Scientific paper

First and Second Derivative Synchronous Fluorescence and Spectrophotometric Spectroscopy for the Simultaneous Determination of Fexofenadine Hydrochloride in Presence of Its Degradation Products. Application to Stability Studies

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Abstract

Two rapid, simple, sensitive, selective and economic derivative spectrophotometric (first [D^1] and second [D^2]) and synchronous spectrofluorimetric (FDSFS and SDSFS) methods have been developed for the analysis of fexofenadine hydrochloride (FXD) in the presence of its different degradation products. Derivative spectrophotometry (D^1) was used to measure FXD at 223 nm in the presence of its alkaline or acidic degradation products, and at 211 nm in the presence of its oxidative degradation product. Derivative spectrophotometry (D^2) was used to determine FXD at 217 nm in the presence of its alkaline or acidic degradation products, and at 215 nm in the presence of its oxidative degradation product; the UV degradation product was measured at 211 nm. Synchronous spectrofluorimetry (FDSFS) was used to measure FXD in the presence of its alkaline or acidic degradation products at 406 nm, and at 367 nm in the presence of its oxidative or UV degradation products. Synchronous spectrofluorimetry (SDSFS) was applied to determine the drug at 225 nm in the presence of its alkaline, acidic, oxidative or UV degradation products. The proposed methods were successfully applied for the determination of the studied compound in its commercial tablets. The results obtained were in good agreement with those obtained by the comparison method.

Keywords: Fexofenadine, derivative spectrophotometry, synchronous fluorimetry, stability study, degradation.

1. Introduction

Fexofenadine, *RS* α, α – dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1 piperidinyl]butyl]-benzene acetic acid[1] (Fig. 1) is the active carboxylic acid analog of the antihistamine terfenadine. It shares the histamine H_1 receptor antagonist and non-sedative properties of the parent compound but does not affect the cardiovascular system. Fexofenadine is a second generation antihistamine drug useful to available treatments of allergic diseases as rhinitis and chronic urticaria, with a wide safety margin.[2]

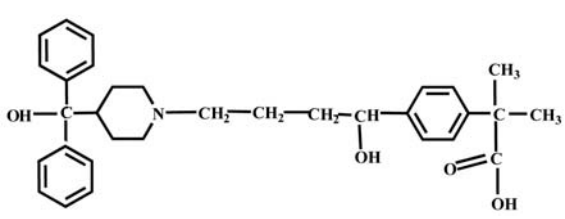

Figure 1: Structural formula of fexofenadine

Several analytical methods have been reported for the determination of FXD, either in pure form or pharmaceutical preparations and biological fluids. These methods included spectrophotometry,[3–5] spectrofluorimetry,[5] and high performance liquid chromatography (HPLC).[6–10]

Derivative spectrophotometry has been widely used over the last few decades for matrix background elimination[11] as well as for multi-component analysis.[12] For this reason, diverse procedures for the resolution of overlapping derivative peaks have been applied. It has a wide range of applications in chemical, pharmaceutical, food, clinical, and environmental analyses;[12,13] in addition they have been reported for the determination of many drugs in

the presence of their degradation products or in multi-component mixtures.[12]

In fluorometric methods, a high sensitivity and selectivity are generally expected. However, problems of selectivity can occur in multi-component analysis because of the overlap of spectra. Synchronous fluorescence spectroscopy (SFS) has been found to have several advantages,[14] such as simple spectra, high selectivity, low interference, etc. Because of its sharp, narrow spectrum, SFS serves as a very simple, effective method of obtaining data for quantitative determination in a single measurement.[15] It has attracted the attention of many researchers and developed rapidly since it was first proposed by LIoyd.[16]

Synchronous fluorescence spectroscopy techniques are classified according to different scanning modes of monochromators into constant wavelength, variety angle, and constant energy. At present, the constant wavelength method, in which a constant difference between the emission and excitation wavelengths is maintained, is used most extensively.

The combination of SFS and derivative is more advantageous than differentiation of the conventional direct spectrofluorimetry in terms of sensitivity, because the amplitude of the derivative signal is inversely proportional to the bandwidth of the original spectrum.[17]

The International Conference on Harmonization (ICH) guidelines[18] entitled "stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The required tests include susceptibility to alkaline, acidic, oxidative and UV degradation.

The aim of the present work is to develop efficient novel derivative spectrophotometric and DSF methods for the determination of FXD in the presence of its different degradation products, and to study the results kinetically so as to indicate that the methods are stability-indicating ones.

Up till now neither derivative spectrophotometry nor synchronous spectrofluorimetry has been reported for the analysis of FXD in presence of its degradation products.

2. Experimental Procedures

2. 1. Apparatus

a) Spectrophotometric analyses were carried out on a Dynamica Halo DB-20 spectrophotometer; UV-Visible double-beam spectrophotometer with matched 1 cm path-length quartz cells. Absorption spectra of the studied drug were recorded at a fast scan speed, setting slit width of 1 nm and an autosampling interval.

b) Spectrofluorometer: Perkin Elmer LS 45 Luminescence Spectrometer, equipped with a 150 W Xenon arc lamp, grating excitation and emission monochromators, and a recorder. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. Derivative spectra were evaluated using Fluorescence Data Manager (FLDM) software, Perkin Elmer Buck, i.e. FL WINLAB (version 400.02).

c) A Consort NV P901 pH meter.

