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Research Article

A Stability-Indicating High Performance Liquid Chromatographic Assay for the Simultaneous Determination of Pyridoxine, Ethionamide, and Moxifloxacin in Fixed Dose Combination Tablets

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Stability indicating reversed phase HPLC method was developed and validated for the simultaneous quantitation of antitubercular drugs, ethionamide (ETH), and moxifloxacin (MOX) with commonly coprescribed vitamin, pyridoxine (PYR) in tablet dosage form. The method was found rapid, precise and accurate. The separation was performed in Hibar 150-4.6, Purospher STAR, RP-18e (5 μm) column, using mobile phase A (0.03 M sodium citrate adjusted to pH 5 with glacial acetic acid) and mobile phase B (100% methanol), ran at variable proportions at flow rate of 1.0 mL/min. The detection was carried out at 320 nm. The method was observed linearly in the range of 2.5–17.5 μg/mL for PYR, 25–175 μg/mL for ETH, and 40–280 μg/mL for MOX with respective limits of detection/quantitation of 0.125 μg/mL/1.28 μg/mL, 0.25 μg/mL/2.56 μg/mL, and 0.35 μg/mL/3.65 μg/mL. The drugs were also subjected to oxidative, hydrolytic, photolytic, and thermal degradation; the degradation products showed interference with the detection of PYR, ETH, and MOX. The proposed method was observed to be effective to quantitate MOX (400 mg), ETH (250 mg), and PYR (25 mg) in fixed dose combination tablet formulation.

1. Introduction

Moxifloxacin is a fluoroquinolones antibacterial agent having potent activity against *M. tuberculosis*, including MDR strains in 400 mg daily dose. Ethionamide is a traditional second line therapy drug for the treatment of tuberculosis in 250–1000 mg daily dose to avoid rapid development of resistance [1–6]. In order to reduce the problems associated with peripheral neuropathy caused by daily high dose of ethionamide therapy, pyridoxine dose ranging from 2.5 to 25 mg is usually added typically along with the above mentioned therapies [7]. In present work, a fixed dose combination of moxifloxacin 400 mg, ethionamide 250 mg, and pyridoxine 25 mg was designed to reduce the duration and neurologic side effects associated with antitubercular therapy (Figure 1) [8, 9].

The literature [8–21] and official monographs of BP [16] and USP [17] present spectrophotometric and HPLC methods for the individual quantitative determination of PYR, ETH, and MOX from the bulk and dosage forms. Therefore, in this study, a rapid, precise, and accurate reversed phase HPLC method was developed and validated for the simultaneous estimation of these drugs. But there is no stability indicating HPLC method available for the determination of these drugs.

A drug and drug product is regarded as stable when its physical, chemical, therapeutics and toxicological attributes remain unchanged according to official monographs. The drug product when subjected to stability testing requires a precise, specific, and accurate analytical method for the quantitation of compounds in the presence of its degradation products, as recommended by International Conference on Harmonization (ICH) [18]. It suggests that the degradation products formed under a number of stress conditions should be identified and segregated from compound(s) of interest in an analytical run. It stated that testing should include the effect of temperature, oxidation, photolysis, and susceptibility

