3: Chromatogram of blank.

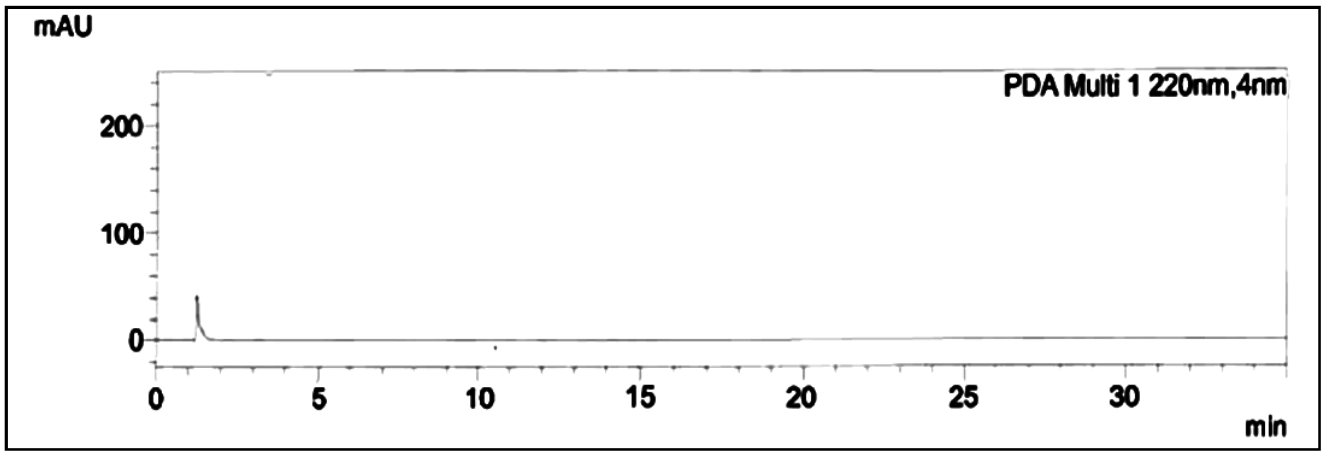

Figure 4: Chromatogram of placebo.

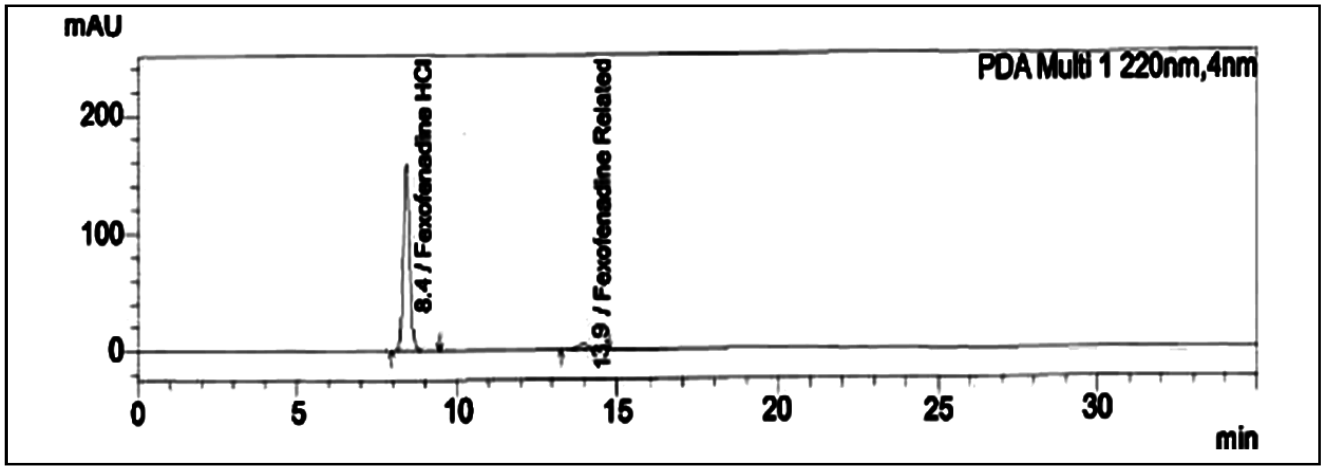

Figure 5: Chromatogram of standard.