2. 2. Materials and Reagents

All reagents and solvents were of analytical reagent grade.

a) Fexofenadine hydrochloride (FXD) of purity 99.68% was kindly provided by El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.

b) Pharmaceutical preparations:
   * Fastofen® tablets (Batch # 7065), labeled to contain 60 mg fexofenadine hydrochloride per tablet, El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.
   * Fastofen® tablets (Batch # 109108), labeled to contain 120 mg fexofenadine hydrochloride per tablet, El-Obour Modern Pharmaceutical Industries Company, Cairo, Egypt.
   * Fexodine® capsules (Batch # 308134), labeled to contain 180 mg fexofenadine hydrochloride per capsule, Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. All were obtained from commercial sources in the local market.

c) Sodium hydroxide (2M solution), hydrochloric acid (2M solution), hydrogen peroxide (6% v/v solution); (BDH, Poole,UK).

d) Methanol (Sigma-Aldrich), HPLC grade.

2. 3. Standard Solutions

A stock solution of FXD was prepared by dissolving 100 mg of the studied compound in 100 ml of methanol and was further diluted with the same solvent as appropriate. The working standard solution was stable for 7 days when kept in the refrigerator.

2. 4. General Procedures

2. 4. 1. Procedure for Method I

Aliquots of FXD standard solutions over the concentration range of 3.0–30.0 μg/ml were transferred into a series of 10-ml volumetric flasks and the solutions were diluted to the mark with methanol and mixed well. The zero-order absorption spectra were recorded against methanol. The first derivative spectra (D^1) of the drug in the presence of alkaline or acidic degradation products were recorded in the wavelength range 200–300 nm using a wavelength interval $\Delta\lambda$ of 6 nm, with the zero crossing point at 223 nm. A wavelength interval $\Delta\lambda$ of 8 nm was used to record the first derivative spectra (D^1) of the drug in the presence of its oxidative degradation product, with the zero crossing point at 211 nm. Second derivative spectrophotometry D^2 used a wavelength interval $\Delta\lambda$ of 8 nm when the drug was assayed in presence of its alkaline or acidic

degradation products where the ^{2}D amplitudes were measured at 217 nm. Under oxidative degradation conditions FXD ^{2}D spectra were recorded at 215 nm using a wavelength interval Δλ of 10 nm. Second derivative spectrophotometry was used to determine FXD in the presence of its UV degradation product at a wavelength interval Δλ of 6 nm and the ^{2}D amplitudes were measured at 211nm. A blank experiment was performed simultaneously. The peak amplitude of either the first or the second derivative technique was plotted *versus* the final concentration of the drug (μg/ml) to obtain the calibration graphs. Additionally, the corresponding linear fitting parameters were derived.

2. 4. 2. Procedure for Method II

Aliquots of FXD standard solutions over the concentration range of 0.2–2.0 μg/ml were transferred into a series of 10-ml volumetric flasks, followed by 3 ml of a borate buffer (pH 8); the solutions were diluted to the mark with methanol and mixed well. The synchronous fluorescence spectra of the solutions were recorded by scanning both monochromators at a wavelength interval Δλ of 80 nm (alkaline or acidic degradation products), 60 nm (oxidative degradation product) or 120 nm (UV degradation products). A scan rate of 600 nm/min using 10 nm excitation and emission windows was used. The first and second-derivative fluorescence spectra of FXD were derived from the normal synchronous spectra using FLDM software. For best resolution and smoothing 99 points were used. The fluorescence intensities of the first and second-derivative spectra were estimated at 406 and 225 nm respectively when FXD was determined in presence of its alkaline or acidic degradation products. When the drug was estimated in the presence of its oxidative or UV degradation products the first and second-derivative spectra were determined at 367 and 225 nm respectively. A blank experiment was performed simultaneously. The peak amplitude of either the first or the second derivative technique was plotted *versus* the final concentration of the drug (μg/ml) to obtain the calibration graphs. Alternatively, the corresponding linear fitting parameters were derived.

2. 5. Applications

2. 5. 1. Procedure for Determination of FXD in the Presence of its Degradation Products

One-ml aliquots of FXD standard stock solution (10.0 mg/ml for method I or 1.0 mg/ml for method II) were transferred into a series of 25-ml volumetric flasks to obtain a final concentration of 400 μg/ml for method I or 40 μg/ml for method II; the volume was made up to the mark with 2 M sodium hydroxide, 2 M hydrochloric acid or 6 % hydrogen peroxide to prepare the alkaline, acidic or oxidative degradation products, respectively. The solutions were left in a boiling water bath for 20 minutes (acidic and alkaline degradation) or for 30 minutes (oxidative degradation). Regarding the UV degradation, the methanolic solution of FXD was exposed to a Deuterium lamp in a wooden cabinet at a distance of 15 cm for 9 h. Aliquot volumes of the degraded solutions equivalent to 3.0–30.0 μg/ml for method I or 0.2–2.0 μg/ml for method II were transferred to a series of 10 ml volumetric flasks, neutralized with 2 M hydrochloric acid or 2 M sodium hydroxide for alkaline and acidic degradation, respectively, and the steps described under "General procedures for method I or method II" were followed. The peak amplitude of the first or the second derivative techniques were plotted *versus* the final concentration of the drug (μg/ml) to obtain the calibration graphs. Additionally, the corresponding linear fitting parameters were derived.

