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Simple and reliable HPLC analysis of fexofenadine hydrochloride in tablets and its application to dissolution studies

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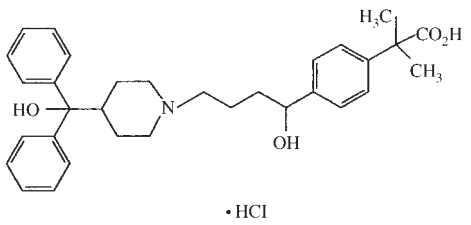
A simple RP-HPLC method using a PDA detector was developed and validated for the analysis and dissolution studies of fexofenadine hydrochloride (FEX) in dosage forms. Mobile phase: triethylamine phosphate 1%, pH 3.2 : acetonitrile(ACN) : methanol (50 : 30 : 20), 210 nm detection, C_{18} Phenomenex® column. The method was validated regarding accuracy/precision (RSD $< 1\%$), linearity ($r^2 = 0.9999$), and robustness. The method was applied to the determination of the drug in commercial tablet preparations and proved to be fast and reliable for quantification and it was also used for the comparison of dissolution profiles of FEX tablets. When we used the factor f_2 as a comparison parameter, all the medium didn't present difference in the formulations, but just in the HCl 0.1 M the formulations showed similar results for the parameters f_1/f_2 and DE allowing to affirm that the two formulations are similar and with the same performance *in vivo*.

1. Introduction

Fexofenadine hydrochloride (±)-4-(1-hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-a, a-dimethyl benzeneacetic acid hydrochloride (FEX) (Merck 2001) is a modern second-generation H_1-antihistamine drug, which does not cross the blood-brain barrier and for which there is no objective evidence of sedation even at doses 2–3 times those normally used. This compound is an active carboxylic metabolite of terfenadine, but does not show its potential cardiotoxicity. Fexofenadine is a suitable option for the first line therapy of seasonal allergic rhinitis and atopic dermatitis (Bender et al. 2003; Simons 2002; Chae and Tharp 2000; Pratt et al. 1999). It was approved by the Food and Drug Administration (FDA) in 1996 (Pinto et al. 1999).



There have been only a few articles published on the analysis of FEX formulations. Gazy et al. (2002) determined FEX in pharmaceutical dosage forms using spectrometric methods, based on ion complex reactions. Radhakrishma and Reddy (2002) used HPLC with UV detection for the determination of FEX and its related impurities, however, this method uses a C_8 column as the stationary phase, which results in a longer retention time (13 min). Breier et al. (2004) analyzed FEX by HPLC with UV detection, using as the mobile phase acetonitrile and 50 mM ammonium acetate buffer, which damages the column if this strong buffer is used routinely. Breier et al. (2005) performed a dissolution test for FEX tablets and capsules using HCl 0.1 and 0.01 M, and phosphate buffer pH 1.2, 4.0 and 6.8 as media. USP 29 2006 gives the method for HPLC with UV detection, using a C18 column, and ACN: phosphate buffer (700 : 300) mobile phase, with detection at 220 nm to evaluate FEX capsules using water as the medium, with apparatus 2 and 50 rpm, samples being collected at 15 and 45 min.

The aim of this work was to develop a new, rapid, sensitive, reliable and direct RP-HPLC procedure with a PDA detector suitable for the determination of the FEX content in tablets and dissolution studies of fexofenadine in pharmaceutical formulations. The RP-HPLC method was thus developed, validated, and applied.

