RP-HPLC Method Development and Validation for Quantification of Fexofenadine in Pharmaceutical Products

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Abstract

Fexofenadine belongs to the antihistamine family of drugs. It is used to treat seasonal allergic rhinitis symptoms. In this study, a rapid, simple and sensitive liquid chromatographic method for the quantitative determination of fexofenadine was optimized and validated. Agilent Extend C_{18} column was used to perform the separation. The optimum chromatographic separation was achieved by the mobile phase consist of acetonitrile and 20 mM KH_2PO_4 solution (pH 7.5) in 35:65 ratios respectively. The flow rate of 1.2 mL min^{-1} with a standard retention time of 3.5 min at a wavelength 220 nm was optimized. The developed method has been validated in terms of their linearity, specificity, precision, accuracy, limits of detection and quantification, and robustness as per I.C.H. guidelines. The developed method has shown excellent linearity, precision, and recovery. There were no spectral or chromatographic interferences from non-medicinal ingredients found. Within a concentration range of 10–60 g mL^{-1}, the correlation coefficient was greater than 0.999. Low relative standard deviation values were obtained in the results of intra-day and inter-day precision tests. The method's accuracy ranged from 99.45 to 100.52 %. The present analytical method can be used to quantify fexofenadine in pharmaceutical preparations.

Keywords: Fexofenadine, analysis, method, validation

Farmasötik Preparatlarda Feksofenadin Kantifikasyonu için RP-HPLC Yönteminin Geliştirilmesi ve Validasyonu

Öz

Feksofenadin, antihistamin ilaç ailesindendir. Mevsimsel alerjik rinit semptomlarını tedavi etmek için kullanılır. Bu çalışmada, feksofenadinin kantitatif tayini için basit, hızlı ve hassas yüksek performanslı sıvı kromatografik yöntem gelitirilmiş ve valide edilmiştir. Ayırma işlemini gerçekleştirmek için Agilent Extend C_{18} kolon kullanıldı. Optimum kromatografik ayırma, hacimsel olarak sırasıyla 35:65 oranlarında asetonitril ve 20 mM KH_2P0_4 çözeltisinden (pH 7.5) oluşan mobil faz ile sağlandı. 220 nm dalga boyunda 3.5 dakikalık standart alıkonma süresi ile 1.2 mL dak^{-1} akış hızı optimize edildi. Geliştirilen yöntemin, lineerliği, özgünlüğü, kesinliği, doğruluğu, saptama ve kantifikasyon limitleri ve sağlamlığı I.C.H. yönergelerine göre doğrulanmıştır. Geliştirilen yöntem iyi bir doğrusallık, kesinlik ve geri kazanım göstermiştir. Tıbbi olmayan bileşenlerden hiçbir kromatografik etkileşim gözlenmedi. Korelasyon katsayısı, 10-60 µg mL^{-1} konsantrasyon aralığında 0,999'dan büyüktü. Gün içi ve günler arası kesinlik testleri sonuçlarında düşük bağıl standart sapma değerleri elde edilmiştir. Yöntemin doğruluğu % 99.45-100.52 aralığındaydı. Mevcut analitik yöntem, farmasötik preparasyonlarda feksofenadin miktarının belirlenmesi için kullanılabilir.

Anahtar kelimeler: Fexofenadine, analiz, metod, validasyon

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1. Introduction

Fexofenadine belongs to the antihistamine family of drugs. It is used to treat seasonal allergic rhinitis symptoms such as runny nose and sneezing. It works by preventing the release of histamine, a chemical in the body that causes allergic reactions [1]. It has non-sedative properties and is a histamine H1 receptor antagonist. This is due to its capacity to exist in the zwitterionic state, which does not penetrate the blood-brain barrier and so does not produce sedation [2,3]. It is a second generation antihistamine with a wide safety margin that can be used to supplement existing allergy treatments [4,5].

It is an anti-inflammatory medication. It also doesn't demonstrate terfenadine's cardiotoxic adverse effects [2,3]. Fexofenadine is quickly absorbed and has a lengthy duration of action, making it suited for once-daily treatment. As a result, it is an ideal antihistamine capable of improving the quality of life of patients suffering from allergic disease. It fully meets the desired basic properties [4,6]. The chemical properties of fexofenadine has been presented in Table 1[7].