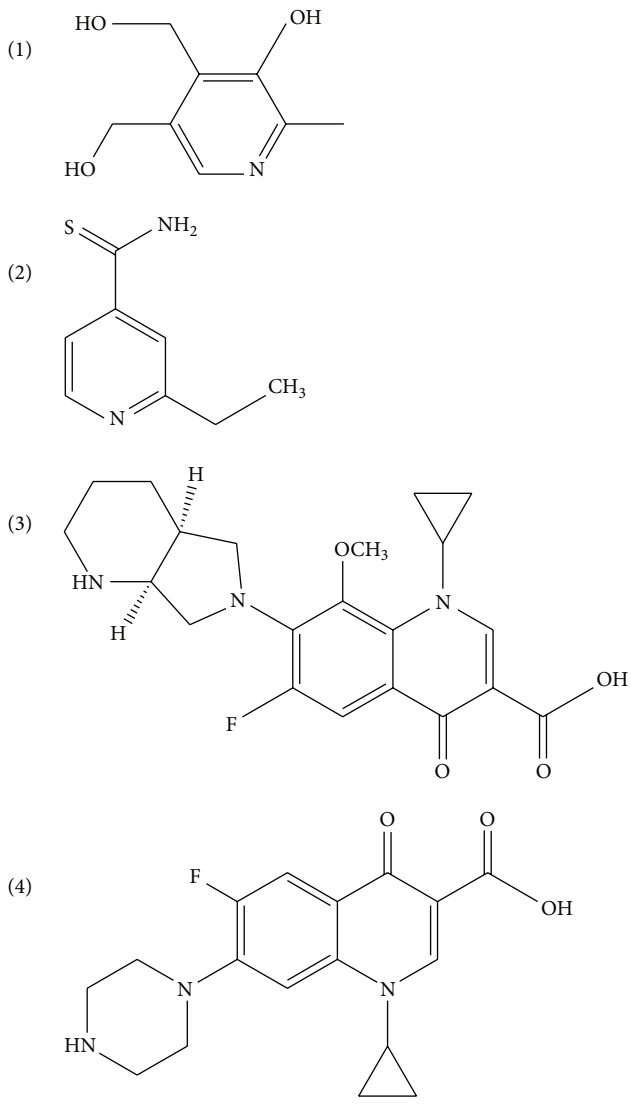

Figure 1: Chemical structures of (1) pyridoxine, (2) ethionamide, (3) moxifloxacin, and (4) ciprofloxacin.

to hydrolysis across acidic and alkaline pH ranges. Therefore an ideal stability indicating method is the one that quantifies the drug and also resolves its degradation products [22].

The objective of this study was to develop and validate a rapid, simple, selective, and sensitive HPLC method that can be easily implemented routinely for simultaneous analysis of pyridoxine, ethionamide, and moxifloxacin in a pharmaceutical solid dosage form (tablet) without interference of their potential degradant(s), excipients, and/or impurities [23]. The applicability of this developed method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) [19]. Ciprofloxacin is used as an internal standard (I.S.) in the method to make the analytical method more robust in terms of HPLC peak detection (through consistent retention time(s) determination) and to ease quantification of the three compounds by eliminating the impact of drug loss due to multiple steps of sample preparation.

2. Experimental

2.1. Chemicals and Reagents. Moxifloxacin was kindly gifted by Getz Pharma, Pakistan (Pvt.), Pyridoxine HCl by GSK, Pakistan, and ethionamide by ShazooZaka Pharmaceuticals, Lahore, Pakistan, while ciprofloxacin (internal standard) was provided by PharmEvo Pvt. Ltd., Pakistan. All solvents used like methanol, dimethyl sulfoxide, glacial acetic acid, hydrochloric acid, and hydrogen peroxide were of HPLC analytical grade (Fischer Scientific, Hampton, New Hampshire, USA; Sigma Aldrich, Switzerland). Merck grade sodium citrate and sodium hydroxide were used (Merck, Darmstadt, Germany). HPLC grade purified water was used to prepare all aqueous solutions.

2.2. HPLC Instrumentation and Conditions. A completely assembled HPLC system of Shimadzu Model LC-20A (Kyoto, Japan) was used and consisted of two isocratic pumps, an autosampler, a solvent degasser, and a UV-Visible tunable absorbance detector. Validated software "LC Solution" provided by Shimadzu (Kyoto, Japan) was used to record, integrate, and evaluate the data of chromatographic analysis. The chromatographic separation was achieved on a Hibar 150-4.6, Purospher STAR, RP-18e (5 μm) column (Merck, Germany) using a mobile phase consisting of 0.03 M sodium citrate buffer (pH 5.0 adjusted with glacial acetic acid) as mobile phase A and 100% methanol as mobile phase B in different proportions at flow rate of 1.0 mL/min. The mobile phase was filtered through 0.45 μm filter prior to use. The sample size was kept 20 μL and eluents were monitored at 320 nm. The column was maintained at ambient temperature. The composition, pH, and the flow rate of the mobile phase were changed to optimize the separation of the three compounds of interest. The initial composition of mobile phases A and B was kept 89 : 11 and given gradient program (Table 4).