2. Investigations, results and discussion

2.1. Method development

A reversed-phase HPLC method was proposed as a suitable method for the determination of FEX in drug dissolution studies and pharmaceutical dosage forms. To obtain the best chromatographic conditions, a suitable detection wavelength, column type and mobile phase composition were selected. Acetonitrile : methanol : triethylamine was preferred to acetonitrile : 5 mM ammonium acetate buffer (Breier et al. 2004) because the latter buffer causes damage to the column in routine use and the mobile phase composition chosen gives a significant retention time (4.15 min) of fexofenadine hydrochloride, thus achieving

Table 1: Intra-day, inter-day and inter-analyst precision data of RP-HPLC for fexofenadine hydrochloride

	Intra-day		Inter-day			Inter-analyst		
Theoretical amount ($\mu g \cdot mL^{-1}$)[a]	Experimental amount ($\mu g \cdot mL^{-1}$)[b]	RSD (%)	Sample	Mean ($\mu g \cdot mL^{-1}$)[c]	RSD (%)	Sample	Mean ($\mu g \cdot mL^{-1}$)[d]	RSD (%)
99.7	99.9	0.64	1	100.2	0.32	1	98.7	1.17
			2	99.6	0.02	2	98.1	0.01
						3	99.3	0.79

(a) Purity of standard; (b) mean of six determinations; (c) mean of three days (n = 5); (d) mean of two analysts (n = 5)

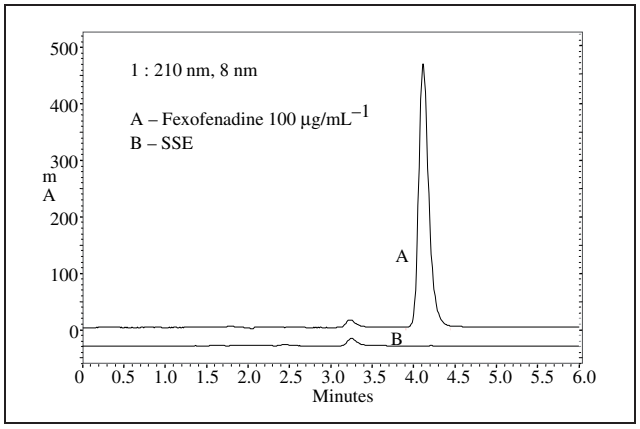


Fig. 1: Typical chromatogram of (A) mobile phase spiked with FEX (100 $\mu L \cdot ml^{-1}$, (B) simulated sample of excipients (SSE)

good selectivity towards any potential interference from the formulation after injection of simulated sample excipients. Selection of the mobile phase was in fact based on peak parameters (symmetry, tailing), run time, and cost. FEX was eluted to form a symmetrical peak, well separated from the solvent front. A chromatogram of the fexofenadine standard is shown in Fig. 1.

2.2. Method validation

Calibration curves for fexofenadine were constructed by plotting peak area versus concentration ($0.02 - 200\ \mu g \cdot ml^{-1}$ range). Linear regression by the least squares method was then applied. The calculated value for the correlation coefficient ($r^2 = 0.9999$) showed excellent linearity of the calibration curve for the method (RSD < 2).

The specificity of the PDA detector and the injection of simulated sample excipients, shown in Fig. 2B, showed that the fexofenadine peak was free from any co-eluting peak, thus achieving good selectivity towards any potential interference from the formulation.

Repeatability (intra-day precision) was studied by calculating the relative standard deviation (RSD) for six determinations of the concentration of $100\ \mu g \cdot ml^{-1}$, performed on the same day and under the same experimental conditions. The results of FEX determinations of the working standard solutions with the relative standard deviation calculated as 0.64% are shown in Table 1.

Intermediate precision includes the estimation of variations in analysis when a method is used within laboratories, on different days (inter-day precision), by different analysts, and on different equipment. The inter-day precision was assessed by analyzing two standard solutions on three different days, when the RSD values obtained were 0.02 and 0.32%. Between-analyst precision was determined by calculating the RSD for the analysis of three standard solutions by two analysts; the RSD values were found to be 0.17, 0.01 and 0.79. The results are presented in Table 1.

