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Original Article

SPECTROPHOTOMETRIC AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FEXOFENADINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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Abstract

*In present study, three new spectrophotometric methods, original UV spectrophotometry, first and second order derivative UV spectrophotometry and a new HPLC method were developed for the determination of fexofenadine HCl (**FEX**) in pharmaceutical preparations. In original UV spectrophotometry, absorbances were measured at 258.7 nm in the zero order UV spectra of the solution of **FEX** in methanol-water (1:1) in the range of 220 - 290 nm. In first derivative UV spectrophotometry, $dA/d\lambda$ values were measured at 270.4 nm in the first derivative UV spectra of the solution of **FEX** in methanol-water (1:1) in the range of 245 - 285 nm ($\Delta\lambda$= 2 nm). In second derivative UV spectrophotometry $d^2A/d\lambda^2$ values were measured at 252.84 nm in the second derivative UV spectra of the solution of **FEX** in methanol-water (1:1) in the range of 245 - 285 nm ($\Delta\lambda$= 2 nm). Linearity range was found as 100.0 – 1000.0 μg/mL in all the spectrophotometric methods. Mean recoveries and the relative standard deviations of the methods were found as 99.55 % and 1.10 % in original UV spectrophotometry, 100.97 % and 1.09 % in first derivative UV spectrophotometry and, 99.25 % and 1.10 % in second derivative UV spectrophotometry respectively. In HPLC method, an isocratic system consisted of an ACE C18 analytical column and a mobile phase composed of methanol - phosphate buffer (pH 3.0, 0.1 M) (95:5, v/v) at a flow rate 1.0 mL/min was used for the optimal chromatographic separation using UV detection at 220 nm. Diflucortolone valerate was used as internal standard. Mean recoveries and the relative standard deviations was found as 100.23 % and 0.54 % in HPLC method. All the methods developed were successfully applied to five tablet formulations commercially available in Turkish drug market and the results were compared statistically with each other.*

Keywords: *Fexofenadine hydrochloride, Spectrophotometry, HPLC, Determination, Pharmaceutical Preparation*

Feksofenadin Hidroklorür'ün Farmasötik Preparatlarda Spektrofotometrik Ve Yüksek Performanslı Sıvı Kromatografik Yöntemlerle Miktar Tayini

*Bu çalışmada feksofenadin HCl (**FEX**)' ün farmasötik preparatlarda miktar tayini için üç yeni spektrofotometrik yöntem, orijinal UV spektrofotometri, birinci ve ikinci türev spektrofotometri,ve yeni bir YPSK yöntemi geliştirilmiştir. Orijinal UV spektrofotometride; absorbans değerleri, **FEX**' in metanol-su (1:1) içerisindeki çözeltilerinin 220-290 nm aralığındaki UV spektrumlarında 258.7 nm de ölçülmüştür. Birinci türev UV spektrofotometride; $dA/d\lambda$ değerleri, **FEX'** in metanol-su (1:1) içerisindeki çözeltilerinin 245-285 nm aralığındaki birinci türev UV spektrumlarında ($\Delta\lambda$= 2 nm) 270.4 nm de ölçülmüştür. İkinci türev UV spektrofotometride; $d^2A/d\lambda^2$ değerleri **FEX** 'in metanol-su (1:1) içerisindeki çözeltilerinin 245-285 nm aralığındaki ikinci türev UV spektrumlarında ($\Delta\lambda$= 2 nm) 252.84 nm de ölçülmüştür. Her üç spektrofotometrik yöntem için de doğrusal çalışma aralığı 100.0 – 1000.0 μg/mL olarak bulunmuştur. Yöntemlerdeki ortalama geri kazanım ve bağıl standart sapma değerleri sırasıyla orijinal UV spektrofotometride % 99.55 ve % 1.10, birinci türev UV spektrofotometride, % 100.97 ve % 1.09 ve ikinci türev UV spektrofotometride % 99.25 ve % 1.10 olarak bulunmuştur. YPSK yönteminde, ACE C18 kolonu, mobil faz olarak 1.0 mL/dk akış hızında metanol – fosfat tamponu (pH 3.0, 0.1 M) (95:5,h/h)' ndan oluşan izokratik sistem ve 220 nm de deteksiyon optimal kromatografik şartlar olarak belirlenmiştir. Diflukortolon valerat iç standart olarak seçilmiştir. YPSK yönteminde ortalama geri kazanım ve bağıl standart sapma değerleri sırasıyla 100.23 % ve 0.54 % olarak bulunmuştur. Geliştirilen tüm yöntemler Türkiye ilaç piyasasında bulunan 5 adet tablet formulasyonuna başarıyla uygulanmıştır. Elde edilen tüm sonuçlar kendi aralarında istatistiksel olarak karşılaştırılmıştır.*