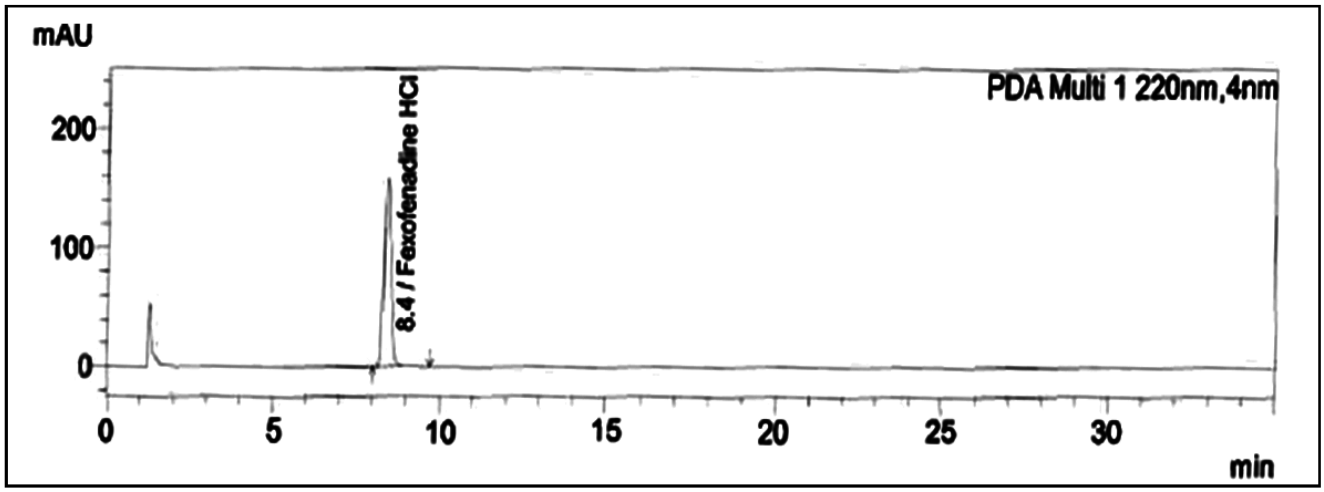

Figure 6: Chromatogram of sample.

3.3.3 Forced degradation study

Typically, for forced degradation investigations, the following stress conditions are employed; however, depending on the predicted level of degradation, stress conditions, kind of exposure, exposure length, strength of the acid/base, and temperature can be specified and optimised. In any one or both conditions of the upcoming stress study, a minimum of 10% or more degradation should be achieved. If necessary, a research under vigorous conditions could be employed to produce the desired degradation. As shown in Table 2 and Table 3.

3.3.4 Acid

Fexofenadine standard used to reflux the 0.1 N HCl at exposure time of 60 min at 60°C reflux for acid degradation, and then it was neutralized with 0.1 N NaOH. After adding 100 ml of neutrazling solution, the solution was further degranted.

3.3.5 Basic

Fexofenadine standard was refluxed with 0.1 N NaOH at exposure time of 60 min at 60°C reflux for basic degradation, and then it was neutralised with 0.1 N HCl. 100 ml of a further degradant and 100 ml of a neutrazling solution were added to the solution.

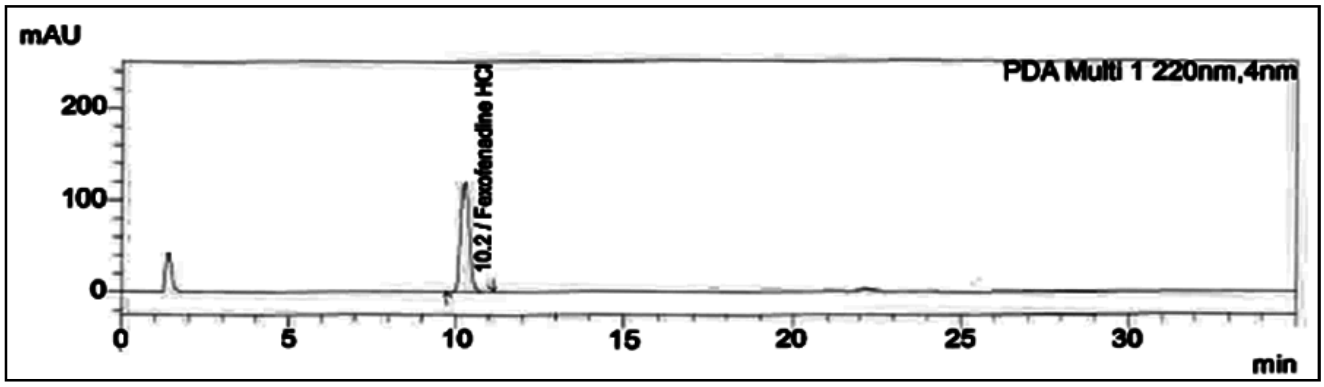

Figure 7: Chromatogram of forced degradation acid stressed sample.