The accuracy of an analytical method is the closeness of the test results obtained by the method to the true value. The accuracy was assessed from five replicate determinations of three concentration levels: 80, 100 and 120 $\mu g \cdot ml^{-1}$. The absolute means obtained were 99.90, 100.02 and 100.15% respectively, with a mean value of 100.03% and RSD of 0.12% as shown in Table 2, and it is evident that the method is accurate within the desired range.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered were: variations in flow rate, pH of the mobile phase, percentage of acetonitrile and methanol in the mobile phase and column temperature. The results and the experimental range of the selected variables are given in Table 3, together with the optimized values. There were no significant changes in the chromatographic pattern

Table 2: Accuracy expressed as percentage of known concentration at three levels of FEX

Theoretical amount ($\mu g \cdot ml^{-1}$)	Experimental amount[a] ($\mu g \cdot ml^{-1}$)	Accuracy[b] (%)	RSD (%)
80	80.15		
100	100.46	100.20	0.32
120	119.78		

[a] Mean of five replicate analyses (RSD = 0.21 – 0.77)
[b] Accuracy: obtained concentration expressed as % of the nominal concentration

Table 3: Chromatographic conditions and range investigated during robustness testing

Variable	Range investigated	Fexofenadine (%)	Optimized value
	0.8	100.74	
Flow rate ($mL \cdot min^{-1}$)	1.0	99.70	1.0
	1.2	98.77	
	30	100.65	
	35	99.95	
Column temperature (°C)	40	99.70	40
	45	99.81	
	10	99.48	
Injection volume (µL)	20	99.70	20
	30	99.05	
Mobile phase (ACN : methanol)	25 : 25	99.22	
	30 : 20	99.70	30 : 20
	35 : 15	99.64	

[a] Mean of three replicates (RSD = 0.06 – 1.17)

when these modifications were made to the experimental conditions, thus showing the method to be robust. The equivalence of the columns evaluated by the system suitability tests also demonstrated no significant column-to-column variability.

The limit of detection (LOD) and the limit of quantitation (LOQ) were obtained from the slope and the standard deviation of the intercept from three calibration curves determined by a linear regression line as defined by ICH. The LOD and the LOQ were found to be 2 and 20 $ng \cdot ml^{-1}$, respectively. These values were used in an experimental assay confirming the values.

2.3. Content of fexofenadine hydrochloride in tablets

The content and uniformity of FEX in 120 and 180 mg tablets were calculated as an average of three determinations by RP-HPLC using the PDA detector. The results obtained were in agreement with the specifications. Tablet contents were 98.1% (RSD = 1.78%) and 102.0% (RSD = 1.05%) and uniformity was 102.1% (RSD = 2.07%) and 101.8% (RSD = 3.92%) respectively for the 120 and of 180 mg formulations.

2.4. In vitro dissolution studies

Dissolution tests for 120 and 180 mg FEX tablets were performed to investigate the release of the drug in different media. Apparatus II was used, the stirring rate was 50 rpm, the volume of medium was 900 ml and the temperature was 37 °C ± 0.5.

The sink condition tested showed that bulk FEX was soluble in distilled water (deaerated), 0.1 M HCl and phosphate buffer pH 4.5 and 6.8 at a stirring speed of 50 rpm. The concentrations of fexofenadine in the dissolution medium were evaluated by the proposed RP-HPLC method and the coefficients of variation ranged from 0.72 to 10.79 (n = 12). The drug release profile curves obtained by plotting the percentage drug-released against time and the results of release from formulations in different media are presented in Fig. 2. To allow the use of mean data it was observed that the calculated coefficient of variation was not more than 20% at earlier time points (e.g., 15 min), and not more than 10% at other time points, except with water as the medium.

The dissolution media tested showed a release profile with the necessary requirements to apply the f-factors. When water and buffer pH 6.8 medium were used, approximately 80% of the fexofenadine was released within 15 min from both dosage forms, in addition, when 0.1 M HCl and the pH 4.5 buffer medium were used, steady state was reached with 80% of fexofenadine release in approximately 60 min. In comparison, when we applied the method suggested by the USP for capsules for the dissolution profile of tablets, there was variation between vessels above the limit to apply the factor -f . When applying the comparison parameters f_1/f_2 and DE (ANOVA) to the analyzed means it was observed that the two formulations are very similar for the 0.1 M HCl medium. For the pH 6.8 buffer medium the formulations did not show difference (f_1 less than 15). The f_2 values were higher than 50 for all media, so the release profiles from both formulations can be considered similar (Table 4).