A review of the literature found several reports on the liquid chromatographic determination of fexofenadine in biological fluids. Among these are those that use LC-MS/MS [8, 9], ultraviolet detection [10], and fluorescence detection. Few methods for measuring FEX in pharmaceutical dosage forms have been reported, including spectrophotometric methods [11], LC methods with ultraviolet detection [12-14], and capillary electrophoresis [15,16]. The fexofenadine monograph is officially available in both the United States Pharmacopoeia and the British Pharmacopoeia describing the high-pressure liquid chromatography (HPLC) technique for the quantification of fexofenadine in bulk powder [17,18].

However, these methods had limitations such as sample preparation, gradient elution, run time. These procedures also need the use of expensive equipment, specialized reagents, and significant volumes of organic solvents. The goal of this research is to create simple, quick, low-cost, and well-validated analytical methods for quantifying fexofenadine in pharmaceutical formulations using LC chromatographic technique. The developed analytical method was validated in accordance with the procedures outlined in ICH guidelines Q2(R1) for analytical method validation [19,20]. Furthermore, the applicability and dependability of this method was evaluated by focusing on routine quality control analyses.

2. Material and Method
2.1. Equipments

The analytical method was developed on an Agilent 1260 HPLC system. HPLC system included Chemstation software, a UV-Vis detector, an autosampler sampler, a quaternary pump, a thermostated column a degasser compartment. The detection wavelength of the analyte was determined in the Shimadzu UV-1800 spectrophotometer device. The spectrophotometer device was dual-beam path and analyzes were made with UV-Probe software using 1.0 cm quartz cell. The ultrapure water used in the analyzes was produced using a Milli-Q water purification system (Millipore).

2.2. Equipments

Fexofenadine HCl Pharmaceutical Secondary Standard (Certified Reference Material) methanol (≥99.9%), and acetonitrile (≥99.9%) was purchased from Sigma-Aldrich Chemie GmbH. Allegro® film tablet (120 mg) was bought from the local pharmacy. Ultrapure water was produced using a Merck Millipore water purification system (Bedford, MA, USA).

2.3. Standard solutions

By dissolving the appropriate amount of fexofenadine HCl in ultra-pure water, a stock standard solution (500 g mL^{-1}) was prepared. To obtain working standard solutions with concentrations ranging from 10 to 60 g mL^{-1} (n=6), serial dilutions of the stock standard solution were prepared using ultrapure water.

2.4. Determination of the detection wavelength

Using ultra-pure water, the spectrophotometer was calibrated to zero. 0.45 micron injector-tip filters were used to filter standard solutions. The spectrophotometer instrument was used to scan the standard solutions in the wavelength range of 200-800 nm, and the wavelength at which fexofenadine absorbed the most was found.

2.5. Calibration curves

Standard and sample solutions were filtered through 0.45 micron syringe filters and injected into the HPLC system at a fixed injection volume (20 µL). A calibration graph was constructed by plotting the concentration versus area of the peak. Slope, intercept and correlation coefficient were calculated from this calibration curve by performing regression analysis.
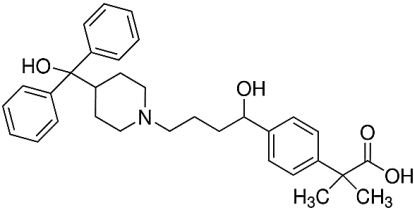
2.6. Assay of fexofenadine in tablets

The ten tablets were each individually weighed precisely and ground into a fine powder in a mortar. Ground tablet powder equivalent to 50 mg of fexofenadine was precisely weighed and transferred to a 100 mL volumetric flask. 50 mL of ultrapure water was added. The contents were sonicated for 5 minutes to thoroughly dissolve the drugs and then diluted to the mark with ultrapure water. A 1.00 mL aliquot was taken from the prepared solution, transferred to a 10 mL volumetric flask and diluted with water up to the mark (50 µg mL-1). 20 µL of sample solution was injected three times into the HPLC system. Sample solutions were analyzed using the proposed method, peak areas were determined for fexofenadine, and active ingredient contents in the tablet were calculated.

