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Investigating the stress effects on Fexofenadine hydrochloride and Montelukast sodium in bulk and tablet dosage form using a validated LC-DAD method

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ABSTRACT

The main aim was to develop RP-HPLC method for the simultaneous estimation of Fexofenadine hydrochloride and Montelukast sodium in tablet dosage form. A mobile phase comprising solvent A as 0.1% v/v trifluoroacetic acid in water and solvent B as acetonitrile with methanol in the ratio of 2:3% v/v was used and the flow was set in a gradient mode with a stationary phase of phenyl-hexyl silica column (150 × 4.6 mm, 5µ i.d).The detection wavelength is 254 nm using PDA detector. The method was validated according to ICH parameters. The results denote that the method is linear and within the range of 60-180 µg/ml for Fexofenadine and 5-15 µg/ml for Montelukast with the r² of 0.9998 and 0.9999 respectively. The degradants peaks non-interfered with main peaks.The developed method is acceptable for the determination of Fexofenadine and Montelukast in routine analysis of dosage form.

Keywords:FEX-Fexofenadine hydrochloride, MON- Montelukast sodium, Co-elution, forced degradation, Gradient elution.

INTRODUCTION

Fexofenadine hydrochloride(FEX) is chemically 2-[4-[1-hydroxy-4-[4-hydroxy (diphenyl) methyl] piperidin-1-yl] butyl] phenyl]-2-methylpropanoic acid (figure 1) with amolecular mass of 501.68 g/mol and log P value of 2.81.¹ It is an second generation antihistaminic agent used for the symptomatic relief of nasal congestion, itching, management and treatment of diseases like chronic urticaria and allergic rhinitis.^{2,3}

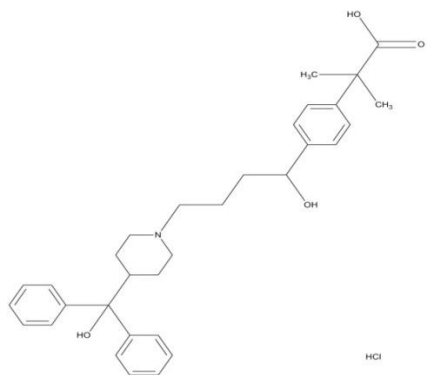


Figure 1. Structure of Fexofenadine hydrochloride

Montelukast sodium (MON) is chemically 2-[1-[[[(1R)-1-[3-[(E)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl]-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanylmethyl] cyclopropyl] acetic acid (figure 2) with a molecular weight of 586.187 g/mol and log P value of 7.9⁴. It is an anti-asthmatic agent and cysteinyl leukotriene receptor antagonist which slackens the inflammation mediating action exerted by immune system and averts broncho constriction as well as inflammation in respiratory diseases⁵. FEX and MON in combined tablet dosage form are used for the treatment of tenacious allergic rhinitis, prurigo nodularis and pemphigoid nodularis. Fexofenadine is a non-sedating agent possessing inflammatory mediating action like MON. Both the drugs in concomitant dosage form are used in the treatment of allergic reactions and inflammatory mediating actions⁶.

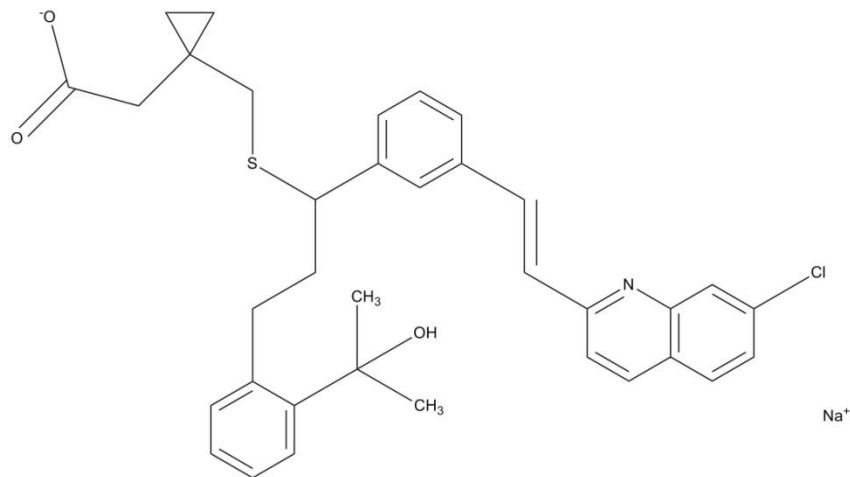


Figure 2. Structure of Montelukast sodium

A number of researchers have reported various analytical techniques for the determination of FEX and MON either alone or in combination from different matrices. These methods include electrometric titration^{7,8}, spectrometric methods such as colorimetry⁸⁻¹³, UV-spectrometry¹⁴⁻²², spectrofluorimetry²³⁻²⁵, chromatographic techniques like Thin Layer Chromatography²⁶, High Performance Thin Layer Chromatography^{27,28}, High Performance Liquid Chromatography^{22,29-56}, Ultra Pressurized Liquid Chromatography^{57,58}, Hyphenated techniques such as Liquid chromatography- mass spectrometry⁵⁹⁻⁶³ and capillary electrophoresis⁶². Official HPLC methods are available for both the drugs individually in USP⁶³. Application of reported USP monograph method of Fexofenadine for the simultaneous estimation of

