Development and Validation of Dissolution Tests for Fexofenadine Hydrochloride Capsules and Coated Tablets

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ABSTRACT. Purpose: This study describes the development and validation of dissolution tests for fexofenadine hydrochloride capsules and coated tablets using an HPLC method. **Method**: The appropriate conditions were determinate after testing *sink* conditions, dissolution medium, and agitation intensity. The apparatus, paddle and basket, were applied to tablets and capsules, respectively. Fexofenadine hydrochloride capsules, products A and B, and coated tablets, products A, B and C were evaluated. The best dissolution conditions tested, for the products in each respective pharmaceutical dosage form were applied to evaluate the dissolution profiles. The parameters of difference factor, similar factor, and dissolution efficacy were employed. **Results**: Optimal conditions to carry out the dissolution tests were 900 ml of 0.01 M hydrochloric acid as dissolution medium, basket at 100 rotation per minute (rpm) stirring speed for capsules and paddle at 75 rpm for tablets. The dissolution profiles for tablets products A, B, and C and for capsules products A and B were not similar. **CONCLUSION**: The developed and validated dissolution tests satisfactorily describes the time-course of the drug release. The obtained results provided adequate dissolution profiles. The HPLC method was validated to quantify fexofenadine capsules and coated tablets from the dissolution tests.

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INTRODUCTION

Fexofenadine,α,α - Dimethyl – 4 - [1-hydroxy – 4 - [4 - (hydroxydiphenyl-methyl) – 1 -piperidinyl] butyl]- benzene acetic acid (1) (Figure 1) is the active carboxylic acid metabolite of terfenadine, and is a non-sedating selective histamine H_1 receptor antagonist. Unlike its precursor, fexofenadine lacks the cardiotoxic potential, since it does not block the potassium channel involved in repolarization of cardiac cells. Fexofenadine is effective in the management of allergic rhinitis and chronic idiopathic urticaria for which it is a suitable option for first-line therapy (2).

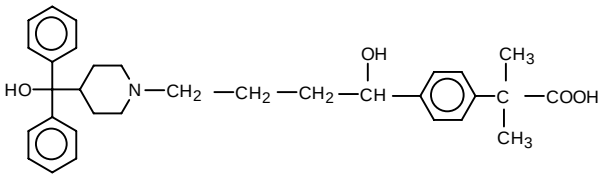

Figure 1: Chemical structure of fexofenadine

Although its distinguish importance in the treatment of common allergic diseases, there is no monograph of this drug in any pharmacopoeia. Moreover, the literature presents few methods related to the quality control of fexofenadine, mainly in its pharmaceutical dosage forms.

The dissolution test has emerged as a valuable quality control tool to assess batch-to-batch product release performance and to assure the physiological availability of the drug (3). Its significance is based on the fact that for a drug to be absorbed and available on the systemic circulation, it must previously be solubilized (4).

Fexofenadine has been determined in biological fluids by HPLC with mass spectrometry detection (5), ionspray tandem mass spectrometry detection (6-7), and fluorescence detection (8). The quantitation of fexofenadine in pharmaceutical dosage forms was realized using spectrophotometric methods, which were based in ion complex reactions (9), and HPLC methods with ultraviolet detection (10-11). The HPLC method developed previously by our research group (10) reported the validation of the method to quantify fexofenadine hydrochloride capsules. There is no dissolution tests describe in literature for fexofenadine hydrochloride in its pharmaceutical dosage forms.

This way, the aim of this work is to present the development and validation of dissolution tests and HPLC method to the quantitation of

fexofenadine hydrochloride capsules and coated tablets in routine quality control and from the dissolution tests, as well as to evaluate the dissolution profiles for capsules and coated tablets.

MATERIALS AND METHODS

Instrumentation

The dissolution tests were performed in a Sotax AT7 multi-bath (n=6) dissolution test system, in accordance with the United States Pharmacopeia (USP) general methods (12).

A Shimadzu liquid chromatograph equipped with a model LC-10ADvp binary pump, SIL-10ADvp autosampler and model SPD-M10Avp UV detector. Detection was made at 220 nm. SCL-10Avp system controller and CLASS-VP chromatography software were used. A CTO-10Acvp oven was used to keep the temperature at 30 °C. The stationary phase was a 250 x 4 mm LiChrospher® 100 RP-18 octadecyl silane column (5 μm particle size) (Merck, Darmstadt, Germany). The mobile phase was prepared by mixing 50 mM ammonium acetate buffer and acetonitrile (50:50, v/v) – pH 3.2 (adjusted with hydrochloric acid 0.1N). The injection volume was 20 μl and the run time was 10 minutes. The mobile phase was filtered using a 0.45 μm membrane filter (Milipore, Milford, MA) and degassed with helium. The mobile phase flow rate was 1.0 ml.min^{-1}.