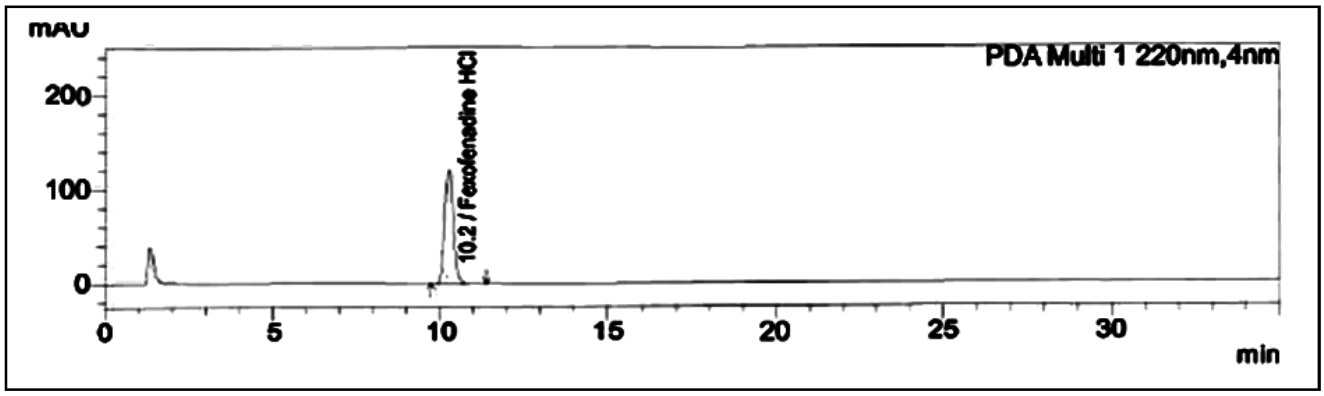

Figure 8: Chromatogram of forced degradation base stressed sample.

3.3.6 Photolytic, thermal, water and humidity degradation

The following solution is exposure to 200 watts per hr per square meter in a photo stability chamber for photolytic degradation of UV. The solution was exposed for 1.2 million lux hr before being analysed for photo stability degradation of light. After being exposed to 105°C for 24 h, fexofenadine's thermal degradation was examined. Fexofenadine standard was refluxed with water for water degradation, and then 100 ml of degradation was added to the solution for its exposure length of 60 min at 60°C. Then analysed following this time. The fexofenadine solution was refluxed with 90% RH and stored at 25°C for 7 days in a desiccator for the investigation on humidity degradation.

3.3.7 Oxidative

Fexofenadine standard was refluxed with 1% H_2O_2 exposure period for 60 min at the bench top for oxidative degradation, and then 100 ml of the required degradant was added.

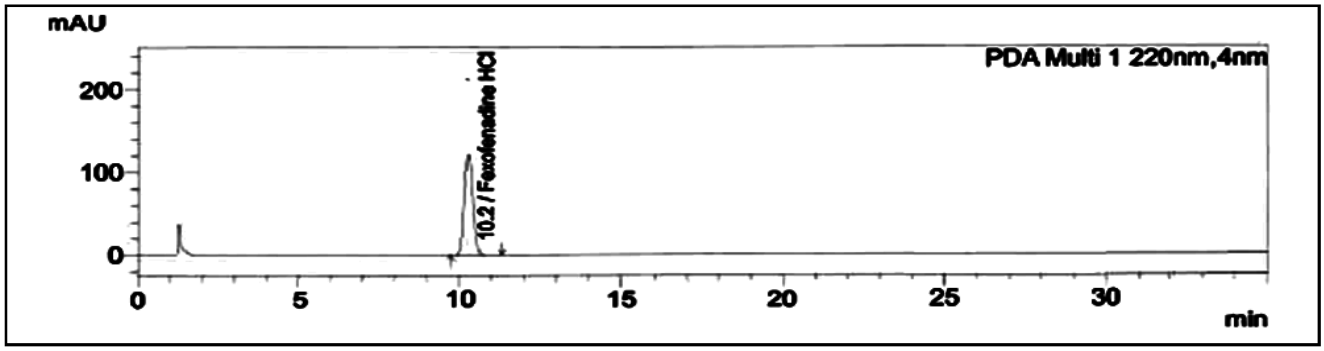

Figure 9: Chromatogram of forced degradation thermal stressed sample.