2. 5. 2. Procedure for Tablets

Twenty tablets were weighed and pulverized. An accurately weighed quantity of the powder equivalent to contain 20 mg of FXD was transferred into a small conical flask and extracted three successive times each with 30 ml of methanol. The extract was filtered into a 100-ml volumetric flask. The conical flask was washed with a few millilitres of methanol, the contents transferred to the volumetric flask and made up to the mark with the same solvent. Aliquots equivalent to the concentration range of 3.0–30.0 μg/ml for method I or 0.2–2.0 μg/ml for method II were transferred into 10 ml volumetric flasks. The steps described under "General Procedures for method I or method II" were followed. The nominal content of the tablets was determined either from the calibration graphs or from the corresponding linear regression equations.

3. Results and Discussion

Zero order absorption spectra for FXD and its degradation products showed overlapping spectra which prevent the direct determination of the drug. This is illu-

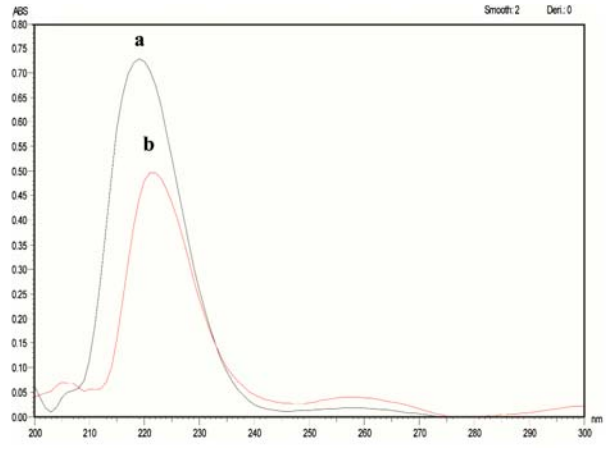

Figure 2: Zero-order spectrophotometric spectra of FXD (a) in presence of its alkaline degradation product (b).

strated in Figure 2 showing FXD in the presence of its alkaline degradation product. Derivative spectrophotometry, based on mathematical transformation of the zero order curves into the derivative spectra can overcome this problem.[19]

First and second derivative spectra of FXD in the presence of its alkaline degradation product were traced with a wavelength interval Δλ of 6 and 8 nm, respectively and used to resolve the spectral overlapping. The zero crossing point for FXD at 223 and 217 nm, for the first and second derivative spectra, respectively, are presented in Figures 3 (a–b) and were used for the determination of the drug in the presence of its degradation product.

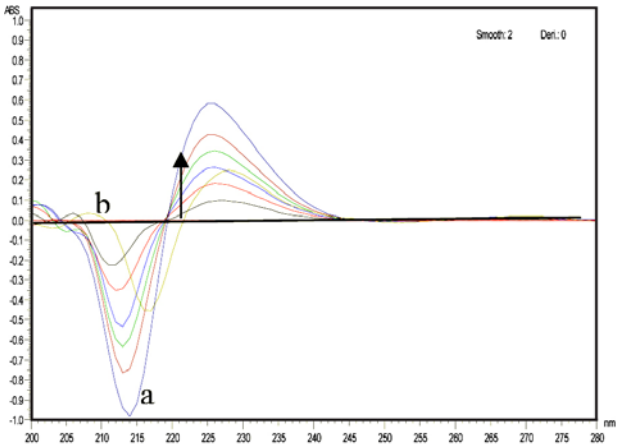

Figure 3a: First-derivative spectrophotometric spectra of different concentrations of FXD (a) in the presence of its alkaline degradation product (b).

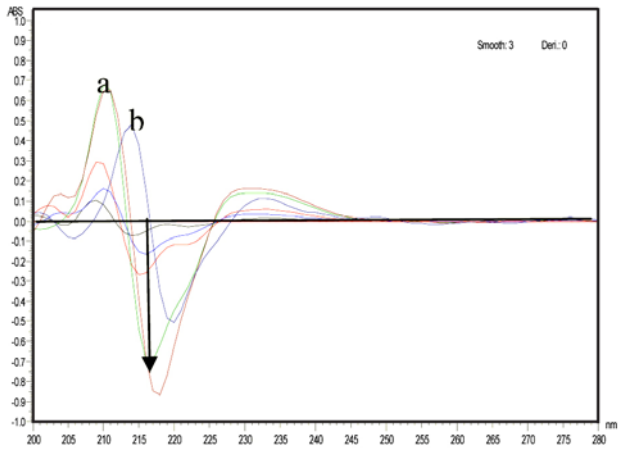

Figure 3b: Second-derivative spectrophotometric spectra of different concentrations of FXD (a) in the presence of its alkaline degradation product (b).

The same zero crossing points were used for the separation of FXD in the presence of its acidic degradation product. The first and second derivative spectra of the studied drug in the presence of its oxidative degradation product illustrate that FXD can be measured at 211 and 215 nm, respectively. The first derivative spectra of FXD in the presence of its UV degradation product show overlapping spectra, hence second derivative spectra can be used for its determination by using a zero crossing point of 211 nm.

It is necessary to record first the normal synchronous fluorescence spectra of FXD in the presence of its different degradation products to derive the first and second-derivative synchronous spectra. The synchronous fluorescence spectra of different concentrations of FXD were recorded at 225 nm in presence of its different degradation products. This is illustrated in Figure 4 showing SFS of FXD in presence of its alkaline degradation product.