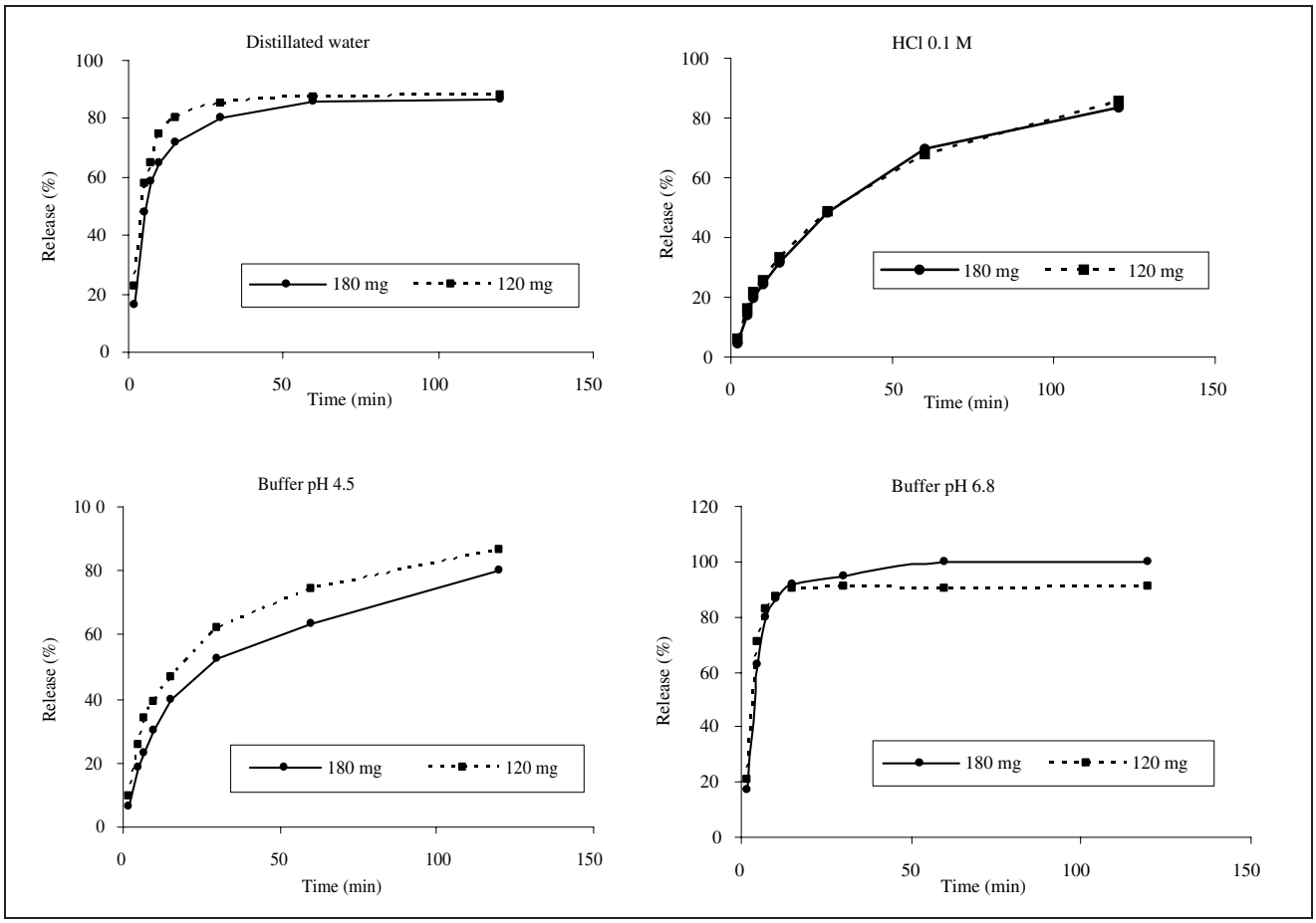


Fig. 2: Dissolution profiles of fexofenadine 180 mg and 120 mg in the four different media