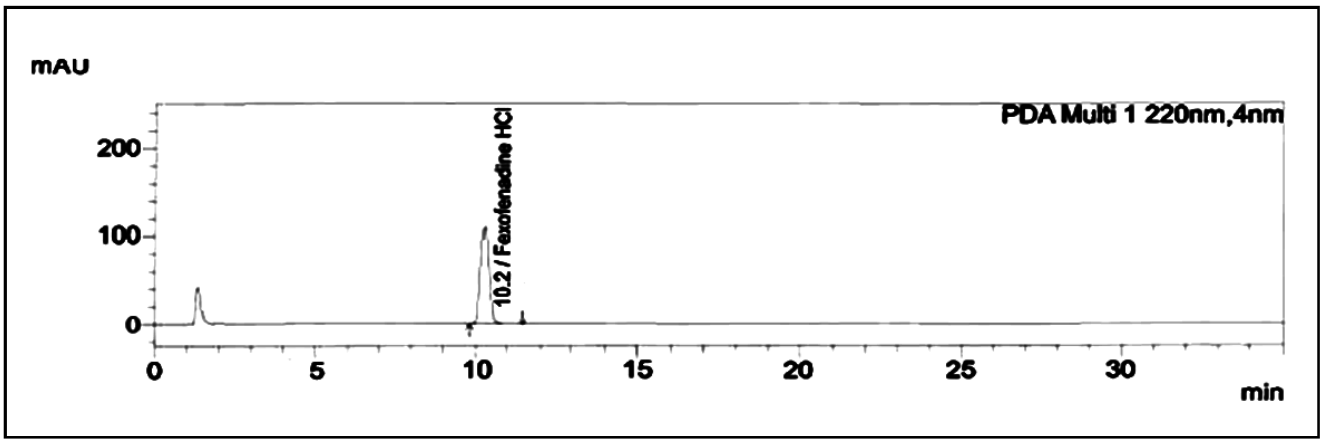

Figure 10: Chromatogram of forced degradation water stressed sample.

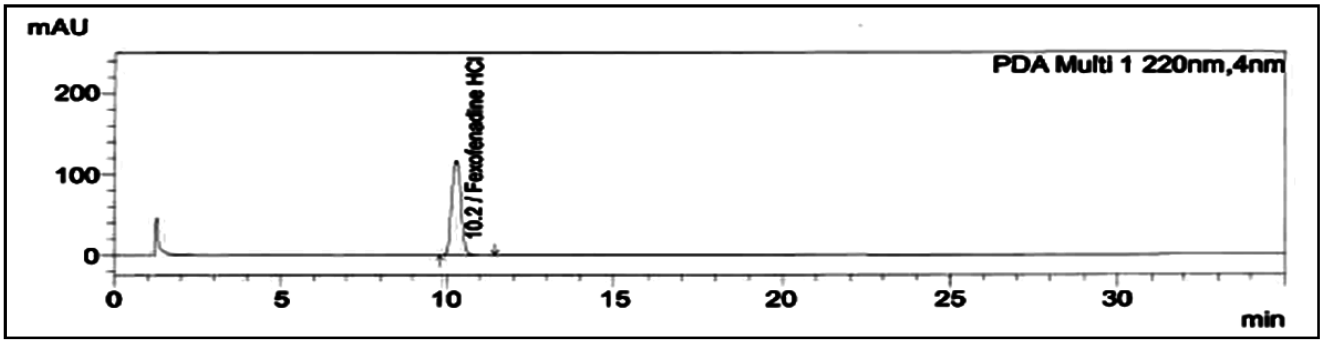

Figure 11: Chromatogram of forced degradation UV sample.

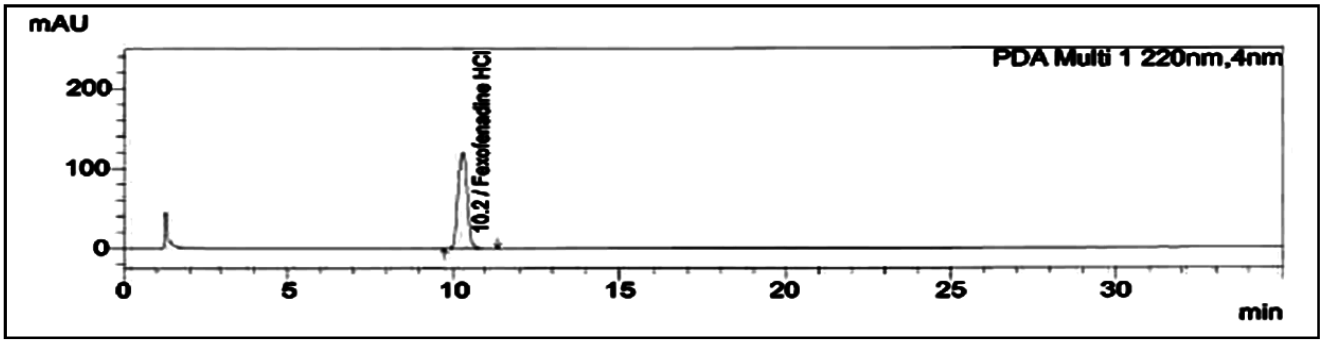

Figure 12: Chromatogram of Forced Degradation Light sample.