Anahtar kelimeler: *Feksofenadin hidroklorür , Spektrofotometri, YPSK, Miktar Tayini, Farmasötik Preparat*

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INTRODUCTION

Fexofenadine (Figure 1) is an oral, second generation antihistamine that is used to treat the signs and symptoms of allergy that are due to histamine. It is similar to the other second generation antihistamines loratadine, cetirizine and azelastine. Histamine is a chemical that is responsible for many of the signs and symptoms of allergic reactions. Histamine is released from histamine-storing cells (mast cells) and then attaches to other cells that have receptors for histamine. The attachment of the histamine to the receptors causes the cell to be activated, releasing other chemicals that produce the effects that we associate with allergy, e.g., sneezing. Fexofenadine blocks the H1 receptor for histamine and thus prevents activation of H1 receptor-containing cells by histamine. Unlike the first generation antihistamines, fexofenadine and other second-generation antihistamines do not readily enter the brain from the blood, and, therefore, they cause less drowsiness.

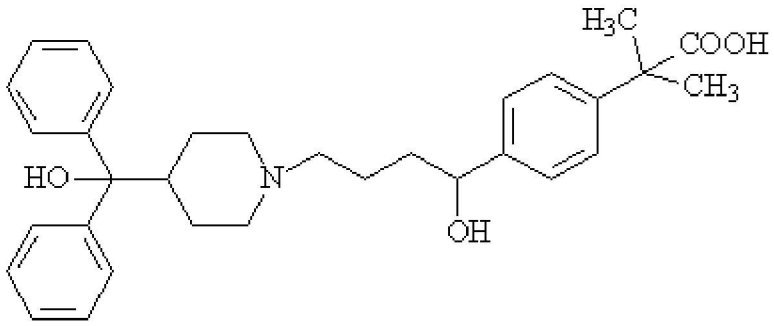

Figure 1. Fexofenadine

In previous studies; the determination of fexofenadine in pharmaceutical preparations containing only fexofenadine was made by using several methods including spectrophotometry (1), HPLC (2,3), voltammetry (4) and CE (5,6). Simultaneous determination of fexofenadine and pseudoephedrine sulfate in tablets was realized by using HPLC (7). The determination of fexofenadine in biological liquids was made by using HPLC (8-12). However, no information concerning with the determination of fexofenadine hydrochloride in pharmaceutical preparations by using classical UV spectrophotometry and derivative UV spectrophotometric methods could be seen in the literatures.

EXPERIMENTAL

Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.

An Agilent Technologies HP 1100 chromatographic system was used equipped with a model series of G13 79A degasser, G1311A quaternary pump, 61313A injector and G1315B DAD detector. ACE C18 column 250 x 4.6 mm, 5 μm particle sized was used.

Materials

Fexofenadine HCl, was kindly donated by FAKO Pharm.Ind., Turkey and used without further purification.

All the materials used in the spectrophotometric and high performance liquid chromatographic analysis were of analytical reagent grade.

Standard solutions

Standard solutions of fexofenadine HCl (500 mg / 250 mL) were prepared in methanol - water (1:1) for spectrophotometric and in methanol for high performance liquid chromatographic methods.

Commercial pharmaceutical preparations assayed

Commercial name	*Content*	*Batch no.*	*Firm*
FEKSİNE	120 mg feksofenadin HCl/film tablet	001	Drogsan
FEXADYNE	120 mg feksofenadin HCl/film tablet	7C121	Ali Raif
FEXOFEN	120 mg feksofenadin HCl/film tablet	09019001	Sanovel
VİVAFEKS	120 mg feksofenadin HCl/film tablet	7010464	Fako
TELFAST	120 mg feksofenadin HCl/film tablet	BNN338MFD	Aventis Pharma

Sample preparation

For spectrophotometric determinations: The content of 20 tablets were accurately weighed and powdered in a mortar. An amount of mass equivalent to one tablet was weighed in 50 mL volumetric flask and diluted to volume with methanol-water (1:1). After 45 min of mechanically shaking and 15 min of standing in the dark the solution was filtered through 4.5 μm milipore filter. Portion of the initial 5 mL was discarded and 10 ml of filtered solution was put into a 50 ml volumetric flask and the volume was completed to 50 mL with the same solvent. Final solution was used for the determinations.

