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DEVELOPMENT AND VALIDATION OF A PRECISE RP-HPLC METHOD FOR SIMULTANEOUS ANALYSIS OF FEXOFENADINE HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN COMBINED TABLET FORMULATION

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ABSTRACT

A precise RP-HPLC method was established and validated for the analysis of a combined tablet formulation containing Fexofenadine hydrochloride and Cetirizine hydrochloride. The study employed a SHIMADZU-1 SERIES LC-2030 system with a Phenomenex Luna C18 100A;1 stationary phase (50mm X 4.6mm). The mobile phase, consisting of 30% ACN, 20% methanol, and 50% pH 3.0 buffer, was held constant in an isocratic manner. The flow rate remained at 1.0 mL/min, and each injection volume was 20 microliters, with detection occurring at 230nm. The RP HPLC analysis yielded distinct separation of peaks corresponding to Fexofenadine hydrochloride and Cetirizine hydrochloride in just 10 minutes. Retention times were measured at 4.121 and 5.72S minutes for Fexofenadine hydrochloride and Cetirizine hydrochloride, respectively. Noteworthy was the high column efficiency, with Fexofenadine hydrochloride having 1,751,807 theoretical plates and Cetirizine hydrochloride having 303,378 theoretical plates, attesting to the column's strong performance. A resolution of 3.976 highlighted the clear distinction between the peaks. The quantitative analysis of the formulated tablet indicated robust method accuracy, showing Fexofenadine hydrochloride at 99.58% and Cetirizine hydrochloride at 99.62%. This method demonstrates its suitability for accurately and reliably determining the presence and quantity of these active pharmaceutical ingredients in the combined tablet dosage form

KEYWORDS: RP-HPLC, Fexofinadine hydrochloride, Cetirizine hydrochloride, Retention time, run time, Buffer, ACN, Methanol.

INTRODUCTION

Cetirizine hydrochloride is an antihistamine derivative [C21H25CIN2O3]. (±)-[2-[4-[(4-Chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid is the formula for chemicals with molar mass of 388.89gmol-1 and a melting point of 145–148°C. Solubility is very soluble in methanol and in ethanol 95% and freely soluble in water. of Cetirizine hydrochloride at is halflife 6.5 to 10hrs. The Cetirizine hydrochloride chemical structure is illustrated in Fig 1.[1]

Fexofenadine hydrochloride is an antihistamine derivative [C32H39NO4]. 2-[4-[1-hydroxy-4-[hydroxy-diphenyl-methyl) piperidin-1-yl] butyl] phenyl]-2-methylpropanoicacid; hydrochloride is the formula for chemicals with molar mass of 538.1gmol-1 and a melting point of 148–150°C. Solubility is freely soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane. Fexofenadine hydrochloride at is half-life 11 to 15hrs. The Fexofenadine hydrochloride chemical structure is illustrated in Fig 2.[2]

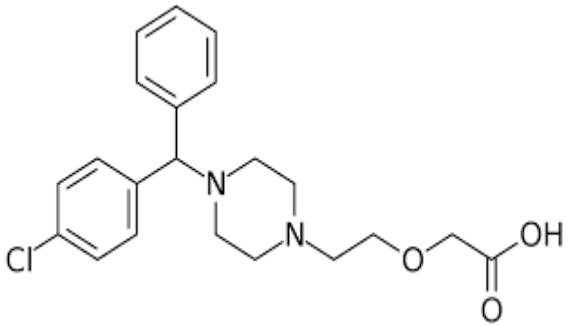

Figure 1: Chemical Structure of Cetirizine hydrochloride.

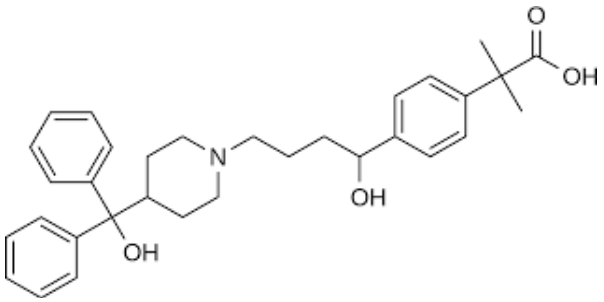

Figure 2: Chemical Structure of Fexofinadine hydrochloride.