Materials and Reagents

Fexofenadine hydrochloride reference substance (99.6%) was obtained from Aventis Pharma (São Paulo, Brazil), whereas the pharmaceutical formulations containing fexofenadine hydrochloride were obtained commercially.

Analytical reagent grade chemicals were used. Buffer solutions pH 1.2, pH 4.0, and pH 6.8 were prepared according USP 28 (12).

Fexofenadine capsules:

Product A- labeled to contain 60 mg of the drug and the following excipients: pregelatinized starch, lactose, croscarmellose sodium, and microcrystaline cellulose. This product is the reference brand in Brazil.

Product B- labeled to contain 60 mg of the drug and the following excipients: lactose, croscarmellose sodium, and microcrystaline cellulose.

Fexofenadine coated tablets:

Product A- labeled to contain 120 mg of the drug and the following excipients: pregelatinized starch, lactose, croscarmellose sodium, and microcrystaline cellulose. This product is the reference brand in Brazil.

Product B- labeled to contain 120 mg of the drug and the following excipients: lactose, croscarmellose sodium, and microcrystaline cellulose;

Product C- labeled to contain 120 mg of the drug and the following excipients: lactose, croscarmellose sodium, and microcrystaline cellulose.

Dissolution tests conditions

Fexofenadine *sink* conditions were determined in different solvents. The solubility of the drug was tested using an amount of fexofenadine hydrochloride equivalent a three times of the dose in the pharmaceutical formulation in 900 ml of HCl 0.1 M, HCl 0.01 M, phosphate buffer pH 1.2, pH 4.0 and phosphate buffer pH 6.8. The solubility in water was not tested, since it is not an ideal dissolution medium. Then, three dissolution medium were chosen to be tested in the drug release percent – 0.1 M hydrochloric acid, 0.01 M hydrochloric acid, and phosphate buffer pH 6.8. Thus, stirring speeds of 75 rpm and 100 rpm for capsules and 50 rpm and 75 rpm for tablets were tested. For dissolution tests, 900 ml of each medium were deaerated in ultrasonic bath for 15 minutes and maintained at 37 ± 0.5 °C and USP apparatus, paddle or basket, were used for tablets and capsules, respectively. The test time was set on 60 min (13).

HPLC

Preparation of standard solutions

The standard solution was prepared using an amount of powder equivalent to 10 mg of fexofenadine hydrochloride that was transferred to a 50 ml volumetric flask with mobile phase (0.2 mg ml^{-1}). Aliquot of 4 ml of this standard solution were transferred to 20 ml volumetric flask and diluted with the same diluent obtaining the final concentration of 40.0 μg ml^{-1}. The solution was filtered in a 0.45 μm membrane filter before the injection in the column.

Preparation of sample solutions

The sample solutions were prepared using amounts of powder equivalent to 10 mg of fexofenadine hydrochloride tablets which were transferred to 50 ml volumetric flask with mobile phase (0.2 mg ml^{-1}). These solutions were kept in the ultrasonic bath for 15 minutes and shaken for 15 minutes. Aliquots of 4 ml of the solutions were transferred to 20 ml

volumetric flasks and diluted with the same diluent obtaining the final concentration of 40.0 μg ml^{-1}. The solutions were filtered in a 0.45 μm membrane filter before the injection in the column.

Dissolution tests and HPLC validation

The dissolution tests were validated to fexofenadine hydrochloride capsules and tablets through the determination of specificity, linearity, intermediate precision, and solutions stability (13-14).

In our previous work (10), the HPLC method was developed and validated for the quantitation of fexofenadine hydrochloride capsules. In this work, in order to validate the HPLC method for coated tablets, the parameters of specificity, linearity, precision, accuracy and robustness were evaluated.

Specificity: the dissolution tests specificity was evaluated by preparing samples of each placebo of the commercial formulation of capsule and tablets (cited in Section 2.2). These samples were transferred to separate vessels with 900 ml of the dissolution medium and stirred for 1 h at 150 rpm using the respective method apparatus. The interference of the excipients of each formulation was evaluated by UV and HPLC. The evaluation of the HPLC method specificity was performed by preparing placebo tablets containing the same excipients of the commercial products (cited in Section 2.2). The solutions were prepared using the same procedure described for the sample solutions (Section 2.4.2) and injected three times.

Linearity: In order to assess the linearity of the method, seven doses of the reference substance (20.0; 30.0; 40.0; 50.0; 60.0; 70.0; and 80.0 μg ml^{-1}) were used at HPLC method for the standard curves. The calculation of regression line was employed by the method of least squares.