For liquid chromatographic determinations: The content of 20 tablets were accurately weighed and powdered in a mortar. An amount of mass equivalent to one tablet was weighed in 50 mL volumetric flask and diluted to volume with methanol. After 45 min of mechanically shaking and 15 min of standing in the dark the solution was filtered through 4.5 μm milipore filter. Portion of the initial 5 mL was discarded and 2.5 ml of filtered solution was put into a 25 ml volumetric flask and the volume was completed to 25 mL with the same solvent. Final solution was used for the determinations.

RESULTS

Spectrophotometric analysis

Original UV spectrophotometry

There are one maxima (258.70 nm) and one shoulder point (253.70 nm) in zero-order UV spectra of the solution of fexofenadine HCl (**FEX**) in methanol - water (1:1) in the range of 220-290 nm (Figure 2). The determination of **FEX** can be realized by measuring the absorbances at this

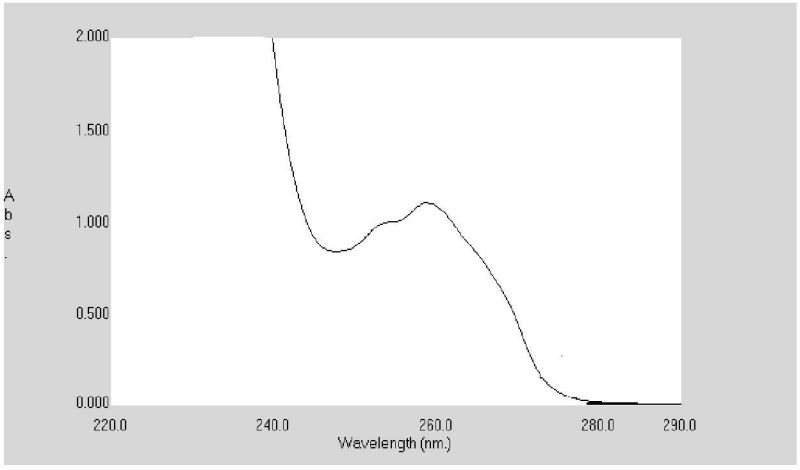

Figure 2. UV spectrum of the 1000 µg/mL solution of **FEX** in methanol - water (1:1) .

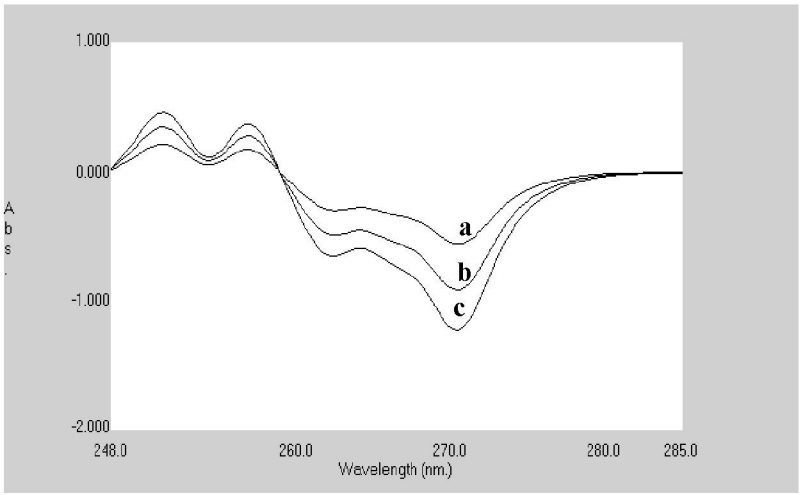

Figure 3. First derivative spectra of the solution of a) 200 µg /mL, b) 700 µg /mL , c) 1000 µg/mL **FEX** in methanol - water (1:1) ($\Delta\lambda$ = 2 nm) (Scaling factor = 10).