There is a great overlap of the spectra of the drug and its degradation products in normal synchronous spectroscopy; this encouraged us to perform first and second derivative synchronous fluorescence spectroscopy technique without a prior extraction or separation step.

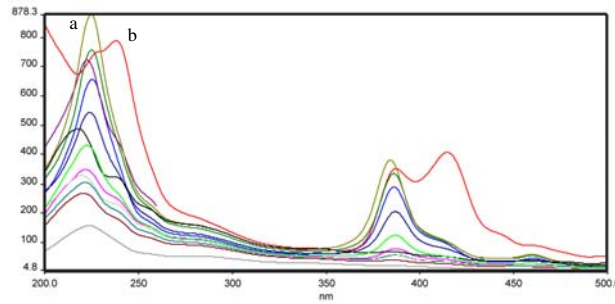

Figure 4: Synchronous fluorescence spectra of different concentrations of FXD (a) in the presence of its alkaline degradation product (b).

First and second derivative synchronous fluorescence spectra of FXD in the presence of its alkaline degrada-

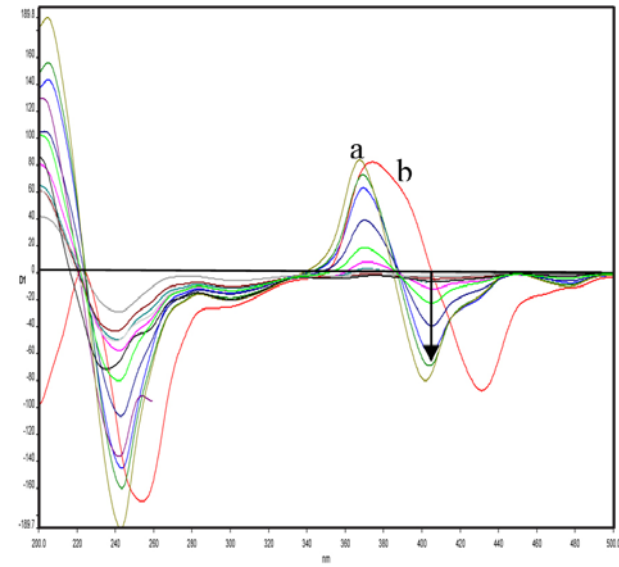

Figure 5a: First-derivative synchronous fluorescence spectra of different concentrations of FXD (a) in the presence of its alkaline degradation product (b).

tion product (Figs. 5 a–b) show that FXD could be measured without interferences at 406 and 225 nm.

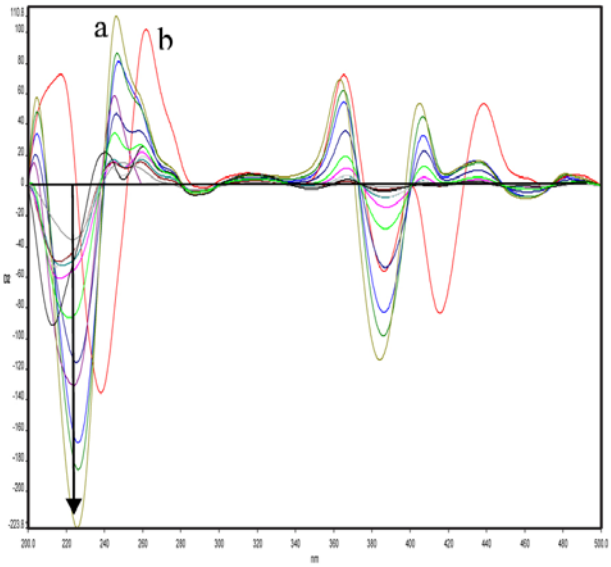

Figure 5b: Second-derivative synchronous fluorescence spectra of different concentrations of FXD (a) in the presence of its alkaline degradation product (b).

Similar results for the separation of FXD in the presence of its acidic degradation product were obtained. FDSFS and SDSFS of the studied drug in the presence of its oxidative and UV degradation products showed that FXD could be determined at 367 and 225.

3. 1. Optimization of Experimental Conditions

Different experimental parameters affecting the stability of the studied compound were carefully studied and optimized. Such factors were changed individually, while others were kept constant. These factors include pH and volume of the buffer, wavelength interval Δλ, type and volume of the diluting solvent and measurement stability.

3. 1. 1. Method I

3. 1. 1. 1. Wavelength Interval Δλ

The optimum Δλ value is very important for performing the derivative scanning technique concerning resolution, sensitivity, and features. It can directly influence spectral shape, bandwidth, and signal value. Wavelength intervals Δλ of 6 and 8 nm were used to determine FXD in presence of either its alkaline or acidic degradation products applying first and second derivative techniques, respectively. When the drug was determined in the presence of its oxidative degradation product, wavelengths intervals Δλ of 8 and 10 were used for first and second derivative spectrophotometric measurements, respectively. On the other hand, a wavelength interval Δλ of 6 was applied to measure the studied drug in the presence of its UV degradation product by second derivative spectrophotometry. These values for Δλ were chosen since they resulted in distinct well separated peaks, with good shape, and highest absorbance values.

3. 1. 2. Method II

3. 1. 2. 1. Buffer Type and pH

Although various types of buffers (phosphate, citrate, or Britton Robinson buffer) under the same pH regime gave the same results, borate and acetate buffers were chosen as having less potential interferences.