Precision: The evaluation of the intermediate precision of the dissolution tests was performed using a well-characterized lot of the drug product of tight content uniformity and compared with the results of the dissolution tests. According USP 28 (12), the content uniformity was evaluated assaying ten capsules or tablets individually and calculating the content of fexofenadine hydrochloride of each one. For the HPLC method, the repeatability (intra-assay) and intermediate precision (inter-assay) were determined by assaying samples of coated tablets, at the same concentration (40.0 μg ml^{-1}), under the same experimental conditions described in 2.4.2 section, during the same day and in three different days, respectively. The intermediate precision (inter-assay) was evaluated by comparing the assays on these three different days. The relative standard deviation (RSD) was determined.

Accuracy: This parameter was determined by the recovery test, which consists in adding known amounts of fexofenadine reference substance to the samples. Aliquots of 2.5, 5.0, and 7.5 ml of a 0.1 mg ml^{-1} fexofenadine hydrochloride standard solution (10.0 μg, 20.0 μg and 30.0 μg, respectively, corresponding to 25.0, 50.0 and 75.0% of the sample concentration) were added to three commercial samples solutions, respectively, prepared as cited in Section 2.4.2. Each solution was prepared in triplicate and each one was injected in triplicate.

Robustness: The robustness was tested by changing the following parameters of the HPLC method (one by one): mobile phase proportion – it was used 50 mM ammonium acetate buffer and acetonitrile (45:55, v/v – pH 3.2) mobile phase; mobile phase pH – it was used pH 2.6 and pH 4.8; stationary phase – it was used a MetaSil octadecyl silane (250 x 4.6 mm, 5 μm – MetaChem Technologies, Torrance, USA); and another liquid chromatograph – the quantitation was performed in a Shimadzu liquid chromatograph equipped with a model LC-10AS pump, Rheodyne injector with a 20 μl loop and model SD-10A UV detector.

Solutions stability: the solutions stability was analyzed over a specified period of time, verifying the response of the sample solution stored at room temperature.

Dissolution profiles

The dissolution profiles were obtained after the determination of the best dissolution conditions tests. Aliquots of 15 ml were withdrawn of each vessel and the same volume of the dissolution medium was replaced to maintain a constant total volume. The times selected were 5; 10; 15; 30; 45; and 60 minutes. Twelve samples were assayed for each dissolution profile. The withdrawn samples were filtered in 0.45 μm and diluted with mobile phase to 40 μg ml^{-1} to HPLC quantitation.

Release dissolution profiles comparison

The dissolution profiles were compared through the calculation of dissolution efficiency (DE) and model-independent simple method. The DE was calculated from the area under the dissolution curve at time t_i (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

The model-independent simple method includes the difference factor (f_1) and the similarity factor (f_2). The f_1 factor measures the percent error between two curves over all time points. The percent error is zero when the test and drug reference profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles.

The f_2 factor is a logarithmic transformation of the sum-squared error of differences between the test and the reference products over all time points. This factor is 100 when the test and reference profiles are identical and tends to 0 as the dissimilarity increases. Two dissolution profiles are declared similar if f_1 is between 0 and 15 and if f_2 is between 50 and 100 (15-16).

RESULTS AND DISCUSSION

The *sink* conditions tested showed that fexofenadine hydrochloride bulk was soluble in HCl 0.1 M, HCl 0.01 M and phosphate buffer pH 6.8. Then, dissolution tests for fexofenadine hydrochloride tablets product A were performed using these three dissolution medium at the stirring speed of 75 rpm, to investigate the drug release in each medium (Figure 2). The results show that HCl 0.01 M was the best dissolution medium, since it provides highest drug release percent.

For capsules, the basket method is routinely used at an agitation speed of 50 to 100 rpm. For tablets, the paddle method is frequently used at 50 or 75 rpm (14). Thus, stirring speeds of 75 rpm and 100 rpm for capsules products A and B (Figure 3) and 50 rpm and 75 rpm for coated tablets product A (Figure 4) were tested. The statistical *t-student* test at 0.05 significance level was applied to compare the drug release percent (DR%), using 75 or 100 rpm for capsules (Table 1) and 50 or 75 rpm for tablets (Table 2). The P-values presented for capsules were greater than the delineated significance level, indicating that there was no statistically significant difference between the drug release percent and suggested that any of the stirring speed could be used, for products A and B. However, it was observed that stirring speed of 100 rpm presents high drug release percent until 30 minutes. The P-value for tablets was smaller than the delineated significance level, indicating that there is statistically significant difference between the drug release percent and suggested that the stirring speed of 75 rpm is better than 50 rpm. Thus, the stirring speed of 100 rpm for capsules and 75 rpm for tablets were chosen.