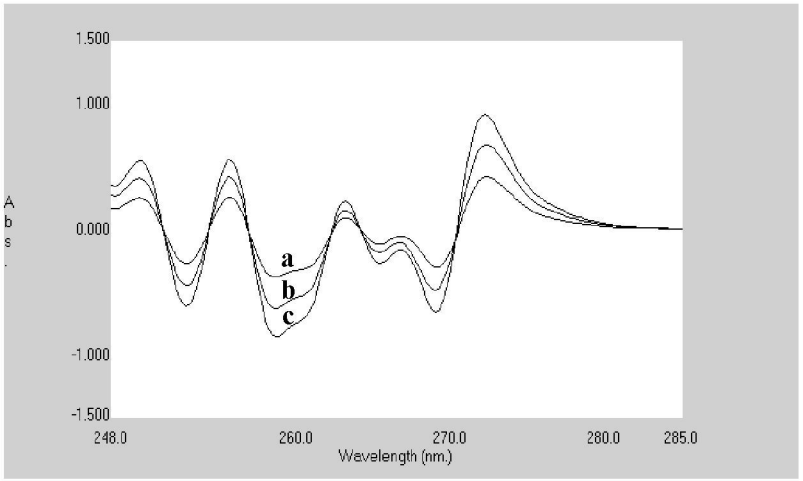

Figure 4. Second derivative spectra of the solution of a) 200 µg /mL, b) 700 µg /mL,c) 1000 µg/mL **FEX** in methanol - water (1:1) ($\Delta\lambda$ = 2 nm) (Scaling factor = 30).

wavelength and using the calibration curve prepared by plotting the absorbances versus ten different concentrations of standard substance. Linearity range according to the Beer's law was found as 100.0 – 1000.0 μg/mL in the method. LOQ was 100.0 μg/mL and LOD was calculated as 33.3 μg/mL by using the following equation; 3.3 SD/m (SD=Standard deviation, m=slope). Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 2.

First derivative UV spectrophotometry

There are two maxima (251.4 and 256.9 nm) and two minimum (262.3 nm and 270.4 nm) in the first derivative spectra of the solution of **FEX** in methanol - water (1:1) in the range of 248 - 285 nm (Figure 3). Different Δλ values were tested and Δλ= 2 nm was found optimal in the method. The determination of fexofenadine can be realized by measuring the dA/dλ values at 251.4, 256.9, 262.3 and 270.4 nm and using the calibration curve prepared by plotting the dA/dλ values versus eight doses of standard substance. Linearity range according to the Beer's law was found as 100.0 – 1000.0 μg/mL in the method. LOQ was 100.0 μg/mL and LOD was calculated as 33.3 μg/mL by using the following equation; 3.3 SD/m (Regression parameters were shown in Table 1). Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 3.

Second derivative UV spectrophotometry

There are four maxima (249.80, 255.62, 263.19 and 272.22 nm) and four minima (252.80, 258.58, 265.40 ve 269.02 nm) in the second derivative spectra of the solution of **FEX** in methanol - water (1:1) in the range of 248-285 nm (Figure 4). Different Δλ values were tested and Δλ= 2 nm was found optimal in the method. The determination of fexofenadine can be realized by measuring the $d^2A/d\lambda^2$ values at the wavelengths mentioned above and using the calibration curve prepared by plotting the $d^2A/d\lambda^2$ values versus ten doses of standard substance. Linearity range according to the Beer's law was found as 100.0 – 1000.0 μg/mL in the method. LOQ was 100.0 μg/mL and LOD was calculated as 33.3 μg/mL by using the following equation; 3.3 SD/m Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 4.

Table 1. Regression parameters in spectrophotometric methods

Methods	λ (nm)	m	n	r	Working range (μg/ml)
original UV spectrophotometry	253.7	0.0011	-0.0616	0.9889	100.0 – 1000.0
	258.7	0.0013	-0.0713	0.9884	100.0 – 1000.0
(^{1}D) First derivative spectrophotometry	251.40	0.005	0.0105	0.9991	100.0 – 1000.0
	256.88	0.004	0.0092	0.9998	100.0 – 1000.0
	262.56	0.0007	0.0029	0.9997	100.0 – 1000.0
	270.45	0.0012	0.011	0.9999	100.0 – 1000.0
(^{2}D) Second derivative spectrophotometry	249.81	0.0005	0.0039	0.9986	100.0 – 1000.0
	252.85	0.0006	0.0014	0.9989	100.0 – 1000.0
	255.62	0.0006	0.0013	0.9996	100.0 – 1000.0
	258.59	0.0009	-0.0017	0,9998	100.0 – 1000.0
	263.19	0.0002	-0.101	0.9959	100.0 – 1000.0
	265.40	0.0003	-0.0031	0.9946	100.0 – 1000.0
	269.02	0.0007	-0.0035	0.9982	100.0 – 1000.0
	272.22	0.0009	0.0045	0.9995	100.0 – 1000.0