The influence of pH on the synchronous fluorescence intensity (SFI) values was investigated over the pH range 3.5–5.6 using acetate buffers and from 6–8.5 using borate buffers. Maximum and constant SFI values were achieved using borate buffer in the pH range 7.5–8.5. A borate buffer at pH 8 ± 0.5 was regarded as optimum in this study.

3. 1. 2. 2. Buffer Volume

The effect of the volume of the borate buffer at pH 8 on the SFI value of the studied drug was also studied. It was found that increasing the buffer volume resulted in a subsequent increase in the SFI values of FXD up to 2.5 ml, after which the SFI remained constant. A volume of 3 ± 0.5 ml borate buffer at pH 8 was regarded as optimum in this study.

3. 1. 2. 3. Wavelength Interval Δλ

The optimum Δλ value can directly influence spectral shape, bandwidth, and signal value. For this reason, a wide range of Δλ (40, 60, 80, 100, and 120 nm) was examined. Wavelength intervals Δλ of 80, 60 or 120 nm were chosen as optimal for separation of FXD in presence of its (alkaline and acidic), oxidative, or UV degradation products respectively, since it resulted in distinct well-separated peaks, with good shape, and highest SFI values.

3. 1. 2. 4. Diluting Solvent

Dilution with different diluents such as water, methanol, acetonitril, isopropanol, dimethylsulfoxide and dimethylformamide was performed. The SFI of FXD increased in methanol more than the other diluents; therefore, it was selected as the best diluent in this study.

3. 1. 2. 5. Volume of Methanol

The effect of the volume of methanol was also studied, and it was found that the SFI of FXD increased gradually by increasing the volume of methanol, then it remained constant after the addition of 5 ml.

3. 1. 2. 6. Measurement Stability

The stability of the fluorescence emission measurement was found to develop instantaneously and remain stable for more than 2 h.

3. 2. Analytical Performance

The peak amplitude – concentration plots for the studied drug by either first or second derivative spectrophotometry (method I) or by FDSFS and SDSFS (method II) in the presence of its different degradation products were linear over the concentration ranges cited in Table 1. The quality of the calibration curves is evident from the close to unity value of the correlation coefficients and the value of the intercept being close to zero.

Table 1. Performance data of the proposed methods.

	Method I						
Paramater	**Alkaline degradation**		**Acidic degradation**		**Oxidative degradation**		**UV degradation**
	First derivative	**Second derivative**	**First derivative**	**Second derivative**	**First derivative**	**Second derivative**	**Second derivative**
Concentration range (µg/ml)	3.0–30.0	3.0–30.0	3.0–30.0	3.0–30.0	3.0–30.0	3.0–30.0	3.0–30.0
LOD(µg/ml)	1.84	1.4	0.42	0.089	1.64	1.42	0.43
LOQ(µg/ml)	5.56	4.25	1.28	0.27	4.96	4.31	1.3
Correlation coefficient (r)	0.9995	0.9997	0.9995	0.9999	0.9996	0.9997	0.9998
Slope	0.013	0.028	0.013	0.026	0.021	0.027	0.029
Intercept	0.0089	0.0139	0.0017	0.00072	0.0177	0.0189	–0.00039
$S_{y/x}$, *S.D. of residuals*	0.0108	0.0178	0.0078	0.00093	0.016	0.0174	0.0058
S_a ,*S.D. of intercept*	0.0071	0.0118	0.00515	0.00061	0.0106	0.0115	0.0038
S_b ,*S.D. of slope*	0.0004	0.00068	0.00029	0.000035	0.00059	0.00065	0.00022

	Method II							
Paramater	**Alkaline**		**Acidic**		**Oxidative**		**UV**	
	FDSFS	**SDSFS**	**FDSFS**	**SDSFS**	**FDSFS**	**SDSFS**	**FDSFS**	**SDSFS**
Concentration range (µg/ml)	0.2–2.0	0.2–2.0	0.2–2.0	0.2–2.0	0.2–2.0	0.2–2.0	0.2–2.0	0.2–2.0
LOD(µg/ml)	0.134	0.048	0.134	0.088	0.111	0.067	0.105	0.063
LOQ(µg/ml)	0.405	0.145	0.405	0.268	0.335	0.204	0.317	0.189
(r)	0.9993	0.9991	0.9994	0.9997	0.9997	0.9998	0.9997	0.9998
Slope	37.75	105.7	34.34	105	40.94	108.8	41.11	104.7
Intercept	0.137	–0.0276	0.0488	–0.0071	0.068	0.00201	0.079	–0.011
$S_{y/x}$	1.97	1.77	1.79	3.62	1.654	2.86	1.704	2.56
S_a	1.53	1.53	1.39	2.81	1.372	2.217	1.302	1.986
S_b	1.26	1.45	1.14	2.31	1.009	1.869	1.009	1.674

3. 2. 1. Sensitivity

The limit of quantification (LOQ) was calculated according to ICH Q2B recommendations[18] according to the following equation: LOQ= 10σ/S where σ is the standard deviation of the intercept of the regression line and S the slope of the calibration curve. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected, and it is calculated according to the following equation[18]: LOD= 3.3 σ/S. The calculated values for both methods are listed in Table 1.