The reversed-phase liquid chromatography method was developed and validated for fexofenadine hydrochloride in coated tablets. The validation analytical parameters described in the guidelines (12, 17) were evaluated. The type of method and its respective use determine which parameters should be evaluated. It is the responsibility of the analyst to select the parameters considered relevant for each method (18).

The specificity of the dissolution test was evaluated through the analysis of placebo capsules and tablets from a dissolution test using the HPLC and UV methods. The analysis by UV shows that the excipients from capsules and coated tablets absorbed at 220 nm (Figure 5), which characterize interference in the analysis. So, the UV method can not be use to quantify fexofenadine hydrochloride capsules and coated tablets from the dissolution tests.

The specificity test by HPLC demonstrated that the excipients from capsules and tablets do not interfere in the drug peak (Figure 6). Thus, the HPLC method is useful to quantify fexofenadine hydrochloride in pharmaceutical formulation from the dissolution tests. The chromatogram obtained through the injection of the placebo solution did not present any other peak in the same retention time (5 minutes) of fexofenadine hydrochloride (Figure 7). The chromatographic peak purity tool was used in order to verify the purity. This tool works analyzing the peak and given a value between 0 and 1. The obtained value was 0.9999, this result shows that the analyzed peak was only fexofenadine hydrochloride, without interference. Thus, it was proved that the

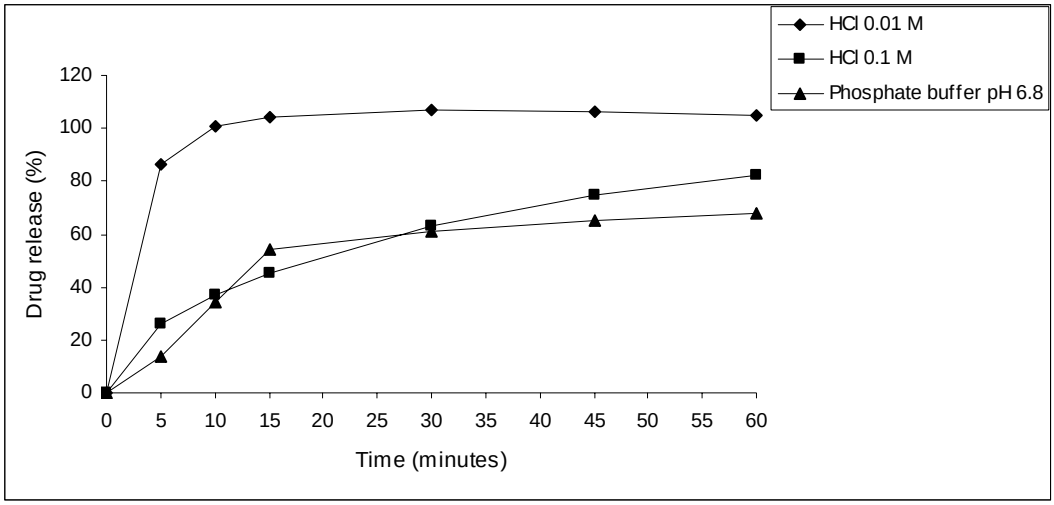

Figure 2. Product A - coated tablets dissolution profiles using HCl 0.01 M, HCl 0.1 M or phosphate buffer pH 6.8 as dissolution medium and paddle at 75 rpm.