m = scope, n = intercept, y = mx + n, r = correlation coefficient.

Table 2. Validation parameters in classical UV spectrophotometry using standard solutions of **FEX** in methanol - water (1:1)

	253.7 nm			**258.7 nm**	
No	Added µg/mL	Found µg/mL	Recovery %	Found µg/mL	Recovery %
1	100.00	101.27	101.27	100.61	100.61
2	200.00	201.38	100.69	199.15	99.58
3	400.00	385.82	96.46	389.46	97.36
4	500.00	494.91	98.98	494.46	98.90
5	700.00	685.82	97.97	698.76	99.82
6	800.00	792.18	99.02	794.76	99.34
7	900.00	890.36	98.89	899.00	99.88
8	1000.00	1006.18	100.62	1009.00	100.90
		$\bar{X}$	99.24		99.55
		SD	1.49		1.09
		RSD	% 1.50		% 1.10

$\bar{X}$= mean, SD= standard deviation, RSD= relative standard deviation

Table 3. Validation parameters in first derivative UV spectrophotometry using standard solutions of **FEX** in methanol - water (1:1)

		251.4 nm		**256.9 nm**		**262.30 nm**		**270.4 nm**	
No	Added µg/mL	Found µg/mL	Recovery %	Found µg/mL	Recovery %	Found µg/mL	Recovery %	Found µg/mL	Recovery %
1	100.00	91.00	91.00	87.00	87.00	98.71	98.71	99.17	99.17
2	200.00	183.00	91.50	189.50	94.75	190.14	95.07	200.83	100.42
3	400.00	357.00	89.25	362.00	90.50	373.00	93.25	404.17	101.04
4	500.00	451.00	90.20	449.50	89.90	464.43	92.89	500.83	100.17
5	700.00	637.00	91.00	654.50	93.50	654.43	93.49	709.17	101.31
6	800.00	729.00	91.13	739.50	92.44	745.86	93.23	820.00	102.50
7	900.00	799.00	88.78	827.00	91.89	837.29	93.03	907.50	100.83
8	1000.00	915.00	91.50	929.50	92.95	944.43	94.44	1023.33	102.33
		$\bar{X}$	90.55		91.62		94.26		100.97
		SD	1.03		2.43		1.95		1.10
		RSD	% 1.14		% 2.65		% 2.07		% 1.09

Table 4. Validation parameters in second derivative UV spectrophotometry using standard solutions of **FEX** in methanol - water (1:1)

		249.80 nm		**252.84 nm**		**255.62 nm**		**258.58 nm**		**263.19 nm**		**265.40 nm**		**269.02 nm**		**272.22 nm**	
No	Added µg/mL	Found µg/mL	Rec. %	Found µg/mL	Rec. %	Found µg/mL	Rec. %	Found µg/mL	Rec. %	Found µg/mL	Rec. %	Found µg/mL	Rec. %	Found µg/mL	Rec. %	Found µg/mL	Rec. %
1	100.00	118.20	118.20	101.00	101.00	89.50	89.50	97.44	97.44	110.50	110.50	80.33	80.33	96.43	96.43	96.11	96.11
2	200.00	220.20	110.10	197.67	98.83	181.17	90.58	190.78	95.39	210.50	105.30	177.00	88.50	187.86	93.93	205.00	102.50
3	400.00	436.20	109.05	392.67	98.17	379.17	94.04	385.22	96.31	415.50	103.90	350.33	87.58	386.43	96.61	411.67	102.90
4	500.00	538.20	107.64	496.00	99.20	456.17	91.23	473.00	94.60	475.50	95.10	450.33	90.07	465.00	93.00	512.78	102.60
5	700.00	754.20	107.74	696.00	99.43	656.17	93.74	676.33	96.62	745.50	106.50	623.67	89.09	636.43	90.92	709.44	101.40
6	800.00	866.20	108.28	799.33	99.92	752.83	94.10	785.22	98.15	770.50	96.31	723.67	90.46	757.86	94.73	805.00	100.60
7	900.00	938.20	104.24	877.67	97.52	851.17	94.57	866.33	96.26	970.50	107.80	860.33	95.59	842.14	93.57	887.22	98.58
8	1000.00	1102.22	110.22	999.33	99.93	931.17	93.12	973.00	97.30	1030.50	103.10	917.00	91.70	925.00	92.50	1003.89	100.40
$\overline{X}$			109.43		99.25		92.61		96.51		103.60		89.17		93.96		100.60
SD			4.01		1.09		1.90		1.15		5.38		4.33		1.93		2.32
RSD			% 3.66		%1.10		%2.04		%1.19		%5.19		%4.85		%2.06		%2.30