FEX and MON fetched no peak for MON. Alternatively application of official USP method of MON for the determination of two drugs simultaneously resulted in poor separation of two peaks. Reported stability indicating assay methods for simultaneous estimation of two drugs by HPLC³³⁻³⁷ using C₁₈ and C₈ analytical column, present the main peaks very near to the dead volume and the separation of degradants peaks were not clear due to co-elution with the main peaks.

In our present work, an ion pair agent was used in HPLC stability indicating assay to get good peak shapes. Reported stability indicating methods make use of C₁₈/C₈ column for the separation of two drugs where there are hydrogen bond interactions. Comparatively in our method phenyl-hexyls silica column⁶⁵ was used which exhibited both hydrogen bond as well as aromatic interactions. In order to obtain the complete separation of degradants peak from the significant main peaks the retention time values of both the analytes should be well separated so that there may not be overlapping of peaks. Also the gradient mode of separation prevents co-elution of degradants with the main peaks. Based on the log P values (non-polar) of both the drugs, it can be predicted that usage of more organic modifiers will result in poor resolution leading to the concurrent elution of the degradants along with the main peaks⁶⁶. The developed method was validated according to ICH guidelines with the parameters like accuracy, linearity, precision, robustness, specificity, system suitability, etc.⁶⁷

METHODOLOGY

Chemicals and Reagents.

Methanol (HPLC grade obtained from Finar, Mumbai, India), Acetonitrile (HPLC grade obtained from Finar, Mumbai, India), Trifluoroacetic acid, Sodium hydroxide, 30% Hydrogen peroxide, Hydrochloric acid (Analytical Reagent grade obtained from Rankem, Mumbai, India) and Milli Q water as double distilled water and membrane filters 0.45µm as membrane filters from Millipores Ltd, Bangalore, India.

Instruments and Software

Chromatography study was performed in Empower software with Waters 2695 separations module with Photodiode array detector 2996 with a quaternary pump and autosampler injections. Separation was carried out using Phenomenex, Phenyl-hexyl silyl column (150 × 4.6 mm, Luna 5µ i.d.) obtained

from Hyderabad, India. Analytical balance (semi-micro) Shimadzu AUW220D from Chennai, India. Ultrasonicator (LMUC-12) Spectrum tek from Chennai, India, Digital pH meter (PH12-5p-920) spectrum tek from, Hot air oven from Inlab equipment Pvt. Ltd. from Chennai, India.

Methods

Pure Standards

FEX and MON were obtained from Vital laboratories Pvt. Ltd, Gujarat, India with percentage purity of 99.07% (CAS No-153439-40-8) and 98.12% (CAS No 151767-02-1) respectively.

Pharmaceutical Formulation

Generic tablets of FEX(120 mg) and MON(10 mg) were obtained from the manufacturer Saimirra innopharm Pvt. Ltd., Chennai, India

Preparation of stock and working solutions

A stock solution of FEX (1.2 mg/ml) and MON (0.1 mg/ml) was prepared by accurately weighing 120.0 mg of FEX and 10.0 mg of MON using Methanol: water (3:1) as diluent. Working standard solution was prepared in the concentration of FEX (120 µg/ml) and MON (10 µg/ml).

Chromatographic conditions

Chromatographic separation was carried out using (Phenomenex) Phenyl-hexyl silyl column (150 × 4.6 mm, Luna 5µ i.d.) in gradient mode. The mobile phase used was Solvent A: 0.1% v/v trifluoroacetic acid in water and solvent B: Acetonitrile: Methanol (2:3). Flow rate of 1.5 ml/min was maintained with an injection volume of 15 µl and the detection was set at 254 nm using PDA detector. Prepared mobile phase was filtered using 0.45 µm Milli pore membrane filter. Total run time is 22.0 min, where the gradient elution is maintained at 0-5 mins solvent A is 48% and solvent B is 52%, at 10-17 mins solvent A is 35% and solvent B is 65% and after 20 min the gradient comes back to initial condition. Chromatographic separation was carried out in ambient condition.

Calibration curve

From the stock solution, serial dilutions were made to prepare working standard solutions of FEX and MON in the concentration range of 60-180 µg/ml and 5-15 µg/ml respectively. A calibration curve is constructed with area versus concentration in microgram/ml.