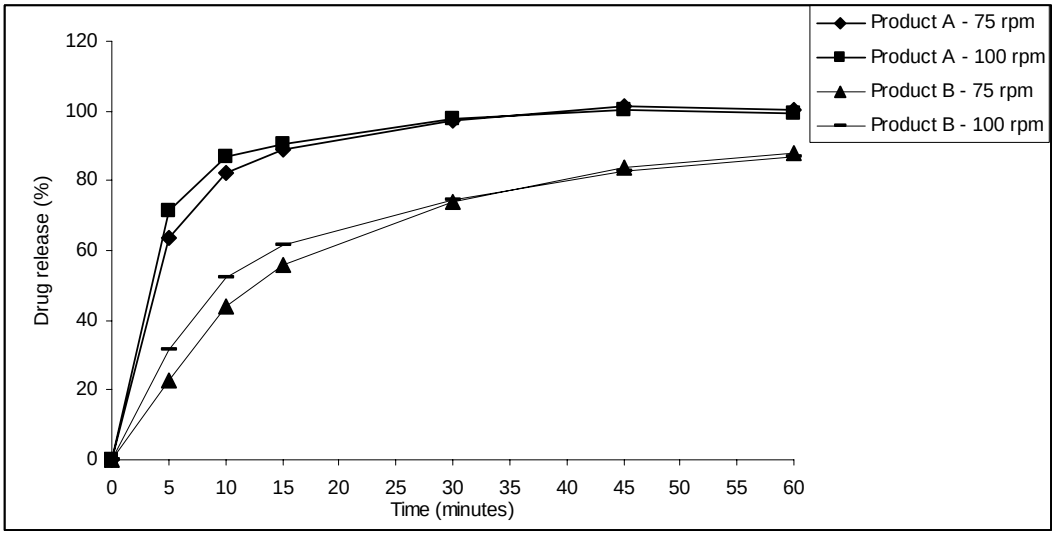

Figure 3: Products A and B - capsules dissolution profiles using HCl 0.01 M, and basket at 75 rpm or 100 rpm.

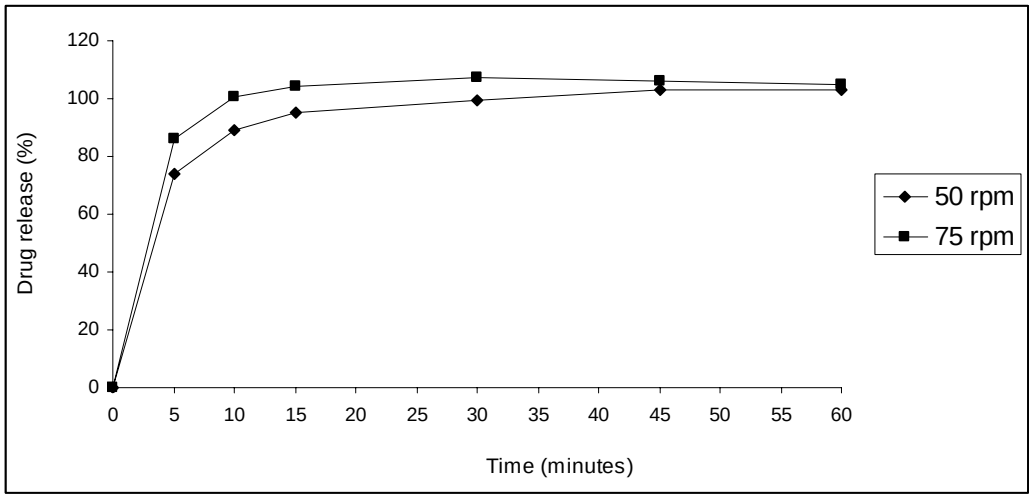

Figure 4: Product A - coated tablets dissolution profiles using HCl 0.01 M, and paddle at 50 rpm or 75 rpm.

peak at 5.0 min was not suffering interference of any excipients from the formulation.

To assess the linearity, three standard curves for fexofenadine hydrochloride were constructed, plotting concentrations (μg ml^{-1}) versus absolute area (mV s) and showed good linearity on the 20.0-80.0 μg ml^{-1} range. The representative linear equation was $y = 31260.64\ x + 31492.44$, where x is concentration and y is the peak absolute area. The correlation coefficient was r = 0.9999, indicating good linearity. The data were validated by means of the analysis of variance, which demonstrated significative linear regression and no significant linearity deviation ($p < 0.05$) (19).

Table 1: Products A and B capsules dissolution tests results (n=12), using different stirring speeds and HCl 0.01M as dissolution medium.

Product	min	DR%		t-test	*p*
		75 rpm	100 rpm		
	0	0	0		
	5	63.85	71.40		
	10	82.32	87.14		
A	15	89.17	90.74	1.468	0.19
	30	97.50	97.82		
	45	101.19	100.57		
	60	100.43	99.29		
	0	0	0		
	5	22.92	31.38		
	10	44.11	52.15		
B	15	56.04	61.40	1.814	0.12
	30	73.77	74.54		
	45	83.69	82.68		
	60	87.94	86.73		

The intermediate precision of the dissolution tests was verified through the comparison of the results of uniformity of content and the percentage drug release. The mean values found for the uniformity of content of product A and B capsules were 111.61% (RSD = 1.80) and 101.20% (RSD = 2.41), respectively. The drug release percent were 99.29%, for product A and 86.73% for product B. The difference between the uniformity of content and drug release percent can be explained by the incomplete dissolution of gelatin wrapping, which kept an amount of the drug inside. However, this effect do not interfere the dissolution test, because more than 70% of drug was dissolved in 30 minutes in all tests.

Table 2: Product A coated tablets dissolution tests results (n=12), using different stirring speeds and HCl 0.01M as dissolution medium.