3. 2. 2. Analysis of FXD in the Presence of its Degradation Products Using the Proposed Methods

Both methods were successfully applied to determine FXD in the presence of its different degradation products presenting a ratio of 30% degradation of the parent drug as stated by ICH guidelines[18]. The amplitude values of the first and the second-derivative spectra (method I) or the synchronous fluorescence intensities of the first and the second-derivative peaks (method II) were also measured. The concentrations of the drug were calculated according to the linear regression equation of the calibration graphs. The results obtained regarding RSD were compared with those obtained using a comparison method[5]. The results indicate a high accuracy of the proposed methods as shown in Table 2.

3. 3. Validation of the Proposed Methods

The methods were tested for linearity, selectivity, accuracy and precision. Linear regression equations were obtained. The regression plots showed that there was a linear dependence of peak amplitude values on the concentration of the drug over the ranges cited in Table 1. The validity of the proposed methods was evaluated by statistical analysis of the regression data regarding the standard deviation of the residual ($S_{y/x}$), the standard deviation of the intercept (S_a), and standard deviation of the slope (S_b)[20]. The results are shown in Table 1. The small statistical errors point to a low scattering of the points around the calibration graph and a high precision of the proposed methods.

3. 3. 1. Accuracy

The accuracy of the proposed methods was evalua-

Table 2. Determination of FXD in the presence of its degradation products by the proposed methods.

	Method I				Method II				
Compound	**taken (µg/ml)**	**% Recovery**		**Comparison method[5]**	**taken (µg/ml)**	**% Recovery**			**Comparison method[5]**
		FDS	**SDS**	**% Recovery**		**SFS**	**FDSFS**	**SDSFS**	**% Recovery**
FXD in presence	3.0	99.57	99.87	99.68	0.2	100.45	99.10	100.50	100.25
of its acidic	5.0	100.42	100.96	100.52	0.5	99.6	100.20	100.40	100.12
degradation	10.0	99.65	99.98	99.35	1.0	99.81	99.70	100.90	100.99
product	15.0	99.63	99.71	1.25	99.84	100.08	99.92		
	20.0	100.18	99.67	1.5	100.67	99.93	100.07		
	25.0	100.50	99.94	1.75	99.83	99.89	99.89		
	30.0	100.59	99.87	2.0	100.90	99.90	99.90		
$\bar{X}$ ± SD		100.08±	100.0±	99.85±		100.12±	99.92±	100.16±	100.40±
		0.45	0.44	0.6		0.48	0.35	0.39	0.47
t test		*0.29	0.26			0.41	0.22	0.59	
F test		*1.78	1.86			1.04	1.80	1.45	
FXD in presence	3.0	100.93	100.40	99.68	0.2	99.50	99.0	99.50	100.25
of its alkaline	5.0	99.70	100.30	100.52	0.5	100.40	100.20	100.40	100.12
degradation	10.0	99.88	99.63	99.35	1.0	100.80	99.70	100.90	100.99
product	15.0	100.83	99.97		1.25	99.92	99.84	99.92	
	20.0	100.18	100.59		1.5	100.13	99.87	100.07	
	25.0	99.86	99.95		1.75	99.94	99.94	99.83	
	30.0	100.39	99.88		2.0	99.90	100.45	99.85	
$\bar{X}$ ± SD		100.2±	100.11±	99.85±		100.0±	99.82±	100.01±	100.40±
		0.49	0.34	0.6		0.39	0.43	0.45	0.47
t test		0.19	0.71			0.41	0.93	0.93	
F test		1.49	3.11			1.31	1.19	1.09	
FXD in presence	3.0	99.87	99.47	99.68	0.2	100.40	100.98	100.45	100.25
of its oxidative	5.0	99.74	99.84	100.52	0.5	99.11	99.40	99.12	100.12
degradation	10.0	100.51	101.13	99.35	1.0	100.20	99.91	99.80	100.99
product	15.0	99.95	99.89		1.25	99.84	99.92	100.08	
	20.0	99.67	99.61		1.5	100.13	99.93	99.81	
	25.0	99.82	100.45		1.75	99.94	100.06	99.88	
	30.0	101.12	99.86		2.0	100.45	99.93	100.23	
$\bar{X}$ ± SD		100.09 ±	100.04 ±	99.85 ±		100.0 ±	100.02 ±	99.91 ±	100.40 ±
		0.53	0.57	0.6		0.43	0.44	0.39	0.47
t test		0.51	0.76			0.49	0.102	0.76	
F test		1.28	1.11			1.19	1.14	1.45	
Compound	**taken (µg/ml)**	**% Recovery**		**Comparison method[5]**	**taken (µg/ml)**	**% Recovery**			**Comparison method[5]**
			SDS	**% Recovery**		**SFS**	**FDSFS**	**SDSFS**	**% Recovery**
FXD in presence	3.0		100.37	99.68	0.2	100.99	100.97	100.45	100.25
of its UV	5.0		100.24	100.52	0.5	100.20	99.80	99.60	100.12
degradation	10.0		101.06	99.35	1.0	99.97	99.80	99.70	100.99
product	15.0		99.75		1.25	99.92	100.08	99.84	
	20.0		99.77		1.5	99.93	100.07	99.87	
	25.0		99.65		1.75	100.06	99.83	99.94	
	30.0		99.77		2.0	100.40	99.95	99.10	
$\bar{X}$ ± SD			100.09 ±	99.85 ±		10.17 ±	100.03 ±	99.83 ±	100.40 ±
			0.51	0.6		0.38	0.40	0.39	0.47
t test			0.54			0.39	0.34	0.18	
F test			1.38			1.53	1.38	1.45	