Preparation of Sample Solution

Twenty tablets were finely powdered and sample solutions were prepared by accurately weighing a weight equivalent to 120 mg of FEX and 10 mg of MON and further dilutions were made to give concentration of 120 µg/ml and 10 µg/ml respectively.

Forced Degradation Studies

Forced degradation is stress testing and the dosage form is subjected to forced degradation using accelerated environmental conditions. Various impurities which arise during the storage of drug products in different environmental conditions can be studied using these stress studies. It also helps to calculate the inherent stability of the molecule, to determine the degradation pathways and endorses the stability indicating assay⁶⁷. In this study the combined dosage form of FEX and MON was exposed to degradation studies like acid hydrolysis, alkaline hydrolysis, thermal degradation, oxidative degradation and photolytic degradation

Acid hydrolysis

Acid hydrolysis was carried out in 807 mg of powdered tablet which is treated with 5 ml of 0.1N hydrochloric acid in a 100 ml volumetric flask. The volumetric flask was heated on a water bath at 60°C for 2 hours and allowed to cool. Neutralized the solution with 5 ml of 0.1N sodium hydroxide and diluted the volume with diluent. Final solution was made up to the concentration of FEX (120 µg/ml) and MON (10 µg/ml) with diluent.

Alkaline hydrolysis

Accurately weighed 807 mg of powdered tablet and transferred to a 100 ml volumetric flask to which 5 ml of 0.1N sodium hydroxide was added. The flask was heated on a water bath at 60°C for 2 hours and

allowed to cool. Neutralization was carried out using 5 ml of 0.1N hydrochloric acid and made up to volume with diluent to give a final concentration of FEX (120 µg/ml) and MON (10 µg/ml).

Thermal degradation

Accurately weighed 807 mg of powdered tablet and it was exposed to 105°C for 2 hours in hot air oven. Sample was allowed to cool and transferred to 100 ml of volumetric flask, the volume was made up with the diluent to give a concentration of FEX (120 µg/ml) and MON (10 µg/ml).

Oxidative degradation

Oxidative degradation was carried out using 5 ml of 30% hydrogen peroxide (H₂O₂) to 807 mg of sample in 100 ml volumetric flask. Heated the flask on water bath for 2 hours at 60°C and allowed to cool. Final concentration of FEX (120 µg/ml) and MON (10 µg/ml) were prepared with diluent.

Photolytic degradation

Accurately weighed 807 mg of powdered tablet sample and it was exposed to sunlight for 2 hours, allowed to cool and transferred to volumetric flask. The final solution was made up with diluent to give a concentration of FEX (120 µg/ml) and MON (10 µg/ml).

All the prepared solutions were injected in chromatographic system and the chromatograms were recorded for degradation studies.

RESULTS AND DISCUSSION

Method development

Chromatographic conditions such as analytical column, mobile phase, flow rate, column temperature, sample temperature, detection wavelength were optimized for better separation of main peaks with degradants peaks.

Various mobile phase compositions, detection wavelength and analytical column from the reported methods³³⁻³⁷ and official monograph methods^{63,64} were tried during method development. While trying

the first two reported methods with mobile phase composition of Phosphate buffer pH 6.0: Methanol (25:75) and 0.1% Triethylamine : Acetonitrile (30:70), good peak shape was obtained in both the methods but the resolution factor and plate count were less showing non-compliant intermediate precision. Third trial was with mobile phase ratio of ortho phosphoric acid and methanol (40:60) using C₈ analytical column, which yielded a poor resolution where there is a possibility of overlapping of degradants peak with that of analytes. After several trials, the chromatographic conditions were optimized as given and used in gradient mode: the mobile phase - 0.1% trifluoroacetic acid in water (Solvent A), acetonitrile with methanol (2:3) (Solvent B). FEX is less non-polar than the MON, so between 0-5 min Fexofenadine got eluted from the column and in 5-17 min MON got eluted due to increased non-polarity in mobile phase. Gradient mode was followed for the better separation of two peaks, so that the degradants peak can be well defined by avoiding co-elution in forced degradation assay. The detection wavelength was set by isobestic point of two drug molecules in UV spectroscopy. The results of quantitative estimation are given in Table 1 and %RSD was less than 2%. The standard chromatogram is shown in the figure 3.