Table 4: Comparison of tablet dissolution profiles in different media by dissolution efficiency (DE), difference factor (f_1 0 − 50) and similarity factor (f_2 50 − 100)

Parameter	Water	HCl 0.1 M	Buffer pH 4.5	Buffer pH 6.8
DE*	79.69 − 83.04	60.61 − 60.70	59.11 − 67.35	87.44 − 93.44
f_1**	NA	04.59 − 14.86	20.01 − 53.37	05.36 − 23.27
f_2**	NA	84.97 − 87,20	53.75 − 73.42	61.62 − 69.55

* DE average of the dissolution profiles of 120 and 180 mg tablets (n = 12)
** Range (n = 12) of f_1/f_2 obtained between 120 and 180 mg tablets
NA = not applicable

2.5. Conclusion

The data validation showed that the RP-HPLC method is accurate and possesses excellent linearity and precision characteristics. This method has been used successfully on a routine basis and allowed the quantitation of FEX without any interference from excipients, being used successfully for the quantification of FEX for monitoring its concentration for *in vitro* dissolution profile studies of pharmaceutical dosage forms. Each method used here for the comparison of dissolution profiles appears to be applicable and useful. However, the methods gave different results as regards the similarity of dissolution profiles. The results show that ANOVA-based methods applied to efficiency of dissolution (ED) are more discriminative than the f-factors. f-Factors seem to be easier to apply and interpret; only one value is obtained to describe the closeness of the two dissolution profiles.

When we used the factor f_2 as a comparison parameter, none of the media presented differences between the formulations, but in the 0.1 M HCl medium only the formulations showed similar results for the parameters f_1, f_2 and DE allowing confirmation that the two formulations are similar and with the same performance *in vivo*. We applied the USP method for capsules (USP 2006) to evaluate the dissolution profile of fexofenadine tablets and found there was variability above the limits for application of the f-factor comparison parameter. Due variability between vessels in the first points of the profile we propose a dissolution profile test using 0.1 M HCl for coatet tablets of fexofenadine 120 and 180 mg. Thus, dissolution testing is useful for batch-to-batch comparison. The need for control of dissolution in drug production is indispensable to ensure quality. The method described is shown to be useful for routine quality control analysis and dissolution studies of fexofenadine tablet formulations.

3. Experimental

3.1. Materials

FEX standard was generously supplied by Aventispharma S. A. with a declared purity of 99.7%. Allegra® tablets (Aventispharma S. A.) of 180 and 120 mg were purchased in a local pharmacy. The following substances were used for the preparation of the mobile phase: methanol and acetonitrile were purchased from Tedia (Fairfield, United States), triethylamine and phosphoric acid (85%) from Merck (Hohenbrerm, Germany). The compounds used in the dissolution medium were 0.1 M HCl and pH 4.5 and 6.8 phosphate buffer, purchased from Merck (Darmstadt, Germany). All chemical products were of analytical grade. Ultra pure water was obtained from a Millipore filtration system. For the dissolution analyses, double-distilled water filtered through a 0.45 μm membrane filter was used.

3.2. RP-HPLC analysis

In the present work, analytical chromatographic separation was carried out on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with SCL-10A_{VP} system controller, LC-10 AD_{VP} pump, DGU-14A degasser, SIL-10-AD_{VP} auto sampler and SPD-M10A_{VP} photodiode array (PDA) detector. Integration of peak areas was done automatically by computer using the Shimadzu Class VP® V 6.12 software program. The experiments were carried out on a Phenomenex (Torrance, USA) Synergi fusion C_{18} reversed phase column (150 mm × 4.6 mm I.D., with a particle size of 4 μm and pore size of 80 Å). A Phenomenex Guard column (4 mm × 3 mm I.D.) was used to protect the analytical column.