Pharmacokinetic profile

Cetirizine is quickly absorbed in the digestive system and substantially excreted via the kidneys. After about an hour, cetirizine's plasma concentration reaches its peak. Usually, it starts working after 20 to 60 minutes, and benefits last for at least 24 hours.[3] Excretion: Cetirizine has an 8.3hour elimination half-life. The kidney is the main organ via which cetirizine is eliminated.[4] Administration: Tablets, capsules, solutions, and orally disintegrating tablets are among forms in which cetirizine is sold. Cetirizine dosage is based on the patient's age. Depending on the severity of the symptoms, an oral dose of 5 or 10 mg per day is advised for adults and kids aged 12 and older.[5] There are 5 mg and 10 mg tablets, a 5 mg/5 ml oral solution, and an elixir available. The ophthalmic formulation comes in 5 mL and 7.5 mL bottles of eye drops containing cetirizine hydrochloride, 0.24%. Depending on the severity of the symptoms, 5 or 10 mg (1 or 2 tablespoons) of syrup once day is advised for children aged 6 to 11.[6] Adverse effects: Cetirizine can be used to treat allergic rhinitis and urticaria and is generally well-tolerated and safe. Although they are rare, its main side effects in adults are sleepiness, lethargy, pharyngitis, vertigo, and dry mouth.[7]

Bioavailability: Fexofenadine hydrochloride, with a bioavailability range of 30% to 41%[8], exhibits rapid absorption following oral intake. In healthy male volunteers, a single dose of two 60 mg capsules led to a mean time to maximum plasma concentration of 2.6 hours post-dose. Administering a single 60 mg capsule to healthy subjects resulted in an average maximum plasma concentration of 131 ng/mL. Similarly, for healthy adult male volunteers, the mean maximum plasma concentrations after a single dose of either the 60 mg or 180 mg tablet were 142 ng/mL and 494 ng/mL, respectively. The tablet formulations show bioequivalence to the capsules when given at equivalent doses. The pharmacokinetics of fexofenadine hydrochloride exhibit linearity for oral doses up to a total daily amount of 240 mg (divided as 120 mg twice daily). The mean elimination half-life of fexofenadine was observed to be 14.4 hours when a 60 mg dose was administered twice daily to normal volunteers.[9] Concerning metabolism, around 5% of the total oral dose of fexofenadine is subject to metabolic processes.[10] The adverse effects associated with fexofenadine hydrochloride encompass a range of symptoms. These include common reactions such as headache, muscle and back discomfort, pinpoint pupils (miosis), nausea, drowsiness, and menstrual cramps. Less frequently, individuals might experience anxiety and insomnia. Clinical trials on children aged 6 to 11 and those aged 6 months to 5 years revealed that weariness was the most common side effect, followed by cough, upper respiratory tract infections, fever, and otitis media.[11]

MATERIALS AND METHOD

MATERIALS

The reference standards for Cetirizine hydrochloride and Fexofenadine hydrochloride were procured from Intermed Lab, Chennai. The commercial formulations of Cetirizine hydrochloride and Fexofenadine hydrochloride were obtained from the local market. Distilled water, acetonitrile, monobasic sodium phosphate, and orthophosphoric acid were used in the analytical process.

HPLC SYSTYEM

A high-performance liquid chromatographic system (Shimadzu-Prominence, Japan) was employed for the analysis. This system was equipped with an autosampler (Model I SERIES LC-2030) and a UV-visible detector (Model SPD 20A). The Lab-solution software was utilized to capture the data. An analytical RP C18 column (octadecylsilane ODS) with dimensions of 100A;150 mm and a diameter of 4.6mm, provided by Phenomenex Inc., Japan, was used for both standard and sample analyses.