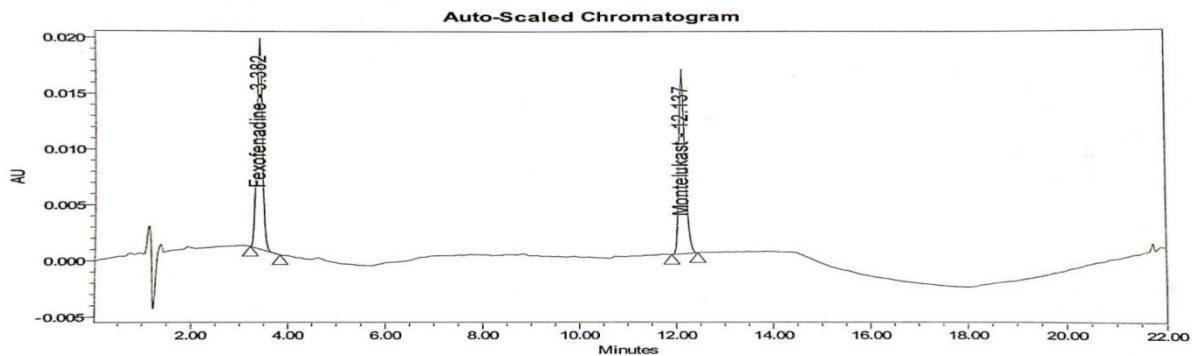


Figure 3. Chromatogram of 100% concentration of (120 µg/ml) Fexofenadine hydrochloride and (10 µg/ml) Montelukast sodium.

Table 1. Quantitative estimation of FEX and MON.

S.No	Sample Name	Retention Time		Amount present (%)	
		FEX	MON	FEX	MON
1	Standard	3.338	12.048	110.31	101.11
2	Sample 1	3.332	12.056	98.25	99.31
3	Sample 2	3.334	12.045	99.56	100.31

Validation of the Method

The developed method was validated according to ICH guidelines⁶⁸.

System suitability

System suitability was carried out by injecting six replicates of standard solution containing 120 µg/ml of FEX and 10 µg/ml of MON. The parameters like retention time (t_R), theoretical plates (N) and peak reproducibility are reported in Table 2.

Table 2. System suitability parameters for FEX and MON.

Parameter	Response	
	FEX	MON
t_R (min)& (RSD %)	3.290&(0.08)	12.006&(0.05)
Theoretical plates	3894	42733
Tailing factor	1.30	1.20

Linearity & Range

Determination of linearity of the method was executed by plotting the calibration curve of peak area versus the concentration range of 60-180 µg/ml FEX and 5-15 µg/ml MON. Result of linearity was statistically examined by correlation coefficient, slope of the regression line and y- intercept. The data are presented in Table 3.

Table 3. Calibration data for FEX and MON.

Parameters	Response	
	FEX	MON
Range	60.00-180.00 µg/ml	5.00-15.00 µg/ml
Slope	12419.70	144000.00
Intercept	670.68	121.30
y-intercept	0.45	0.08
R^2	0.9998	0.9999

Accuracy

Accuracy of the method was determined by preparing three known concentrations of FEX and MON comprising 60 & 5 µg/ml, 120 & 10 µg/ml and 180 & 15 µg/ml respectively representing 50%, 100% and 150% of working standard solution. Prepared solutions were injected into HPLC in triplicate manner and the results are reported in Table 4. Results show that relative standard deviation (%RSD) was found to be less than 2.

Table 4. Accuracy of the proposed method at three different concentrations of FEX and MON.

Concentration %	Amount added (mg)		Amount found (mg)		Recovery (%)		Mean % Recovery (%RSD)	
	FEX	MON	FEX	MON	FEX	MON	FEX	MON
50%	0.060	0.005	0.059	0.005	98.33	100.23	98.32% (0.03%)	102.75% (1.73%)
	0.058	0.005	0.057	0.006	98.27	104.01		
	0.061	0.005	0.060	0.005	98.36	104.01		
100%	0.121	0.010	0.119	0.010	99.16	98.05	99.71% (1.41%)	98.71% (0.47%)
	0.120	0.011	0.122	0.010	101.66	99.09		
	0.120	0.010	0.118	0.009	98.33	99.00		
150%	0.183	0.015	0.185	0.015	101.09	102.03	100.36% (1.03%)	99.60% (1.73%)
	0.182	0.016	0.180	0.015	98.90	98.12		
	0.181	0.015	0.183	0.014	101.10	98.66		
Mean							99.46% (0.82%)	100.35% (1.31%)

Precision

The precision of the method was calculated by executing intra-day and inter-day precision at 100% concentration (120 µg/ml of FEX and 10 µg/ml of MON). Six injections of the above solutions were injected into HPLC system at some other time of the same day for intra-day precision. For inter-day precision, 100% solutions were injected six times by different analyst on different day. The results showed that the % relative standard deviation (%RSD) was less than 2% and are reported in Table 5.

Table 5. Precision of the proposed method for FEX and MON.