Rec.= Recovery

High performance liquid chromatographic analysis

An new isocratic programme was developed for optimal separation of **FEX.** In the method, ACE C18 analytical column and mobile phase composed of methanol - phosphate buffer (pH 3.0, 0.1 M) (95:5, v/v) at a flow rate 1.0 mL/min and detection at 220 nm were found for the optimal chromatographic separation. Diflucortolon valerate (**DIF**) was selected as internal standard. Under the chromatographic conditions employed, **FEX** and **DIF** were well resolved and their retention times were found to be 2.64 and 3.48 min, respectively. A typical chromatogram of the drugs and internal standard was illustrated in Fig. 5. The values of suitability test are in the range of expected values which means that HPLC method used in this study is appropriate for the measurement of concentration of **FEX** using **DIF** as internal standard.

The calibration curves were established with ten different concentrations in the range of 20.0 - 400.0 μg/mL for standard solutions of **FEX**. A triplicate injection was carried out from each standard solution and the peak areas were measured at 220 nm. The ratios of the peak areas of investigated substances to that of internal standard were calculated for each injection. Regression equation was established by plotting the ratio of peak areas to the concentration of each substance. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression method. Regression equation was;

$y = 0.1356\ x + 0.4354)$ ($r^2 = 0.999$)

where x is the concentration of **FEX** as μg/mL, y is the ratio of peak areas.

LOQ was 20.0 μg/mL and LOD was calculated as 0.34 μg/mL by using the following equation; 3.3 SD/m (SD=Standard deviation, m=slope)

Mean recoveries and relative standard deviations calculated for standard solutions were shown in Table 5. Statistical values in the table indicate that the method is appropriate for determination of **FEX** with optimum recovery.