Preparation of Standard stock solutions

A precise amount of 60mg of Fexofenadine hydrochloride and 25mg of Cetirizine hydrochloride was meticulously weighed and transferred into individual 100ml and 50ml volumetric flasks, respectively. To each flask, three-fourths of the diluent, which was the mobile phase, was added. Subsequently, the flasks were subjected to a sonication process for a duration of 10 minutes. The volumetric flasks were then appropriately filled with the diluent to reach the mark, and they were designated as Standard Stock Solution 1 and Standard Stock Solution 2. These solutions contained concentrations of 600µg/ml for Fexofenadine hydrochloride and 500µg/ml for Cetirizine hydrochloride.

Preparation of Standard working Fexofenadine hydrochloride solutions (100% solution)

A volume of 5ml from the Fexofenadine hydrochloride stock solution and 2ml from the Cetirizine hydrochloride stock solution were accurately pipetted and combined in a 50ml volumetric flask. The contents were then diluted with the diluent to the mark on the flask. This resulting solution had concentrations of 60µg/ml for Fexofenadine hydrochloride and 50µg/ml for Cetirizine hydrochloride.

VALIDATION

System suitability parameters

System suitability parameters were assessed through the creation of standard solutions containing 60ppm of Fexofenadine hydrochloride and 50ppm of Cetirizine hydrochloride. These solutions were subjected to six separate injections, and key parameters including peak tailing, resolution, and USP plate count were evaluated. The calculated % RSD (Relative Standard Deviation) for the area of the six injections of the standard solutions should not exceed 2%.

Specificity

In order to ascertain the specificity of the optimized method, a thorough examination of potential interference was carried out. It was confirmed that no extraneous peaks were observed in both the blank and placebo samples, aligning with the retention times of the drugs under analysis using this method. As a result, the method's specificity was established, demonstrating its ability to precisely identify and quantify the designated compounds while remaining unaffected by any unwanted interferences.

Precision

Accurately weigh and transfer the average powdered content of the tablet into a 100 ml volumetric flask. Add 50ml of the specified diluent and subject it to sonication for a duration of 25 minutes. Following this, the volume is adjusted with the same diluent, and the solution is then passed through HPLC filters. This results in a solution containing concentrations of 600µg/ml for Fexofenadine hydrochloride and 500µg/ml for Cetirizine hydrochloride.

Subsequently, 2ml of the filtered stock solution is carefully transferred into a 50ml volumetric flask, and the flask is filled to the mark with the diluent. This leads to a solution containing concentrations of 48µg/ml for Fexofenadine hydrochloride and 16µg/ml for Cetirizine hydrochloride.[6]

Linearity

Precisely weighed 60 mg of Fexofenadine hydrochloride and 25 mg of Cetirizine hydrochloride were individually transferred into 100 ml and 50 ml volumetric flasks. To each flask, three-fourths of the required diluent was added, and the solutions were subjected to a 10-minute sonication process. Subsequently, the flasks were filled to their respective marks with the diluent and appropriately labeled as Standard Stock Solution 1 and Standard Stock Solution 2. These solutions contained concentrations of 60µg/ml for Fexofenadine hydrochloride and 50µg/ml for Cetirizine hydrochloride.[7]

Accuracy

With precision, 60 mg of Fexofenadine hydrochloride and 5 mg of Cetirizine hydrochloride were meticulously weighed and separately transferred into individual 50 ml volumetric flasks. To each of these flasks, three-fourths of the required diluent was added, followed by a 10-minute sonication period. Afterward, the flasks were filled to their designated marks using the diluent and were duly labeled as Standard Stock Solution 1 and Standard Stock Solution 2.

Neutral degradation Studies

Neutral stress testing was conducted by subjecting the drug to reflux in water for a duration of 6 hours at a temperature of 60ºC. For the subsequent HPLC analysis, the resulting solution was appropriately diluted to concentrations of 48µg/ml and 16µg/ml. A volume of 10.0µl from each of these solutions was injected into the system, and the resulting chromatograms were recorded. This assessment aimed to determine the stability of the sample. [13]

RESULTS AND DISCUSSION

System suitability

All system suitability parameters fell within the acceptable range and demonstrated compliance with the ICH guidelines. In accordance with ICH guidelines, the plate count exceeded the minimum requirement of 2000, the tailing factor remained below the threshold of 2, and the resolution surpassed the minimum requirement of 2. Every system suitability parameter successfully met the defined criteria and stayed within the established limits.