Parameters	Amount added (mg)		Amount found (mg)		Recovery (%)		Mean % Recovery (%RSD)	
	FEX	MON	FEX	MON	FEX	MON	FEX	MON
Intra-day precision	0.120	0.011	0.118	0.010	98.33	99.09	99.17 % (1.27%)	98.92 % (0.96%)
	0.122	0.010	0.119	0.009	97.54	98.01		
	0.118	0.012	0.117	0.012	99.15	100.82		
	0.123	0.011	0.124	0.011	100.81	99.14		
	0.122	0.010	0.120	0.010	98.36	98.14		
	0.120	0.012	0.121	0.011	100.83	98.34		
Inter-day precision	0.123	0.010	0.125	0.011	101.62	101.85	101.10 % (0.91%)	100.45% (1.27%)
	0.117	0.012	0.119	0.012	101.70	101.62		
	0.120	0.010	0.121	0.010	100.83	100.94		
	0.121	0.012	0.123	0.012	101.65	100.80		
	0.120	0.011	0.119	0.011	99.16	99.15		
	0.120	0.012	0.122	0.012	101.66	98.37		
Mean							100.14% (1.09%)	99.68% (1.12%)

Robustness

Robustness was calculated by making intentional small changes in the parameters of assay method. It was checked by changes in flow rate (± 0.2 ml/min) and wavelength (± 2 nm). The results are reported in Table 6 and it is shown that the intentional changes do not affect the method.

Table 6. Robustness of the proposed method for FEX and MON.

Variation parameters	Accuracy % ^a ±SD		Relative Standard Deviation (%RSD)	
	FEX	MON	FEX	MON
Flow rate (±0.2 ml/min)				
1.3 ml/min	101.82 ± 1.4	101.04 ± 0.8	0.86%	0.49%
1.7 ml/min	100.49 ± 1.6	99.57 ± 1.7	0.73%	0.74%
Wavelength (± 2nm)				
252 nm	103.34 ± 1.8	106.11 ± 1.5	0.87%	0.68%
256 nm	101.61 ± 1.2	101.17 ± 0.8	0.63%	0.66%

Specificity

Specificity is the capacity of the method to determine univocally the analyte in the sample in presence of excipients, impurities and degradation products. In present study specificity study was conducted by the forced degradation of the analyte molecule. All the samples were exposed to stress conditions such as acid hydrolysis, alkaline hydrolysis, thermal degradation, oxidative degradation and photolysis for about 2 hours. The results are reported in Table 7 with % of degradation and recovery of analyte with peak purity. The forced degradation chromatograms are featured in figure 4-8. Though various unknown peaks, placebo peaks and a blank peak appeared in the chromatogram, the peak purity values show that the degraded products do not affect the purity of principle peaks of both analytes. Unknown peak 1 was found to be Fexofenadine related compound A with reference to the spectral index results of Radhakrishna and co-workers.⁶⁹

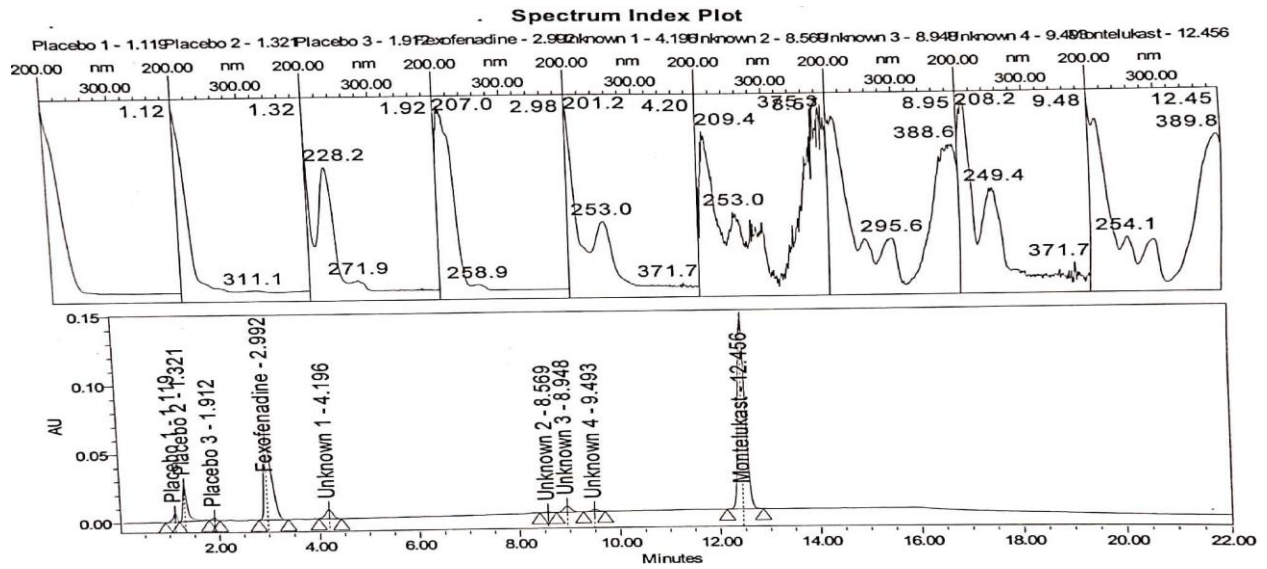


Figure 4. Chromatogram after acid degradation.