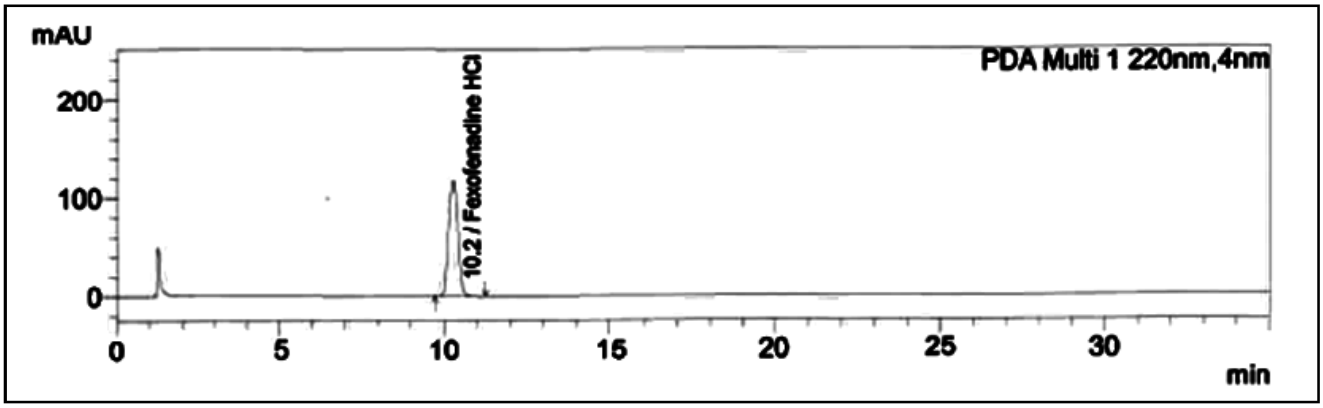

Figure 13: Chromatogram of forced degradation humidity sample.

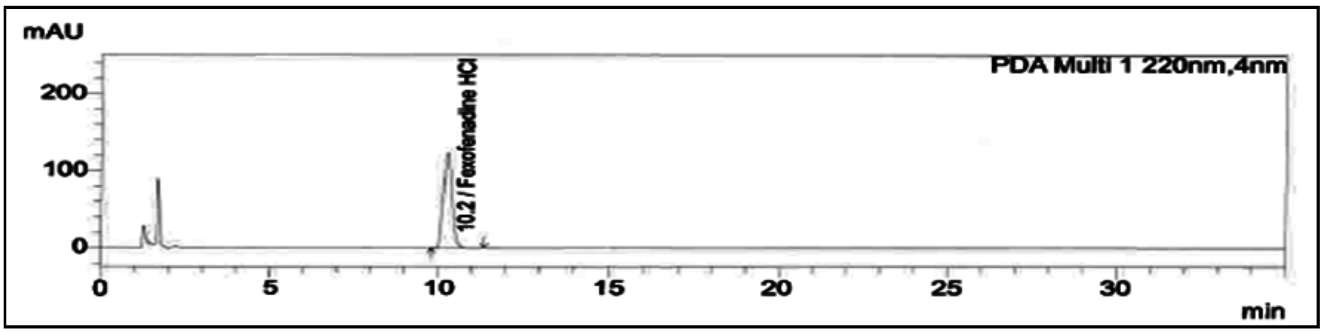

Figure 14: Chromatogram of forced degradation oxidation sample.

Table 2: Content of fexofenadine and its degradation

Stressed conditions	Content in % of fexofenadine	Fexofeandine HCl % of degradation
Unstressed condition	100.6	NA
Acid hydrolysis	94.3	6.3
Base hydrolysis	95.7	4.9
Oxidation	99.7	0.9
Thermal	98.7	1.9
Water	95.3	5.3
UV	98.5	2.1
Light	100.5	0.1
Humidity	100.8	-0.2

Table 3: Forced degradation study

No. of injection	Fexofenadine Rt in min	Keto fexofenadine Rt in min	Area of fexofenadine mAU	Area of keto fexofenadine mAU
1	10.2	17.6	2225922	89039
2	10.3	17.6	2226590	88893
3	10.2	17.6	2225977	88586
4	10.2	17.5	2226742	88793
5	10.2	17.5	2226048	88671
6	10.2	17.5	2226227	89004
Average	10.2	17.6	2226251	88831
SD	0.0	0.0	341	181
% RSD	0.1	0.1	0.0	0.2

3.3.8 Linearity and range

From 50% to 150% of the working concentrations of substances, there are six test solutions, are used to determine the linearity of the procedure. In relation to the working concentration of 100%, the standard solutions were prepared at concentrations of 50%, 80%, 100%, 120%, and 150%. The remaining concentrations of 80%, 100%, and 120% were injected into the HPLC system in three replicate injections for 50% and 150%, respectively, A graph between Area and concentration is drawn according to the median area at each concentration. As shown in Table 4.