Product	min	DR%		t-test	*p*
		50 rpm	75 rpm		
	0	0	0		
	5	74.12	86.28		
	10	89.25	100.59		
A	15	95.36	103.97	3.57	0.01
	30	99.55	107.29		2
	45	103.10	106.12		
	60	103.01	105.05		

The mean values found for uniformity of content to product A, product B, and product C coated tablets were 107.01% (RSD=0.51), 103.03% (RSD=1.11), and 103.87% (RSD=1.2), respectively. The drug release percent were 105.05%, 103.85%, and 105.06%, for products A, B, and C, respectively. In all tests, almost all drug was dissolved. These results show the good precision of the dissolution tests.

The experimental values obtained for the determination of fexofenadine hydrochloride in samples are presented in Table 3. The low relative standard deviation (RSD) of 1.67 (intra-day precision), and 0.12 (inter-day precision) showed the good precision of the method.

The accuracy expresses the agreement between the accepted value and the value found. The mean recovery was found to be 99.94% for the coated tablets (Table 4). This value shows the good accuracy of the proposed method.

The robustness of the method evaluated by changing the mobile phase proportion, 50 mM ammonium acetate buffer and acetonitrile (45:55, v/v; pH, 3.2) demonstrated an increase on the retention time of the drug. The use of pH 2.6 resulted in a decrease in the retention time. The method was robust with these two modifications. When pH 4.8 was used, the retention time was about 4.3 min, but the peak become wide, probably because in this pH the drug is in the ionizated form. The effect of using MetaSil octadecyl silane (250 x 4.6 mm, 5 μm) as stationary phase has increased the retention time in two minutes. Even so, the method was robust. The last experiment was the quantitation in another liquid chromatograph **(**Shimadzu equipped with a model

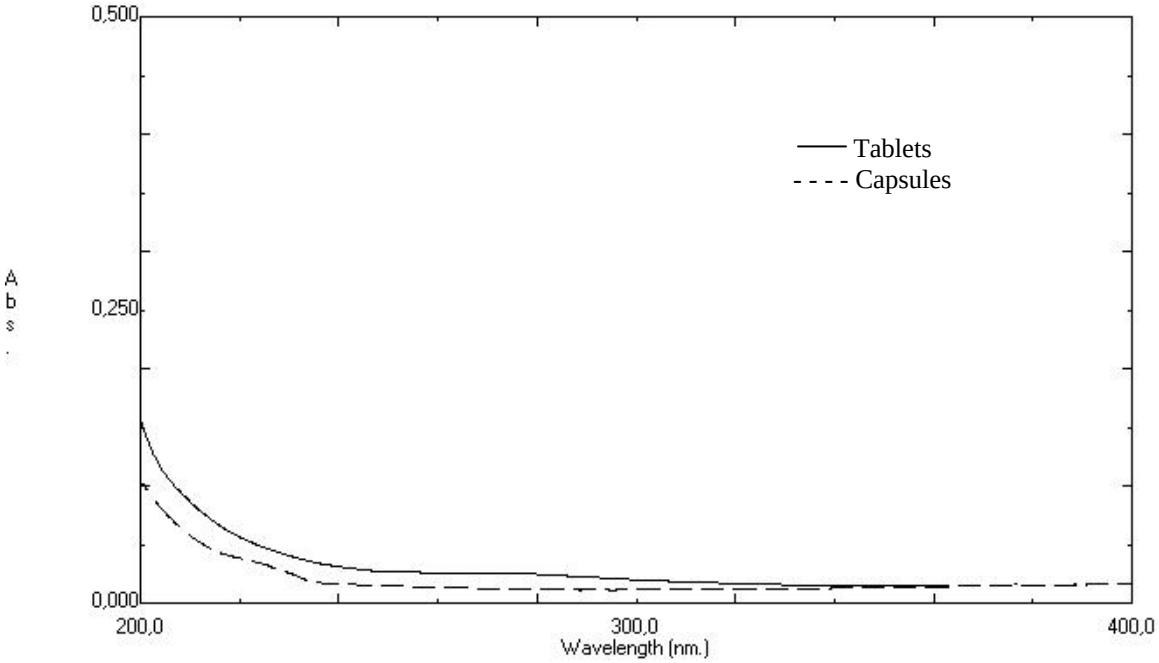

Figure 5: Absorbance vs wavelength specificity of fexofenadine hydrochloride capsules and coated tablets from the dissolution test by UV.