2.7. Analysis method conditions

Chromatographic analyzes were carried out using Agilent brand 1260 model liquid chromatography device with UV-Vis detector. The mobile phase was determined as a 20 mM KH_2PO_4 solution at a flow rate of 1.2 mL min^{-1}. The mobile phase was filtered using a 0.45 mm membrane filter before use and degassed in a sonicator for 5 min. Chromatographic separation was performed on an ODS (4.6 mm x 250 mm, 5 µm particle size) column held constant at 25 °C. Detection of fexofenadine was done with a UV detector at 220 nm. The operating time under these conditions was determined to be 10 minutes.

Table 1. Chemical properties of fexofenadine

Molecular formula	$C_{32}H_{39}NO_4$
Molecular weight	501.6564 g mol^{-1}
IUPAC name	4-[1-hydroxy-4-[4- (hydroxydiphenylmethyl)-1-piperidinyl] butyl]-α,α-dimethyl-benzeneacetic acid.
log P	5.02/2.94
pKa (Strongest acidic)	4.04
pKa (Strongest basic)	9.01
Solubility	Slightly soluble in water.
Chemical structure	

2.8. Validation of Analytical Method

Analytical method was validated in accordance with the procedures outlined in ICH guidelines Q2(R1) for analytical method validation [19,20]. Accuracy, linearity, system compatibility tests, sensitivity, precision, robustness, originality, and stability studies were examined as validation parameters. For the validation of the chromatographic method, fexofenadine standard solutions were prepared in triplicate with ultrapure water in the concentration range of 10-60 µg mL^{-1}. Then, Each standard solution (20 µl) was injected into the chromatographic system, and the retention time and fexofenadine peak area and were measured and recorded. A calibration curve was created by plotting the plot of the peak area versus the concentration of standard fexofenadine solutions. Linearity was determined using regression analysis. Intercept, slope and correlation coefficient values were determined by regression analysis. The accuracy of the analytical method was determined using the standard addition method to the sample solution. Three different levels of standard (120%, 100%, and 80% of concentration) were added to the sample solution analyzed earlier. The samples were analyzed using the developed analysis method. Recovery% and RSD% values were calculated for each concentration. The reproducibility (within-day) and intermediate precision (between-days) of the analytical method were used to evaluate its precision. In intraday precision studies, fexofenadine standard solution at three different concentrations (40, 50 and 60 g mL-1) was injected into the system six times in the same day, and the responses were determined. In between-day precision studies, standard solutions were injected into the system three times in three consecutive days and the responses were determined. Detection (LOD) and quantification (LOQ) limit were used to evaluate the sensitivity of the chromatographic method. LOD and LOQ values were calculated from the regression analysis using the following equations.

$$LOD = 3.3 x \sigma/S \text{ and } LOQ = 10 x \sigma/S,$$

where, σ: standard deviation of y intercept of calibration curve and S = slope of the calibration curve.

The specificity of the fexofenadine peak was determined from chromatograms at three regions (start, peak, and end of the peak).

The robustness of the analytical method was evaluated by looking at how small but deliberate changes in method parameters affected the analysis results. For this, the same standard solution was analyzed under different conditions such as changes in detection wavelengths (2 nm), and mobile phase flow rate (0.1 ml min^{-1}). System suitability parameters were determined.

For system compatibility tests, standard fexofenadine solution at a concentration of 30 g mL^{-1} was injected into the chromatographic system six times. Fexofenadine peak areas, tailing factors, capacity factors, theoretical plate numbers and asymmetric factors were recorded. For stability studies; Fexofenadine standard solution was stored under laboratory bench conditions for up to two days and in the refrigerator (4 °C) for up to five days. Afterwards, These solutions were analyzed once every eight hours with the developed chromatographic method.

3. Results and Discussion

A new analytical method was described for the quantification of fexofenadine in pharmaceutical products.