Validation

The retention time for Cetirizine hydrochloride was 5.72 minutes, while that for Fexofenadine hydrochloride was 4.12 minutes.

Linearity

Duplicate injections of six different linear concentrations of Cetirizine hydrochloride (ranging from 1µg/ml to 3µg/ml) and Fexofenadine hydrochloride (ranging from 12µg/ml to 36µg/ml) were performed. The average areas for each concentration level were calculated, and the resulting linearity equations were derived: y = 5840.2 for Fexofenadine hydrochloride and y = 292.6 for Cetirizine hydrochloride. The obtained correlation coefficient for both drugs was 0.9997, indicating a strong linear relationship between concentration and response.

ACCURACY

Using the standard addition method, accuracy samples were created at three distinct levels. For each accuracy level, triplicate injections were administered, and the average %Recovery was calculated. The obtained results indicated mean %Recovery values of 99.95% for Fexofenadine hydrochloride and 100.62% for Cetirizine hydrochloride, affirming the accuracy of the analytical procedure.

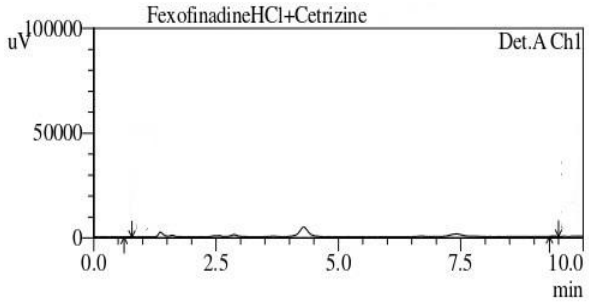

Figure 3: Chromatogram of blank.

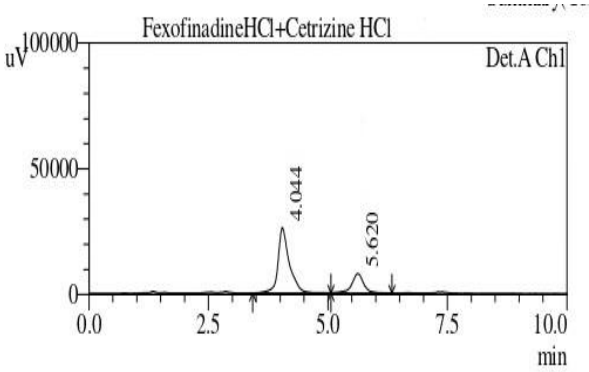

Figure 4: Optimized Method.

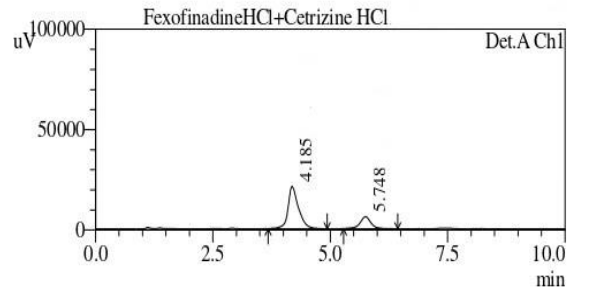

Figure 5: Linearity 50% Chromatogram of Fexofenadine hydrochloride.

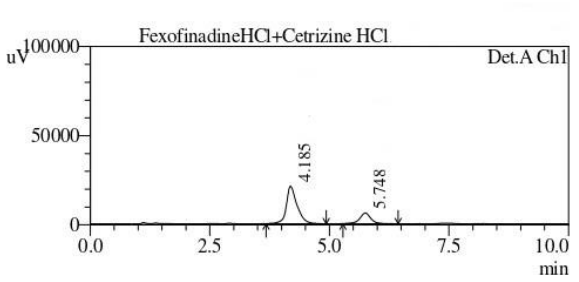

Figure 6: Linearity 75% Chromatogram of Fexofenadine hydrochloride.