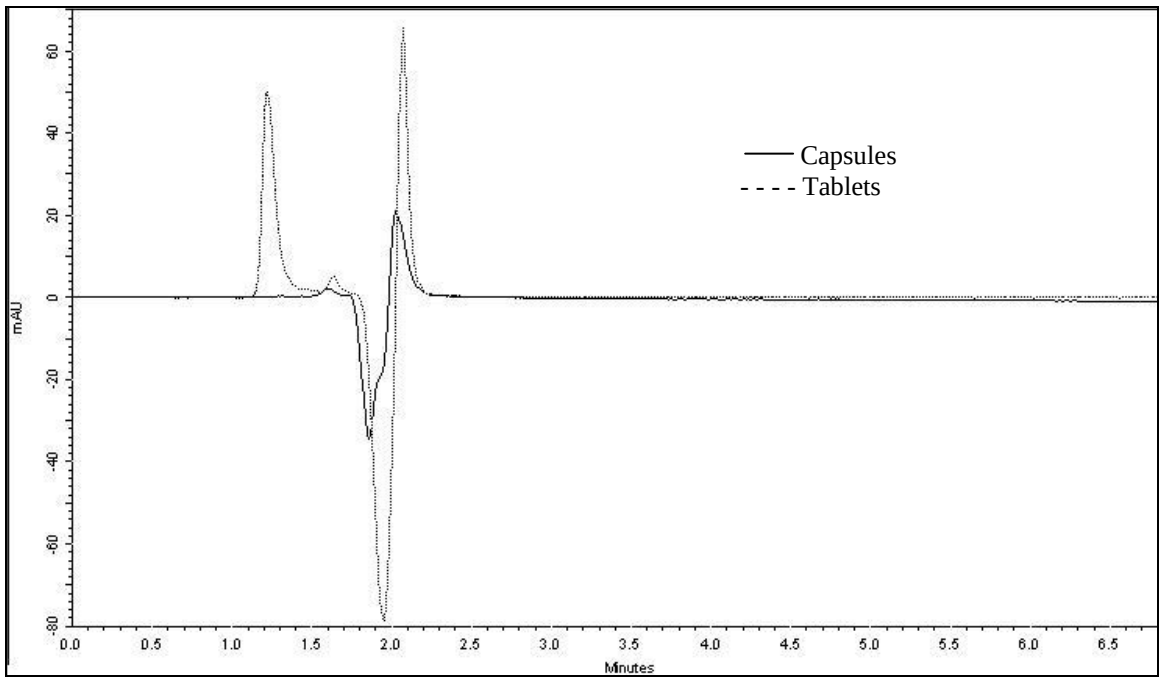

Figure 6: Specificity test for fexofenadine hydrochloride capsules and coated tablets from the dissolution test by HPLC.

LC-10AS pump, Rheodyne injector with a 20 µl loop and model SD-10A UV detector) where the retention time was a little high (about 5.4 minutes), but it was possible to quantify the drug satisfactorily, confirming the robustness of the method. At that rate, it was possible to demonstrate that the developed method was robust with all the changes employed, except for the use of pH 4.8 in the mobile phase.