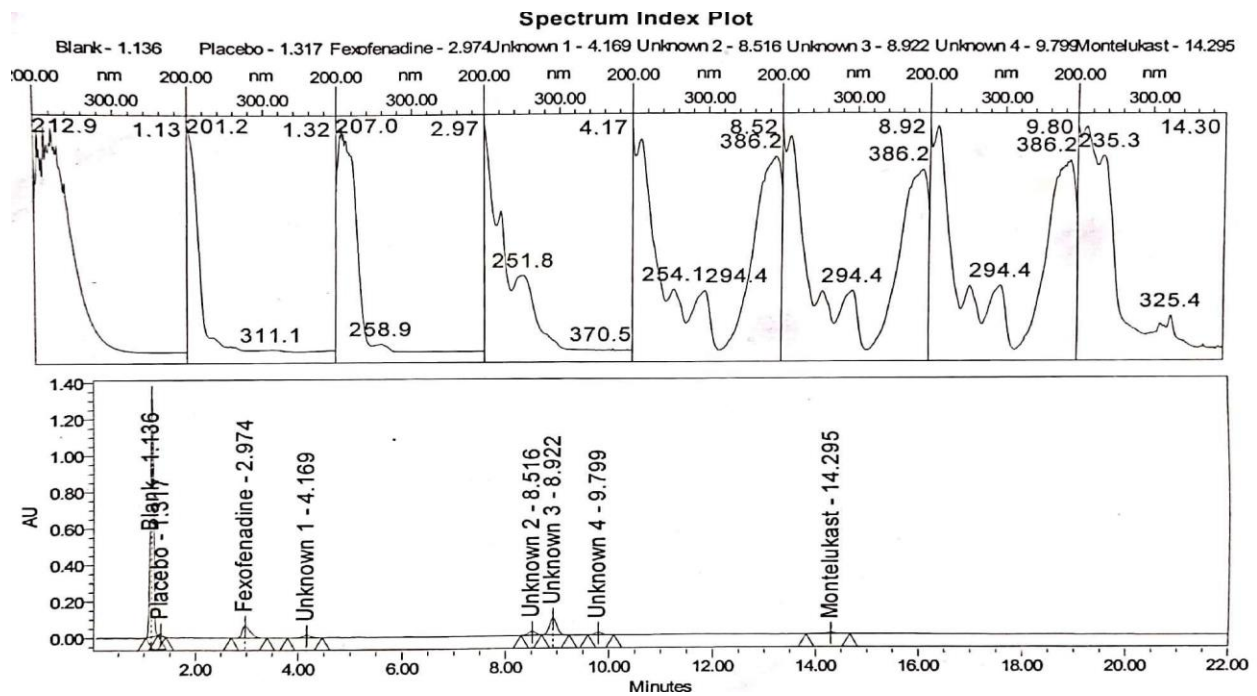


Figure 5. Chromatogram after alkaline degradation.