3.1. Determination of λ$_{max}$

Standard solutions were scanned in a spectrophotometer device in the wavelength range of 200-800 nm. The λ$_{max}$ value for all standard solutions was determined as 220 nm. The overlap spectrum of standard solutions is presented in Figure 1.
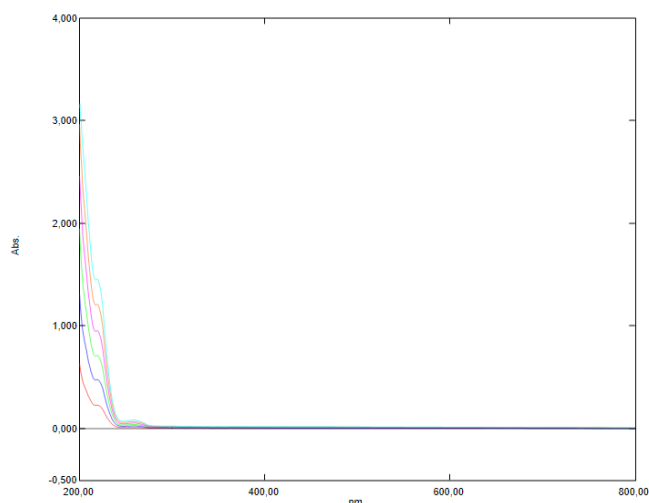
Figure 1. The overlay spectrum (standard solutions)

3.2. HPLC Method

Chromatographic conditions were optimized after meticulous work. The chemical composition, pH, flow rate and pH of the mobile phase were determined depending on the analysis time, peak shape (symmetry and tailing factor), cost of solvents, and baseline shift. Several mobile phases were tried to achieve a perfect separation. In the end, 20 mM KH_2PO_4 (pH 7.50) solution in water and acetonitrile was decided to be the best mobile phase for short working time, symmetrical peak and acceptable K value.

Overlapping chromatograms of standard solutions are given in Figure 2. The linearity graph of the standard solutions is presented in Figure 3. The retention time of Fexofenadine was determined as 3.50 minutes under the specified conditions. The oproposed analytical method was validated in terms of system suitability, accuracy, linearity, specificity, sensitivity (LOD and LOQ), precision, and robustness. Fexofenadine was found to have a purity of 0.9999 at its peak, and the proposed approach was found to be unique.

By performing linear regression analysis, it was determined that the analyte concentration and peak area had a perfect correlation. The calibration curve for the standards was linear in the concentration range of 10 to 60 µg mL^{-1} and the correlation coefficient was determined as 0.9999±0.0005, the linear regression equation was determined as Y = 23.685 X - 14.987. The detection and quantification limits were determined as 1.50 µg mL-1 and 4.50 µg mL-1, respectively, which indicates the sensitivity of the method.

The findings and statistical assessments in Table 2 demonstrate the method's excellent accuracy and precision. Recovery experiments revealed high accuracy and minimal relative standard deviations (R.S.D. %). Because the R.S.D. percent values for repeatability and intermediate precision studies were 2 as indicated by the ICH criteria, the devised HPLC technique was judged to be exact. R.S.D.% of repeatability (intra-day) and intermediate precision (inter-day) ranged from 0.1240 to 0.1745 and 0.2130 to 0.2855 respectively. The validation and regression parameters of fexofenadine using propoed method was shown in Table 2.

Table 2. Validation parameters (n=6)

Parameter		Value
Range of concentration (µg mL^{-1})		10-60
Detection limit/quantificaion limit (µg mL^{-1})		1.50/4.50
Slope		23.685±0.008
Standard error of slope		3.624
Intercept		-14.987±0.005
Standard error of intercept		4.385
Correlation coefficient		0.9999±0.0007
Standard deviation (residuals)		0.41
Recovery (%)		100.10±0.88
Precision (R.S.D.%)	Intraday	0.1240-0.1745
	Interday	0.2130-0.2855

Intentional minor modifications to the method conditions did not have a high event effect on peak symmetry and did not significantly alter the fexofenadine retention time. R.S.D.% values for each parameter of the method were calculated and it was seen that all of them were less than 2. The amount of fexofenadine in the reference material analyzed by the developed method was determined as 100.10±0.88. System suitability tests have shown that the chromatographic method developed for fexofenadine analysis in routine laboratory studies is suitable. The capacity factor for the fexofenadine peak was determined as 10.20 with Chemstation software. The tailing factor of the fexofenadine peak was also determined by the firmware to be 1.158 showing good peak symmetry. The theoretical plate number was determined as 4975, showing high column efficiency. System suitability tests are given in Table 3, revealing that the analytical method developed meets these requirements. Data obtained from studies to determine the stability of standard solutions have shown that standard solutions are stable for 48 hours at room conditions.