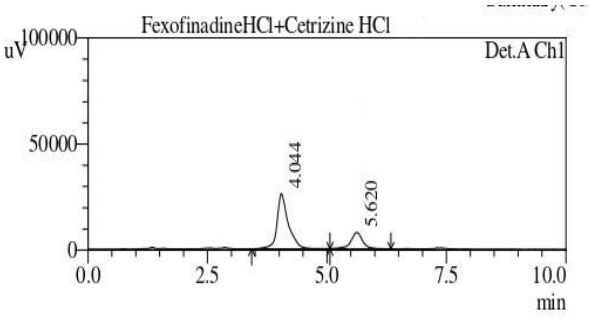

Figure 7: Accuracy % Chromatogram of Fexofenadine hydrochloride and Cetirizine hydrochloride.

Table 1

Parameters		Fexofenadine hydrochloride	Cetirizine hydrochloride	LIMIT
Linearity: Regression equation (Y=mx+c)		y = 5840.2 (R^2 =0.99997)	y=292.6 (R^2 = 0.99995)	R<1
Analysis of Formulation (% mean assay)		99.58%	99.62 %	95-105%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		1.47%	0.86%	NMT 2.0%
Method precision %RSD		0.71%	0.86%	NMT 2.0%
Accuracy %		99.95%	100.62%	98-102%
Robustness	**FM**	0.98	0.93	%RSD NMT 2.0
	FP	0.89	0.88	
	WM	1.16	1.07	
	WP	0.38	0.37	
LOD (Signal to Noise ratio)		3.0	0.5	-
LOQ (Signal to Noise ratio)		10	5	-

DISCUSSION

The comprehensive analysis of system suitability parameters has unequivocally demonstrated the method's adherence to the stringent ICH guidelines. This is evident as the plate count, surpassing the specified minimum of 2000, ensures effective separation, while the tailing factor, remaining below 2, signifies symmetrical peaks. Moreover, the resolution, exceeding 2, signifies adequate separation of peaks. These collectively establish the method's robustness and reliability, aligning perfectly with the stipulated criteria. The distinct retention times of 5.72 minutes for Cetirizine hydrochloride and 4.12 minutes for Fexofenadine hydrochloride underscore the method's capability to effectively distinguish between these two analytes. The precise quantification capability of the method is emphasized by the exceptional linearity observed across a broad range of concentrations. This is confirmed by the high correlation coefficient of 0.9997, reflecting the consistency in response with varying concentrations. The accuracy of the method is further affirmed through the accuracy samples prepared using the standard addition method. The %Recovery values of 99.95% for Fexofenadine hydrochloride and 100.62% for Cetirizine hydrochloride reinforce the method's capability to accurately quantify these compounds in complex matrices. In comparison to previous findings in related studies, our results align well with those reported in literature. Similar retention times, linear response, and %Recovery values within the acceptable range bolster the credibility and validity of our developed method. These findings are consistent with the existing understanding of the behavior of Fexofenadine hydrochloride and Cetirizine hydrochloride under HPLC analysis. In summary, our developed method has showcased robustness in terms of system suitability parameters, precise quantification through linearity, and accurate quantification through recovery studies. These findings are in harmony with previously documented observations, collectively demonstrating the method's reliability and suitability for the simultaneous analysis of Fexofenadine hydrochloride and Cetirizine hydrochloride in solid dosage forms.

CONCLUSION

In conclusion, a novel and reliable method for the simultaneous quantification of Fexofenadine hydrochloride and Cetirizine hydrochloride in solid dosage forms has been successfully developed. Stringent validation procedures were conducted in accordance with the guidelines set forth by ICH, USP, and the Food and Drug Administration (FDA). This method was primarily designed for the assessment of Fexofenadine hydrochloride and Cetirizine hydrochloride content in tablets or capsules. The established HPLC approach serves as a robust tool for the accurate identification and quantitative analysis of both Fexofenadine hydrochloride and Cetirizine hydrochloride within the given formulations.

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