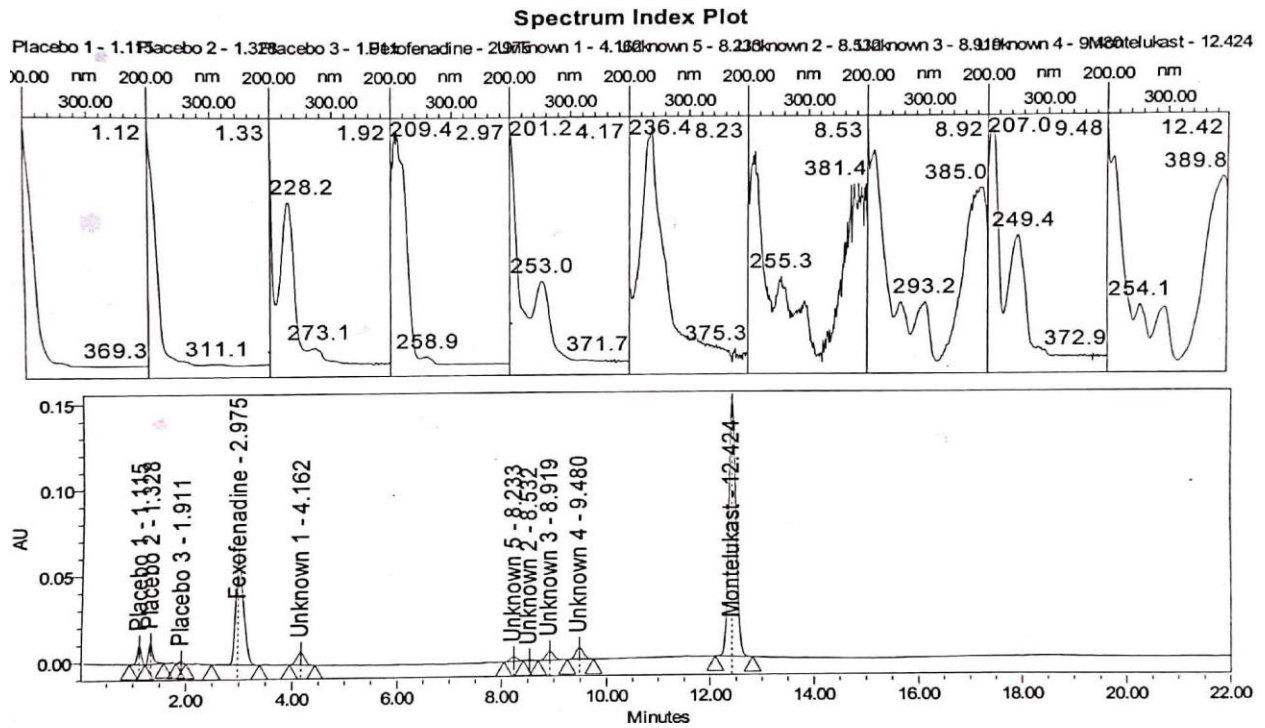


Figure 6.Chromatogram after Oxidative degradation.

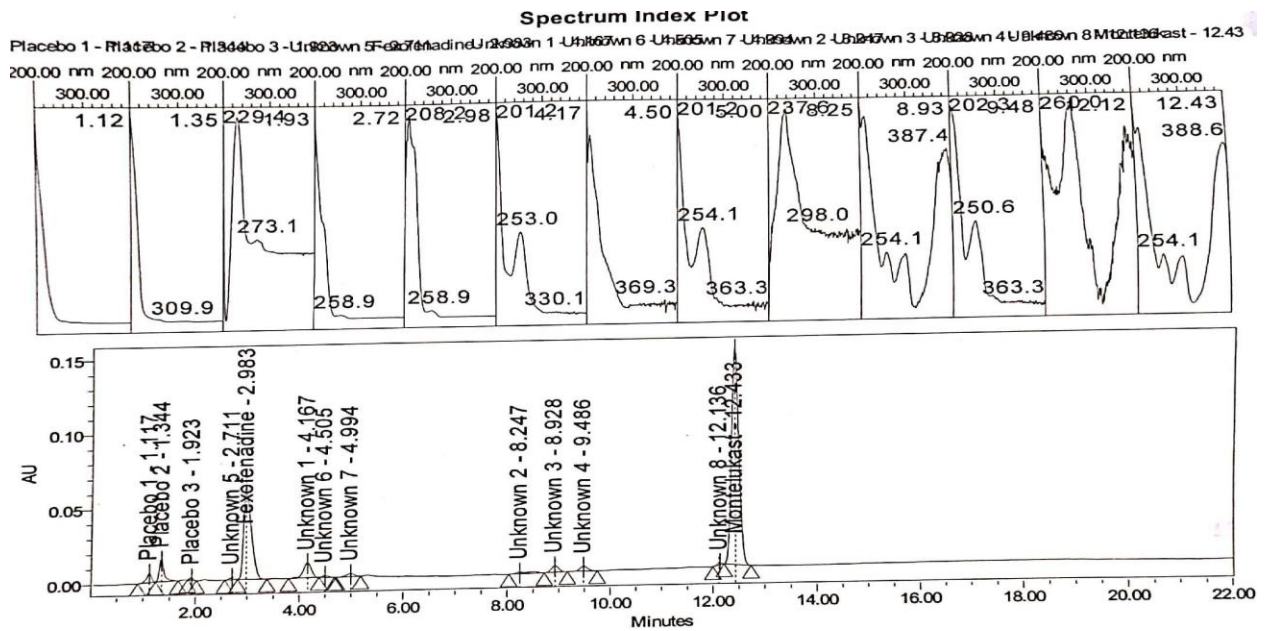


Figure 7.Chromatogram after Photolytic degradation.