Table 4: Linearity studies for fexofenadine

Conc in %	Average area mAU	Conc in ppm µg/ml
50	1227353	45.2867
80	1969093	72.4587
100	2444816	90.5734
120	2940711	108.6881
150	3663857	135.8601

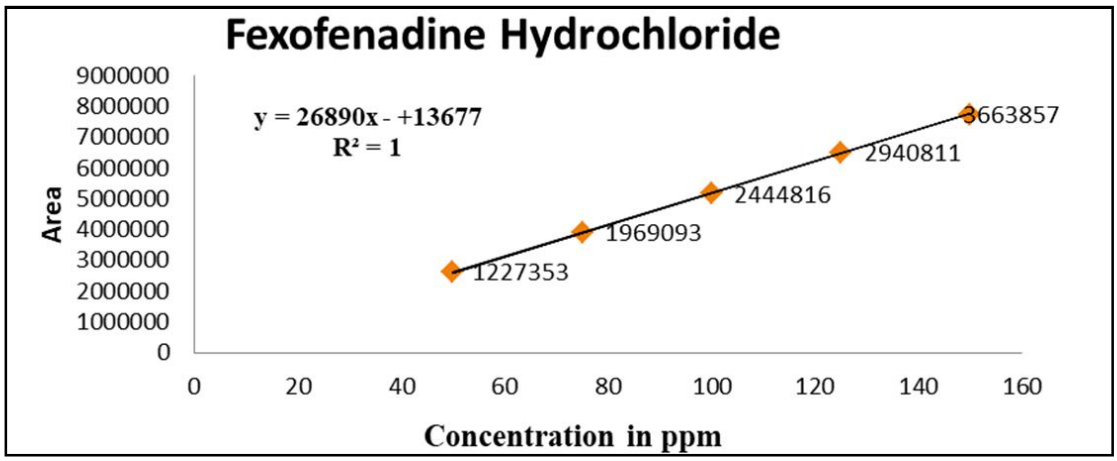

Figure 15: Fexofenadine linearity.

Table 5: Accuracy studies for fexofenadine

Sample	Area mAU	Amountadded (ppm)	Amountrecovered (ppm)	% Recovery	% Average
50%	1124137	45.0497	44.4486	98.6656	98.6
	1124041	45.0796	44.4448	98.5918	
	1123382	45.1145	44.4187	98.4577	
100 %	2306411	89.8499	91.1960	101.4981	101.3
	2301479	89.9597	91.0010	101.1575	
	2304565	89.9996	91.1230	101.2482	
150 %	3419516	134.7449	135.2084	100.3439	100.2
	3417386	134.9545	135.1242	100.1257	
	3419932	135.0194	135.2248	100.1521	

Table 6: Precision studies for fexofenadine

No. of injection	Retention time in min		Area in mAU	
	Fexofenadine	Keto fexofenadine	Fexofenadine mAU	Ketofexofenadine mAU
1	8.3	13.9	2303092	95062
2	8.3	13.9	2304912	94767
3	8.3	13.9	2303145	94793
4	8.3	13.9	2305287	95116
5	8.4	13.9	2305881	94902
6	8.4	13.9	2304293	94841
Average	8.3	13.9	2304435	94914
SD	0.0516	0.0	1143.0	144.0
% RSD	0.6	0.0	0.0	0.1

3.3.9 Accuracy

Accuracy is a level to which test findings acquired using a procedure are near to the actual value. Accuracy is frequently expressed as a percentage recovery by the presence of known, additional analyte concentrations. The degree of exactness in an analytical process is measured by accuracy. Utilizing three concentrations 50%, 100%, and 150% accuracy was evaluated. The concentrations of the standard and spiked sample solutions are created at 50%, 100%, and 150%. The area acquired for each concentration is used to compute the percentage of recovery. As shown in Table 5.

3.3.10 Precision

3.3.11 System precision

Six duplicates of the standard solution of fexofenadine hydrochloride will be injected to determine the system's precision. 2 replicates of

fexofenadine hydrochloride check standard solution and Calculate % RSD of peak area and RT, % recovery and resolution. As shown in Table 6 and Table 7.

3.3.12 Method precision and intermediate precision:

Through performing 6 replicate examinations, the method's accuracy will be proved. Fexofenadine hydrochloride capsules 180 mg, soft, sample preparations for the fexofenadine hydrochloride content.

Analysis of the same batch of fexofenadine hydrochloride capsules 180 mg, soft as in precision using six duplicate samples, a different facility, a different analyst, a different equipment, and a different column on a separate day indicates intermediate precision (reproducibility).

3.3.13 Robustness

The reliability of the analytical method for the 180 mg of fexofenadine hydrochloride included in the capsules, soft is demonstrated with quiet but intentional modifications in pH variation, flow rate and mobile phase composition. The outcomes meet the standards for approval. In consideration of this, it can be said that the procedure is robust with regard to tiny deviations. In flow rate, pH variation mobile phase composition. As shown in Table 8.

3.3.14 Filter validation

This filter validation was shown by filtering the centrifuging the sample solution to acquire the final concentration of the sample without filtering, and then filter through 0.45 µm nylon and through 0.45 µm PVDF**.** Validation of the analytical method using filters will be performed check for homogeneity preparing a sample (without filtration and with filtration) solution. The test findings % RSD will be determined. By centrifuging the sample preparation and testing it without filtration, the filter validation will be given, filtering through 0.45 µm nylon and 0.45 µm PVDF. As shown in Table 9.