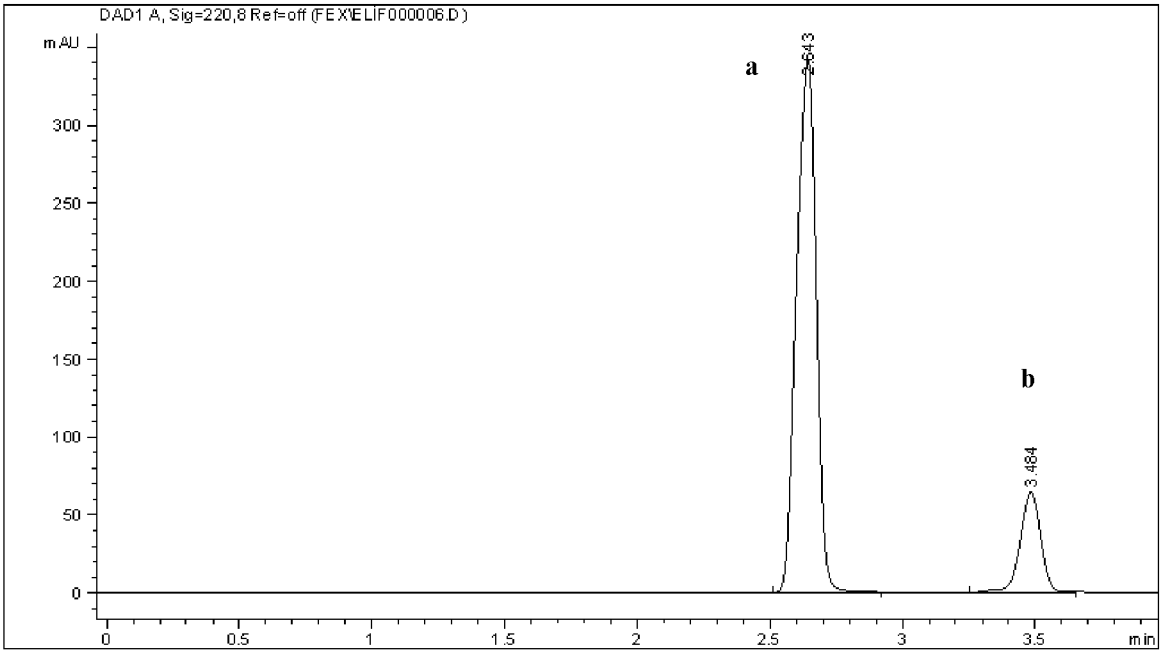

Figure 5. Chromatogram of the solution of a) 40 μg/mL **FEX** and b) 10 μg/mL **DİF** (IS) in methanol

Table 5. Validation parameters in high performance liquid chromatography using standard solutions of **FEX**

No	Added µg/mL	Found µg/mL	Recovery %
1	40.00	39.71	99.28
2	80.00	79.86	99.83
3	120.00	120.06	100.05
4	160.00	160.30	100.19
5	200.00	202.08	101.04
6	240.00	240.98	100.40
7	280.00	282.62	100.94
8	320.00	321.13	100.38
9	400.00	399.86	99.97
		$\bar{X}$	100.23
		SD	0.54
		RSD	% 0.54

Selectivity

According to official validation guidelines, in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product for determining recovery. For this reason, in order to know whether the excipients in the pharmaceutical preparation show any interference with the analysis, the recovery test was done by the standard addition method by adding known amounts of **FEX** at three different concentrations corresponding to 10, 25 and 50 % of the label claims. Each solution was prepared in triplicate and the methods were applied. According to the recoveries obtained for the amount of the added **FEX** (99.5 – 100.7 % for all the formulations selected) when applied three spectrophotometric methods at selected wavelengths (at 258.7 nm in original UV spectrophotometry, at 270.4 nm in first derivative UV spectrophotometry and at 252.84 nm in second derivative spectrophotometry) and for the amount of the added **FEX** (99.2 – 101.1 % for all the formulations selected) when applied high – performance liquid chromatographic method, it was concluded that there was no interference from the ingredients placed in the formulations.

Accuracy and Precision

Accuracy in the methods was determined by the recovery studies using standard solutions of **FEX.** In original UV spectrophotometry**:** the mean recoveries were found as 99.24 and 99.55 % at 253.7 and 258.7 nm respectively. Relative standard deviations at these wavelengths were found as 1.50 and 1.10 % respectively (Table 2). In first derivative UV spectrophotometric method; the mean recoveries were found as 109.42, 99.25, 94.26 and 100.97 % at 251.4, 256.9, 262.3 and 270.4 nm respectively. Relative standard deviations at these wavelengths were found

as 1.14, 2.65, 2.07 and 1.09 % respectively (Table 3). In second derivative UV spectrophotometric method; the mean the mean recoveries were found as 99.43, 98.37, 92.61, 96.51, 103.60, 89.17, 93.96 % and 100.60 % at 249.8, 252.84, 255.62, 258.58, 263.19, 265.40, 269.09 and 272.22 nm respectively. Relative standard deviations at these wavelengths were found as 3.66, 1.10, 2.04, 1.19, 5.19, 4.85, 2.06, 2.30 % (Table 4). In HPLC, mean recovery and relative standard deviation for **FEX** are 100.23 % and 0.54 % respectively.

Robustness

For spectrophotometric determinations, robustness was tested by changing the percentage of methanol and water. No significant difference was observed for any change. We selected methanol: water (1:1) for the methods proposed.

For high – performance liquid chromatographic analysis, robustness was tested by changing the percentage of methanol and phosphate buffer, pH and the concentration of phosphate buffer in mobile phase. Finally, we selected methanol-phosphate buffer (pH:3, 0.1 M) (95:5, v/v) as mobil phase for the method proposed.

Solution Stability

Solution of **FEX** in methanol - water (1:1) and in methanol is stable over three days at room temperature.