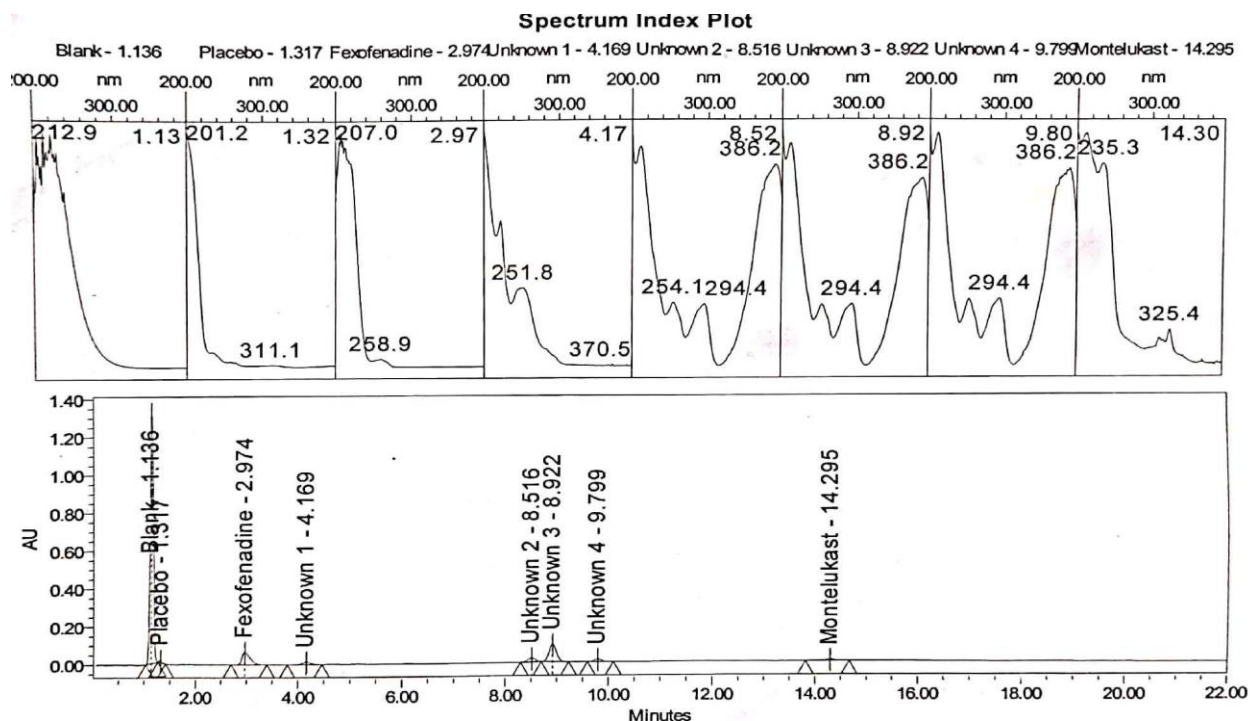


Figure 8.Chromatogram after Thermal degradation.

Table 7. Specificity of the proposed method FEX and MON after forced degradation.

Degradation condition	Recovery %		Degradation % ^a		Peak purity		Threshold purity	
	FEX	MON	FEX	MON	FEX	MON	FEX	MON
Acid	92.50	88.05	7.50	11.95	0.879	0.585	1.125	0.712
Alkali	94.70	96.20	5.30	3.80	0.778	0.545	1.058	0.705
Oxidative	86.50	91.30	13.50	8.70	0.895	0.515	1.180	0.762
Thermal	96.70	97.20	3.30	2.80	0.775	0.543	1.111	0.722
Photolysis	97.20	84.66	2.80	15.34	0.787	0.589	1.021	0.798

Gampa vijaya kumar and co-workers³³ did not observe any additional peaks in the chromatogram of degraded FEX and MON. In the present study FEX highly degraded in oxidative degradation condition (30% hydrogen peroxide solution) as similar to Maher and Co-partner work²⁹. Rameezuddin and collaborators³⁴ reported a very less oxidative degradation of 5.3% due to the usage of 3% hydrogen

peroxide solution for FEX. Rajeev kumar and confederates³⁵ did not observe any supplementary peaks in the chromatogram of degradation whereas in the same degradation conditions unknown additional peaks were observed in the chromatogram of present study. Montelukast sodium is highly degraded in photolytic degradation study which is showing a similar response like the report of Juliana roman and co-partners³². The reason for high photo-degradation is that MON is a photosensitive compound⁷⁰

Solution stability

Solution stability is the stability of standard and sample solutions which are injected into the HPLC system. The solvent used for sample solution has chances to decompose the chemical compound during sample preparation process. Normally, a degradation of 2% is allowed for solution stability from its initial condition⁷¹. Both the standard and sample solutions were found to be stable up to 25 hours.

Filter integrity

Filter integrity study is used to determine that the filters are used for the intended purpose and does not interfere in analysis. Filters such as nylon membrane, Teflon, Polyvinylidene fluoride, Polytetrafluoroethylene membrane filters were used during the sample preparation. None of the above filters used were found to interfere with study. Results are reported in Table 8

A simple, rapid, precise stability indicating assay method using RP-HPLC for the determination of FEX and MON in tablet dosage form was developed. The proposed method was validated according to ICH guidelines with the validation parameters such as accuracy, precision, robustness, intra-day, inter-day precision and specificity. The percentage degradation was within the limit for commercial tablets and the degraded peaks do not interfere with significant main peaks. The proposed method can be used for routine analysis and quality control analysis of pharmaceutical preparations. The identification of unknown impurity peaks may be taken up as further research in this study.

Table 8. Filter integrity of the method.

S.No	Filter Integrity	% Deviation from the initial area	
		FEX	MON
1	Centrifuge	-	-

2	PVDF 0.45 μm (Polyvinylidene fluoride)	2.40	0.84
3	Nylon 0.45 μm	2.33	1.23
4	PTFE 0.45 μm (Polytetrafluoroethylene)	2.73	1.29

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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