3.3.15 Stability of analytical solution and mobile phase stability

By introducing standard and sample solutions at various intervals from the time of injection while using the same mobile phase, stability of the mobile phase, standard, and sample solutions will be shown. Solutions must be kept at room temperature and administered intravenously every 12 h for up to 48 h. The area obtained at various solution time intervals and mobile phase days will be used to determine the solution's stability. Report the hr up to which the solution is judged to be stable and stop the test if the findings do not fulfill the acceptance requirements within the time frame given. For mobile phase stability, the mobile phase must be physically checked for haziness; if haziness or an odour is found, the study must be stopped the test the solution and record the number of hr it remains steady. The solution stability has been shown by injecting the reference and test solution for up to 48 hr. Below is a calculation of the percentage RSD of the areas of the standard and sample solutions. Mobile phase stability also demonstrated by checking visually for clarity and odour of the solution from initial to 48 h. As shown in Table 10.

Table 7: Method precision and intermediate precision studies for fexofenadine

Preparation	Method precision in %	Intermediate precision in %
1	99.8968	99.8417
2	99.1890	99.8131
3	97.8081	99.8390
4	99.3271	99.8865
5	97.6802	99.8155
6	99.5153	99.8847
Average	98.9	99.8
SD	0.9293	0.0323
% RSD	0.9	0.0
Confidence limit	0.7	0.0

Table 8: Robustness studies for fexofenadine

Parameter	Parameter varied	Average content	% RSD
Flow rate ml/ min	1.3 ml/min	98.6	0.9
	1.5 ml/min	99.0	0.9
	1.7 ml/min	98.6	0.8
pH variation	1.8	98.7	1.2
	2.0	99.0	0.9
	2.2	98.9	1.1
Mobile phase variation	600 ml : 1400 ml	101.4	0.1
	700 ml: 1300 ml	99.0	0 9
	800 ml: 1200 ml	96.8	0.2

Table 9: Filter validation studies for fexofenadine

Parameter	Observation		% recovery	Limit
The average content of fexofenadine	Centrifuging the sample solution		97.6	93.0 to 105.0
	0.45 µm nylon	1 ml discarded	97.3	
		2 ml discarded	97.0	
		3 ml discarded	97.3	
	0.45 µm PVDF	1 ml discarded	97.4	
		2 ml discarded	97.4	
		3 ml discarded	97.4	
Absolute % difference of assay result between centrifuged and 2 different types of filtered sample solution	Centrifuging the sample solution		NA	NMT ≥ 2.0
	0.45 µm nylon	1 ml discarded	0.3	
		2 ml discarded	0.6	
		3 ml discarded	0.3	
	0.45 µm PVDF	1 ml discarded	0.3	
		2 ml discarded	0.2	
		3 ml discarded	0.2	

Table 10: Stability studies for fexofenadine

Time interval	Fexofenadine hydrochloride area mAU			Fexofenadine related compound A mAU		
	Standard	%RSD	Sample	%RSD	Standard	%RSD
Initial	2447202	NA	2390632	NA	94985	NA
12 th hr	2439630	0.2	2381429	0.2	96998	1.4
24thhr	2438314	0.1	2379832	0.2	96582	1.1
36th hr	2440341	0.1	2381457	0.2	98368	1.4
48thhr	2448115	0.1	2382654	0.1	96585	1.2
Average	2442720	NA	2383200	NA	96703	NA
SD	4577.5	NA	4273.4	NA	1208.2	NA

4. Discussion

The stability-indicating test technique, the HPLC processes were improved. Injections of pure drug and its degradation derivatives products were made, and several solvent systems were used. A preliminary screening needs to be done in order to identify significant levels of fexofenadine. Acetonitrile was shown to successfully separate the drug products with excellent resolution when used in amounts greater than 40%. Methanol, when utilized over 50%, provided a flawless peak and resolution in addition to working as an organic phase. However, in this case, methanol, an organic phase, can take the lead in after fifteen min, time for retention. As a result, acetonitrile at a 50% concentration was utilised as the ideal threshold for drug detection. At first, various ratios of methanol and water were examined. It was found that the degradants began to elute in dead volume as the mobile phase methanol concentration increased. As a result, the methanol content was lower and the resolution was better. When choosing a buffer, pH was crucial for how easily products would dissolve. According to their pKa value, buffers are most effective when they are close to their pH value. Additionally, a pH balance must be preserved because a higher pH can harm the column.

It was determined that a mobile phase with a buffer and acetonitrile (65:35, v/v) concentration, a pH adjustment to 2.0 with OPA, and a flow rate of 1.5 ml/min yielded a satisfactory retention time of 8.3 min of fexofenadine and 13.1 min of keto fexofenadine, theoretical plates, and good resolution of drug and degradation products. After several experimental trials. There were well separated identical peaks. When the reaction time of the effluent was measured at the optimal conditions. Performance studies were conducted using columns zorbax SB Phenyl, L11, 250 mm x 4.6 mm x 5 µm column or equivalent. When fexofenadine HCl UV detector response was examined, the optimal wavelength that showed the highest sensitivity was discovered to be 220 nm.