* 1.94, 5.14 are the tabulated t and F values at P = 0.05[20]

ted by analyzing standard solutions of the studied drug. The results obtained by the proposed methods were favorably compared with those obtained by a comparison method[5]. The percentage recoveries (Table 2) show excellent accuracy. Statistical analysis[20] of the results obtained by the proposed and comparison methods using student's t-

test and variance ratio F- tests, revealed no significant differences between the performance of both methods (Table 2). The comparison method[5] recommended a spectrophotometric determination of FXD through formation of an ion pair complex with eosine in the presence of Mclivaine's citric acid phosphate buffer at pH 3 with measurements carried out at λ_{max} 540 nm (for method I), and also a spectrofluorimetric determination of FXD through quenching the fluorescence of eosine and carrying out measurements at a λ_{em}/ λ_{ex} ratio of 545 nm/310 nm (for method II).

3. 3. 2. Precision

3. 3. 2. 1. Repeatability

The repeatability was evaluated through the replicate analysis of different concentrations of the drug, either in pure drug or in dosage forms. The mean percentage recoveries based on the average of three separate determinations for pure and dosage forms are abridged in Table 3.

3. 3. 2. 2. Intermediate Precision

Intermediate precision was obtained through replicate analysis of different concentrations of the drug, either in pure or dosage forms on three successive days. The percentage recoveries are based on the average of three separate determinations. The results are shown in Table 3. The data indicate that the proposed methods are highly precise during one run and between different runs.

3. 5. Degradation Kinetics Study

For the kinetic study, 2 M sodium hydroxide, 2 M hydrochloric acid, or 6% hydrogen peroxide were used for alkaline, acidic, or oxidative degradation of the drug. Regarding the UV degradation, the methanolic solution of FXD was exposed to a Deuterium lamp in a wooden cabinet at distance of 15 cm for different time intervals. The degradation was found to be temperature-dependent as represented by the alkaline degradation in Figure 6.

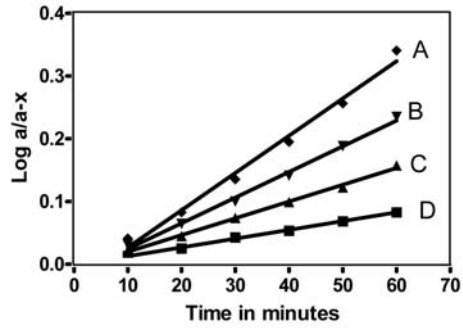

Figure 6: Semilogarithmic plot of FXD (20μg/ml) versus different heating times in the presence of 2M NaOH (A = 80 °C, B = 70 °C, C = 60 °C, and D = 50 °C).

The first order degradation rate constant and the half life time at each temperature were calculated (Table 5) according to the following equations:

Table 3. Validation of the proposed methods for determination of FXD in pure and dosage forms.

Preparation	Method I		Method II	
	Repeatability, % Recovery	Intermediate precision, %Recovery	Repeatability, % Recovery	Intermediate precision, %Recovery
Fexofenadine pure form	(3.0 μg/ml)	(15.0 μg/ml)	(1.0 μg/ml)	(1.5 μg/ml)
$\bar{X}$ ± SD	99.89 ± 0.65	100.16 ± 0.61	100.14 ± 0.63	100.05 ± 0.63
Fastofen® tablets (60 mg FXD / tablet)	(10.0 μg/ml)	(20.0 μg/ml)	(0.75 μg/ml)	(2.0 μg/ml)
$\bar{X}$ ± SD	99.67 ± 0.57	99.92 ± 0.83	99.64 ± 0.57	100.12 ± 0.56
Fastofen® tablets (120 mg FXD / tablet)	(30.0 μg/ml)	(7.0 μg/ml)	(0.5 μg/ml)	(1.25 μg/ml)
$\bar{X}$ ± SD	100.36 ± 0.57	99.65 ± 0.51	99.58 ± 0.49	100.11±0.71
Fexodine® capsules (180 mg FXD / cap.)	(25.0 μg/ml)	(10.0 μg/ml)	(1.2 μg/ml)	(0.75 μg/ml)
$\bar{X}$ ± SD	100.58 ± 0.51	99.98 ± 0.65	100.44 ± 0.36	99.32 ± 0.29

3. 4. Application to Pharmaceutical Preparations

The proposed methods were successfully applied to the determination of FXD in its commercial tablets. The results are summarized in Table 4. After testing different constituents of tablets matrix such as talc, magnesium stearate, lactose, starch, etc.., no interference from the sample matrix was observed on the proposed methods. The results were found to be in good agreement with the labeled amount.

ln a/a-x = Kt, where a is the initial concentration of the drug, x is the concentration of the resulting degraded solution after time t, and K is the reaction rate constant. The half life time was calculated from $t_{1/2}$ = 0.693/K.

Through plotting log K values *versus* 1/T, the Arhenius plot was obtained as exemplified by Figure 7, representing the alkaline degradation. The activation energy for each type of degradation was also calculated from ln K = – Ea/RT + ln A, where Ea is the activation energy, K is first order reaction rate constant, R is the gas constant, and T is the temperature (K).

Table 4. Determination of FXD in its dosage forms by the proposed methods.