Table 3. System suitability test results

Parameters of system suitability tests	Value
Retention time of fexofenadine	3.95
Peak purity index for	0.9985
Peak asymetry	0.8480
Tailing factor for fexofenadine	1.1584
Capacity factor of fexofenadine peak	10.20
Theoritical plates number	4975

3.3. Application of analytical method to pharmaceutical products

Developed analytical method was applied to pharmaceutical products. Analysis results for tablet containing fexofenadine are given in Table 4. It has been observed that the analysis results are very close to the amounts stated on the labels of pharmaceutical products.

Table 4. Application results

Commercial Formulation	Label claim (mg/tablet)	Found Fexofenadine (mg/tablet)	Assay % ± S.D.
Allegro® film tablet (120 mg)	120	119.85	99.88 ± 0.88

3.4. Stability of standard solutions

Over the course of a 48-hour period, stability of the reference standard solutions was examined. For this purpose, standard solutions were injected into the HPLC system with 8-hour periods and the retention time and peak area were recorded. Table 5 shows the results of the stability study. The R.S.D.% was determined as 0.060 for peak area and 0.217% for retention time. No significant changes in the concentration of the active pharmaceutical ingredient in the standard solution were observed.

Table 5. Standard solution stability

Time, hours	Peak area	Mean	S.D.	R.S.D. %	Retention time (min)	Mean ± S.D.	S.D.	R.S.D. %
	1175.6				3.489			
0	1174.8	1175.5	0.7	0.060	3.491	3.486	0.008	0.217
	1176.2				3.477			
	1175.1				3.485			
24	1175.6	1175.0	0.7	0.056	3.491	3.486	0.004	0.119
	1174.3				3.483			
	1174.6				3.482			
48	1175.3	1175.2	0.6	0.051	3.495	3.488	0.007	0.188
	1175.8				3.487			

Figure 2. Overlay chromatograms (standard solutions)
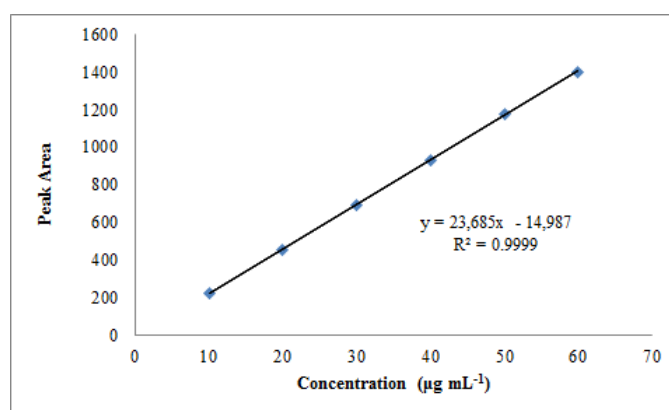
Figure 3. Linearity graph (standard solutions)

$y = 23,685x - 14,987$
$R^2 = 0.9999$

To assess the quantity of fexofenadine in pharmaceutical formulations, chromatographic method was established in this study. The chromatographic conditions of the developed analytical method were optimized and validated. Various analytical methods have been previously reported for the quantification of fexofenadine in pharmaceutical formulations. Some of these methods are quite complex. These methods use expensive equipment, large amounts of organic solvents and special reagents. Analysis times are long. It requires a complex sample preparation process.

4. Conclusions and Recommendations

The results show that developed chromatographic method is sufficient methods for the quantification of fexofenadine in parmaceutical formulations. No interfering peaks were observed during the retention time of fexofenadine in the chromatographic method. Because these analytical method is rapid, simple, precise, specific and accurate, it can be applied successfully for routine quality control analysis of fexofenadine in pharmaceutical preparations.

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