The mobile phase consisted of triethylamine phosphate 1%, pH 3.2 adjusted with phosphoric acid 85%, acetonitrile and methanol in the ratio 50 : 35 : 15 (v/v/v). The mobile phase was prepared daily and degassed by sonication under reduced pressure and filtered through a 0.45 μm membrane filter (Millipore®). The column was thermostated at 40 °C.

The HPLC system was operated isocratically with a flow rate of 1.0 mL · min^{-1}, the injection volume was 20 μL and the wavelength for PDA detection was 210 nm. All determinations were carried out in triplicate or more. The total analysis time was 6 min. The statistical analysis was performed by MS Excel 2000.

3.2.1. Preparation of standard stock solutions and calibration curves

The stock solution of FEX was prepared by dissolving 20 mg of reference standard in 20 ml of methanol, obtaining a concentration of 1 mg · ml^{-1} from which nine standard solutions in the range of 0.02–200 μg · ml^{-1} were prepared by appropriate dilution with mobile phase.

3.2.2. Validation parameters and procedures

The method was validated according to the ICH Guidance for Industry, Q2B Validation of Analytical Procedures Methodology 1997. These tests included determination of linearity, sensitivity, accuracy, precision, limit of detection, and limit of quantification.

3.3. Application of the method

3.3.1. Determination of fexofenadine hydrochloride content in tablets

Twenty Allegra® tablets of 180 mg and twenty of 120 mg were weighed, combined and thoroughly crushed. An amount of tablet powder equivalent to about 180 mg and 120 mg of FEX was accurately weighed and transferred to a 100 ml volumetric flask, made up to volume with methanol, and placed in an ultrasonic bath for 15 min. After filtration, the methanol solutions were diluted with mobile phase to obtain a concentration of about 100 μg · ml^{-1}. The uniformity of the dosage units was determined by placing a whole tablet of 180 and of 120 mg in each volumetric flask, for a total of 10 100 mL volumetric flasks, which were made up to volume with methanol and placed in an ultrasonic bath for 30 min. After filtration, the methanol solutions were diluted with mobile phase to obtain a concentration of about 100 μg · ml^{-1}. The drug concentration was determined in triplicate by RP-HPLC, and calculated with a calibration curve.

3.3.2. In vitro dissolution studies

A Vankel VK7010 (Vankel Technology Group, Cary, USA) type II apparatus was used integrated with a VK 8000 dissolution sampling station, and a VK type bi-directional peristaltic pump. Drug release was determined in four different media: distilled water (deaerated), 0.1 M HCl and pH 4.5 and 6.8 phosphate buffer. The stirring rate was 50 rpm, the volume of medium was 900 ml and the temperature of the medium was 37 °C ± 0.5 using a thermostatic bath. Dissolution was determined for twelve tablets each of 120 mg and 180 mg for each dissolution medium. At predetermined time intervals (0, 2, 5, 7, 10, 15, 30, 45, 60 and 120 min), an exact volume of sample was withdrawn from each vessel and immediately replaced by an identical volume of fresh medium. A correction factor was included in the calculation to account for the drug lost in the samples. The concentrations of FEX in all the dissolution media were determined by the proposed RP-HPLC method with PDA detection.

The dissolution profiles obtained using the four different media were compared between the 120 mg and 180 mg formulation by calculation of the dissolution efficiency (DE) and assessed by analysis of variance – ANOVA, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time, and by the model-independent simple

method which includes the difference factor (f_1) and the similarity factor (f_2). The f_1 (0–15) factor measures the percentage error between two curves over all time points and the f_2 (50–100) factor is a logarithmic transformation of the sum-squared error of differences between the test and the reference products over all time points (Costa and Lobo 2001; Shah et al. 1999; Moore and Flanner 1996; Rolim et al. 2005).

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