Preparation		Method I			Method II	
	Amount taken (µg/ml)	% Recovery	Comparison method[5], % Recovery	Amount taken (µg/ml)	% Recovery	Comparison method[5], % Recovery
Fastofen® tablets	5.0	100.46	99.63	0.25	99.60	100.25
(60 mg FXD / tablet)	10.0	99.98	99.21	0.5	99.60	99.34
	15.0	100.97	100.45	1.0	100.90	100.12
	20.0	100.48		1.5	99.80	
	25.0	99.86		1.75	99.89	
	30.0	100.21		2.0	100.50	
$\bar{X}$ ± SD		100.33 ± 0.41	99.76 ± 0.63		100.05 ± 0.53	99.90 ± 0.49
t test		*0.13			0.49	
F test		*2.36			1.17	
Fastofen® tablets	5.0	99.92	100.32	0.25	100.40	100.32
(120 mg FXD / tablet)	10.0	100.54	100.96	0.5	100.40	100.97
	15.0	100.15	100.06	1.0	99.80	100.05
	20.0	100.51		1.5	99.93	
	25.0	100.46		1.75	99.83	
	30.0	100.55		2.0	100.45	
$\bar{X}$ ± SD		100.36 ± 0.26	100.45 ± 0.46		100.14 ± 0.312	100.45 ± 0.47
t test		0.03			0.59	
F test		3.1			2.27	
Fexodine® capsules	5.0	99.94	100.65	0.25	99.21	99.97
(180 mg FXD / cap.)	10.0	100.22	100.12	0.5	100.10	99.21
	15.0	100.71	99.84	1.0	100.80	100.15
	20.0	100.33		1.5	99.80	
	25.0	99.86		1.75	99.71	
	30.0	99.77		2.0	99.85	
$\bar{X}$ ± SD		100.14 ± 0.35	100.21 ± 0.41		99.91 ± 0.52	99.78 ± 0.49
t test		0.61			0.71	
F test		1.37			1.13	

* 2.31 and 5.79 are the tabulated t and F values at P = 0.05[20]

Table 5. Effect of temperature on the kinetic parameters of FXD.

Temperature (°C)	Alkaline degradation		Acidic degradation		Oxidative degradation	
	K ($min.^{-1}$)	$t_{1/2}$ (min.)	K ($min.^{-1}$)	$t_{1/2}$ (min.)	K ($min.^{-1}$)	$t_{1/2}$ (min.)
50	0.001400	495	0.002028	342	0.001400	495
60	0.00267	260	0.004929	141	0.00267	260
70	0.004089	169	0.009751	71	0.004089	169
80	0.005943	117	0.01338	52	0.005943	117
Ea = ($K.Cal.mol^{-1}$)	**10.7**		**14.46**		**10.7**	

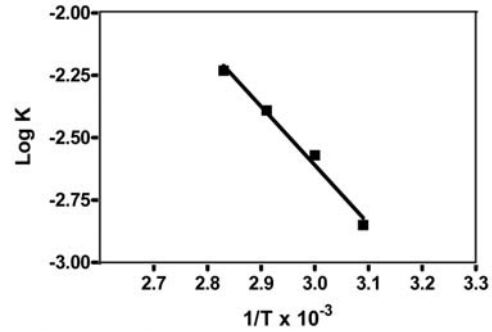

Figure 7: Arrhenius plot for the degradation of FXD (20µg/ml) in 2 M NaOH

4. Conclusion

Derivative spectrophotometry and synchronous spectrofluorimetry were successfully applied for the analysis of fexofenadine hydrochloride in the presence of its different degradation products using simple procedures. The proposed methods were also used for the determination of the studied compound in commercial tablets. The results obtained were in good agreement with those obtained by the comparison method. Detailed kinetic study showed that the proposed methods were stability-indicating.

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Povzetek

Za analizo feksofenadin hidroklorida (FXD) v prisotnosti različnih razgradnih produktov sta bili razviti dve hitri, enostavni, občutljivi, selektivni derivativni spektrofotometrični ([D^1] in [D^2]) in spektrofluorometrični metodi (FDSFS in SDSFS). Za merjenje FXD pri 223 nm v prisotnosti njenih kislih in bazičnih razgradnih produktov in pri 211 nm v prisotnosti oksidacijskih razgradnih produktov je bila uporabljena derivativna spektrofotometrija (D^1). Za merjenje FXD pri 217 nm v prisotnosti njenih kislih in bazičnih razgradnih produktov in pri 215 nm v prisotnosti oksidacijskih razgradnih produktov je bila uporabljena (D^2) derivativna spektrofotometrija; UV razgradni produkt je bil merjen pri 211 nm. Sinhrona spektrofluorometrija (FDSFS) je bila uporabljena za merjenje FXD v prisotnosti njenih bazičnih ali kislih razgradnih produktov pri 406 nm in pri 367 nm v prisotnosti oksidativnih ali UV razgradnih produktov. Sinhrona spekrofluorometrija (SDSFS) je bila uporabljena za določanje učinkovine pri 225 nm in v prisotnosti njenih kislih, bazičnih, oksitacijskih ali UV razgradnih produktov. Predstavljena metoda je bila uspešno uporabljena za določanje proučevane učinkovine v komercialno dostopnih tabletah. Dobljeni rezultati se dobro ujemajo s primerjalno metodo.