The parameters for method validation display the outcome of the placebo and blank solutions should not exhibit any peaks at the Fexofenadine hydrochloride retention time.

Standard and sample peak purity indices should both be greater than or equal to 0.99. The retention time of fexofenadine hydrochloride is

not interfered with by the peaks of the blank, placebo, or stressed sample solutions, according to the chromatograms of these solutions. Standard and sample solutions peak purity index values shouldn't be less than 0.99. With no interference from blank, placebo, or degradant peaks, it can therefore be said that the approach is specific to estimating the amount of fexofenadine hydrochloride.

Fexofenadine hydrochloride should have a correlation coefficient (r) of 0.999 is not less than 0.99 per cent and a y-intercept of 5.0 since the method is linear from 50% to 150% of the working concentration.

To evaluate the HPLC method's robustness, a number of parameters were systematically altered. The factors included the rate of flow variation, acetonitrile and buffer percentages in the mobile phase, and variations in pH. As a consequence, multiple injections (n=3) of the standard solution at three concentration levels were performed while six chromatographic parameters (factors) were gently altered. The findings show that minor changes in these parameters had little effect on the chosen variables. The parameters' findings showed that there is no difference that is significant. Additionally, it was discovered that acetonitrile from various lots produced by the same manufacturer had no appreciable impact on the outcome. Less variation in retention time and insignificant differences in peak areas were noted.

A benzene acetic acid group with a side chain of a carboxylic acid group that was hydrolyzed is what gives fexofenadine its unique properties. The original chemical and its significant degradation products were kept apart. When kept for 1 h at room temperature, the medication became unstable under simple stress conditions. The substance was degraded nearly to 95.7%. Additionally, it eliminated stability in acidic conditions after 1 hour at room temperature. The drug was degraded approximately to 94.3%. The medication was destroyed to about 99.7% when subjected to oxidative stress conditions with 1% H_2O_2 for 1 h at room temperature. The quantity of fexofenadine in the stock solution was quantified, and its stability was assessed by comparing it to a freshly produced standard. The stock solution reaction to the freshly created standard did not alter much.

The results obtained by centrifugation, 0.45 µm Nylon filter and 0.45 µm PVDF (with discarding volumes of 1 ml, 2 ml and 3 ml) are well within the results. Hence, it is recommended to use centrifugation/ Nylon filter/PVDF filter with discarding volume of 3 ml during regular analysis.

Mobile Phase visually observed for clarity and odour, found to be clear for Initial to 48 h and no irregular odour observed during the study. % cumulative RSD result for standard and sample solutions produced over various time intervals are found to be within the acceptance criteria. Hence, the solution stability and mobile phase stability are stable upto 48 h for both standard and sample at room temperature.

5. Conclusion

Keto fexofenadine (impurity A) and other compounds linked to fexofenadine HCl were analysed), has been designed and confirmed using an easy, precise, and accurate RP-LC conduct with a UV/PDA detector. It was not necessary to employ a chiral substance in the stationary phase or add chiral chemicals to the mobile phase, making the procedure exceedingly cost-effective. Mobile phase and a C_{18} RP-LC column were both employed, an inexpensive buffer, triethylamine, and acetonitrile was used as the organic modifier; these are available in every chromatography laboratory. According to ICH Q2 R1 criteria, the new examine was thoroughly evaluated. For determining the presence of fexofenadine and related substances in bulk powder and pharmaceutical capsules, the process provides an interference-free, easy, precise, and stability-indicating test, from the excipients and in the presence of water, humidity, acidic, basic, oxidative, thermal, and photolytic processes. The ability of the approach to indicate stability has been shown by the complete separation of all of the degradation products from the therapeutic components. Consequently, the technique developed is a stability-indicating assay that is applicable to a fexofenadine and its associated contaminants have undergone an extensive range of regular analyses in both bulk and pharmaceutical capsules, all without any interference.

The assay technique for estimating the content of fexofenadine hydrochloride in soft by HPLC method is suitable, specific, linear, accurate, precise, robust, and stable, according to the findings of analytical method validation. As a result, this approach may be used for routine analysis and is regarded verified.

Acknowledgements

The authors thank the management and staffs, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS). Soft Gel Health Care PVT, Pudhupakkam for providing us the raw material to carry out this work,

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **M. Vimal raj and M. Sumithra (2023). Analysis of second-generation anti histamine fexofenadine soft gelatin capsules and its related compound by using RP-HPLC. Ann. Phytomed., 12(1):616-627. http://dx.doi.org/10.54085/ap.2023.12.1.80.**