The behavior of the analyte remained unchanged up to about 3 days from their preparation. Results showed that samples are stable at least for one month, and changes during sample preparation and time of reading are found to be negligible.

Table 6. Assay results of commercial formulations for **FEX.**

Methods **Pharmaceutical preparatons**	**^{1}D** Mean (mg) ± SD (% RSD)	**^{2}D** Mean (mg) ± SD (% RSD)	**Original UV** Mean (mg) ± SD (% RSD)	**HPLC** Mean (mg) ± SD (% RSD)
FEKSİNE®	125.13 ± 2.21 (% 1.77)	124.43 ± 0.70 (% 0.56)	124.30 ± 1.81 (% 1.46)	124.53 ± 2.09 (% 2.38)
FEXADYNE®	123.45 ± 0.44 (% 0.36)	123.08 ± 0.92 (%0.75)	123.12 ± 0.40 (% 0.32)	123.66 ± 0.85 (% 0.67)
FEXOFEN®	119.30 ± 1.96 (% 1.64)	119.01 ± 0.76 (% 0.64)	118.74 ± 0.79 (% 0.67)	120.00 ± 1.13 (%0.93)
VİVAFEKS®	119.65 ± 1.29 (% 1.08)	119.16 ± 0.92 (% 0.77)	118.95 ± 0.84 (% 0.70)	119.34 ± 2.53 (%2.12)
TELFAST®	117.93 ± 0.40 (% 0.34)	117.80 ± 0.85 (% 0.72)	117.54 ± 0.43 (% 0.36)	116.58 ± 3.12 (% 2.68)

* Mean of ten replicates
** SD = Standard deviation,
*** RSD = Relative Standard deviation

Analysis of Pharmaceutical Preparations

Developed three methods were applied to the determination of **FEX** in pharmaceutical preparations selected 5 film tablets. Each pharmaceutical preparation was analyzed by performing ten independent determinations. In application, 258.7 nm in original UV spectrophotometry, 270.4 nm in first derivative spectrophotometry and 252.84 nm in second derivative spectrophotometry were selected by their lowest RSD values in the validation studies, Table 2-4. Satisfactory results were obtained for **FEX** and were found to be in agreement with the label claims (Table 6). The results obtained by the developed methods were compared with each other statistically by using Student's *t* test and no significant difference was observed between them by the fact that *t* values calculated were lower than that of tabulated (theoretical) values for P = 0.05 level (Table 7).

Table 7. Calculated *t* values for the results obtained by the methods developed for the pharmaceutical preparations selected

Pharmaceutical preparations	**Original UV – HPLC**	**^{1}D– HPLC**	**^{2}D– HPLC**	**Original UV – ^{1}D**	**Original UV – ^{2}D**	**^{1}D–^{2}D**
FEKSİNE®	0.27	0.23	0.26	0.76	0.17	0.19
FEKSADYNE®	0.40	0.30	0.77	1.48	0.11	0.97
FEXOFEN®	1.75	0.80	1.38	0.69	0.65	0.36
VİVAFEKS®	0.40	0.30	0.18	1.20	0.43	0.83
TELFAST®	0.80	1.12	0.99	1.76	0.71	0.38

*Tabulated value of *t* is 2.11 for P = 0.05

CONCLUSION

Three methods, original UV spectrophotometry and, first and second derivative UV spectrophotometry, and a new HPLC method were developed and they were successfully applied to the determination of **FEX** in 5 different formulations, film tablets, after their optimization and validation. Proposed spectrophotometric methods are original and very simple methods for the determination of **FEX** in pharmaceutical preparations. The obtained results are accurate and precise and confirmed by statistical parameters. There was no interference of the excipients. So, these methods can be applied accurately and precisely for the analysis of **FEX** in the pharmaceutical preparations mentioned above without prior separation procedure and for the routine analysis of the formulations. These are an excellent alternative to HPLC method for the determination of **FEX** in pharmaceutical formulations. It is very efficient and offers high sample throughput results. Therefore, it undoubtedly renders in-time data turnaround during formulation development. Advantages of the developed HPLC method are having an internal standard and having a wide range of linearity that makes it suitable for all in vitro studies of the tablet formulations when compared with the HPLC methods shown in literatures. By the fact that there was no official method proposed for the assay of **FEX** the results obtained were compared with each other and no significant difference was observed statistically among them.

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