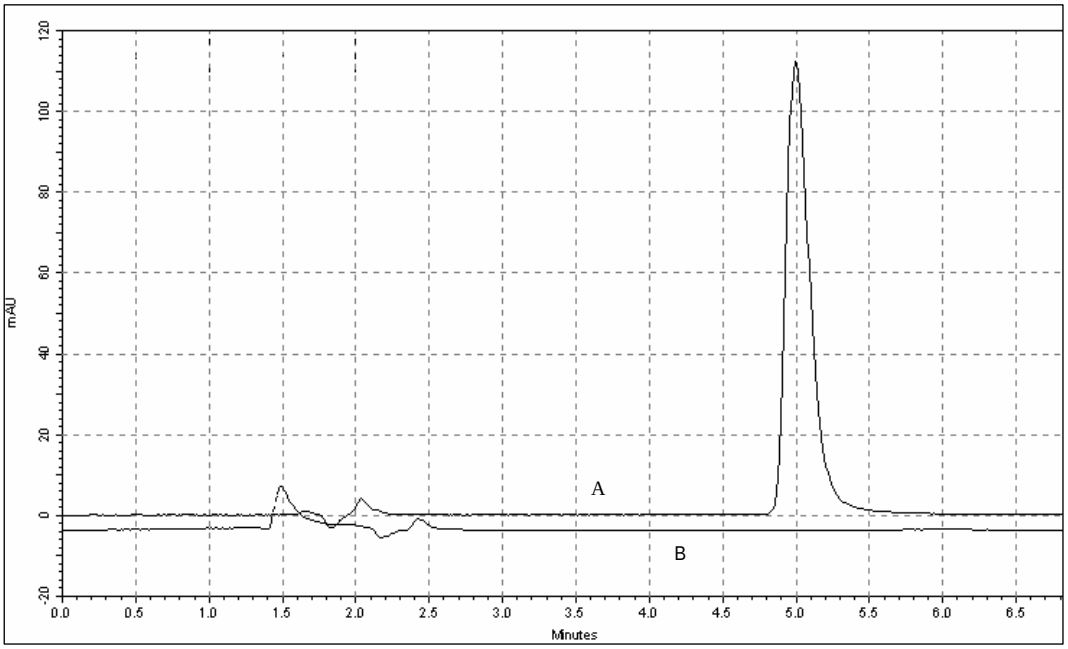

Figure 7: Chromatograms of fexofenadine hydrochloride coated tablets sample solution 40 μg ml^{-1} (A) and placebo solution (B). Chromatography conditions: acetonitrile, 50 mM ammonium acetate buffer (50:50, v/v) at pH 3.2 mobile phase; flow rate of 1,0 ml ml^{-1}; Lichrospher® 100 RP-18 (250 × 4.0 mm, 5 μm) stationary phase; ultraviolet detection at 220 nm; temperature of 30 °C; injection volume of 20 μl.

Table 3: Experimental values of fexofenadine hydrochloride coated tablets obtained in commercially available sample, using the HPLC method.

Sample	Precision, Intra-assay		
	1 st day	**2 nd day**	**3 th day**
1	97.86	100.02	99.24
2	100.83	99.35	99.12
3	96.91	98.58	98.83
4	100.09	99.21	99.79
5	100.66	99.28	99.92
6	98.14	98.01	99.01
Mean	99.08	99.24	99.32
RSD	1.67	0.48	0.44
Precision Inter-assay	99.21		
RSD	0.12		

The stability test of the solutions shows that fexofenadine hydrochloride was stable in HCl 0.01 M at least 24 hours at room temperature and this way it can be analyzed with precision during the dissolution assay.

The comparison of the dissolution profiles for the different products cited in section 2.2 was realized. The results of dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2) are presented in Tables 5 and 6 for coated tablets and capsules, respectively. Since product A is the reference brand, the factors f_1 and f_2 were calculated between product A and B for tablets and capsules. Two dissolution profiles are declared similar if f_1 is between 0 and 15 and if f_2 is between 50 and 100. The results of f_1 and f_2, 36.23 and 17.45, respectively, for the comparison of product A and B, showed that the profiles are not similar. For product C (coated tablets) these factors were not calculated, because the dissolution was very fast (more than 85% in 15 minutes). The dissolution efficiency was calculated for all products capsules and tablets. The analysis of variance of the DE values shows that the profiles are not similar for tablets and capsules.

Typical acceptance criteria for the amount of drug dissolved are in the range of 75% to 80% dissolved. Acceptance criteria including test times are

Table 4: Experimental values obtained in the recovery test for fexofenadine hydrochloride coated tablets, using the HPLC method.

Sample	Amount of reference (mg)		Recovery*	*Mean*	*RSD*
	Added	Recovered	%		
R1	0.25	0.251	100.35		
R2	0.50	0.491	98.10	99.94	1.67
R3	0.75	0.760	101.36		

usually established on the basis of an evaluation of the dissolution profile data (14). In this article, it was observed that for all products a dissolution of 70% /30 min. So, this acceptance criterion was utilized.

Table 5: Comparison of coated tablets dissolution profiles through the dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2).

Parameter	Product A (reference)	Product B	Product C
DE	103.85	101.64	78.85
f_1^*	36.23		-
f_2^*	17.45		-

* calculated between products A and B.

Table 6: Comparison of capsules dissolution profiles through the dissolution efficiency (DE), difference factor (f_1) and the similarity factor (f_2).

Parameter	Product A (reference)	Product B
DE	95.19	73.07
f_1^*	28.90	
f_2^*	27.62	

*calculated between products A and B.

CONCLUSIONS

In this work, it was developed and validated dissolution tests and evaluated dissolution profiles for fexofenadine hydrochloride in capsules and coated tablets. The use of 900 ml of 0.01 M hydrochloric acid at 37 °C, basket at the stirring speed of 100 rpm and paddle at the stirring speed of 75 rpm as apparatus for capsules and coated tablets, respectively, and 60 min of test provided satisfactory results for all products. The comparison of the obtained dissolution profiles was realized by DE and the factors f_1 and f_2 and show that the profiles were not similar neither for capsules products A and B, nor for tablets products A, B and C. However, for all products the drug delivery was satisfactory, since at least 70% was dissolved in 30 minutes. The HPLC method was validated to the routine quality control of fexofenadine hydrochloride in coated tablets and was satisfactory in the quantitation of fexofenadine hydrochloride capsules and coated tablets from the dissolution tests. The UV method could not be used, since it lacks in specificity.

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