2.3. Preparation of Stock and Standard Solutions. Stock and working solutions of PYR, ETH, and MOX were prepared as mentioned in Figure 5. Ciprofloxacin was used as internal standard. The working solutions were protected from light using actinic glass wares and stored for four weeks at 4°C with no evidence of decomposition.

2.4. Preparation of Tablets for Assay. Trial batches of fixed dose combination were prepared using different proportions of Avicel PH-102 (microcrystalline cellulose) as diluent, croscarmellose sodium (Ac-Di-Sol) as disintegrant, and magnesium stearate as lubricant. Assay of trial batches was performed by the developed and validated method mentioned above. The following procedure was adapted.

Twenty tablets were weighed, crushed, and mixed in a mortar and pestle to fine powder. A portion of powder equivalent to the weight of one tablet was accurately weighed into two separate 100 mL volumetric flasks and about 50 mL of 0.5 mg/mL ciprofloxacin in diluent was added to each flask. The volumetric flasks were sonicated for 20 minutes with occasional manual stirring to affect complete dissolution of the three APIs and the solutions were then made up of

volume with 0.5 mg/mL ciprofloxacin in diluent. Aliquots of the solution were filtered through a 0.45 μm filter and 10 mL of the filtered solution was transferred to a 25 mL volumetric flask and made up of volume with 0.5 mg/mL ciprofloxacin in diluent. 5 mL of this solution was diluted to 50 mL with mobile phase A to give the final concentrations of 10 μg/mL of PYR, 100 μg/mL of ETH, and 160 μg/mL of MOX. The concentration of ciprofloxacin (I.S.) in the final solution was 50 μg/mL.

2.5. Forced Degradation Studies of API. In order to develop a stability indicating HPLC method for the quantification of PYR, ETH, and MOX, the active pharmaceutical ingredients were stressed under various conditions to conduct forced degradation studies [18]. PYR is freely soluble in water and methanol but slightly soluble in alcohol and insoluble in ether [20]; ETH is soluble in methanol; sparingly soluble in alcohol and in propylene glycol; slightly soluble in water, chloroform, and ether, while MOX is soluble in 0.1 N Sodium Hydroxide; sparingly soluble in water and methanol; slightly soluble in 0.1 N hydrochloric acid, dimethyl formamide, and alcohol; practically insoluble in methylene chloride, acetone, ethyl acetate, and toluene; insoluble in tert-butyl methyl ether and n-heptane [20].

Dimethyl Sulfoxide: Methanol: Sodium Citrate buffer pH 5.0, were used as cosolvents in the ratio of 1 : 1 : 2 in forced degradation studies. The solutions were prepared by dissolving active pharmaceutical ingredients in diluent and aqueous degrading agents, aqueous hydrogen peroxide, aqueous hydrochloric acid, or aqueous sodium hydroxide separately [24]. After degradation, these solutions were diluted with mobile phase (A) to yield starting concentrations of 10 μg/mL of PYR, 100 μg/mL of ETH, and 160 μg/mL of MOX.

2.5.1. Oxidation Studies. Hydrogen peroxide is commonly used oxidizing agent to produce oxidative degradation in concentration range of 3–30% at a temperature not exceeding 40°C for 2–8 days [24]. The degradation products may arise as minor impurities during long-term stability studies (Figure 2).

Solutions of PYR, ETH, and MOX were prepared separately in 100 mL diluent for oxidation studies keeping Hydrogen peroxide 3% at the initial stage for 2 h, followed by 30% exposure of hydrogen peroxide at room temperature for 72 h.

2.5.2. Acid/Alkali Degradation Studies. Initially forced degradation studies were performed by using 1 N hydrochloric acid and sodium hydroxide as stressors. Aliquots of stock solutions of PYR, ETH, and MOX were mixed with 10 mL of 1 N hydrochloric acid and sodium hydroxide in 100 mL volumetric flasks separately to see the impact of acid and alkali on the degradation of the active pharmaceutical ingredients. The solutions were left at room temperature for 2 h, then diluted to mobile phase (A), and analyzed immediately after preparation. The procedure was repeated and samples were left for 6 h to analyze the further degradation.

None of the three active pharmaceutical ingredients showed degradation therefore further subjected to extreme stress conditions in the presence of 5 N hydrochloric acid and 5 N sodium Hydroxide (5 mL of each to make final volumes of 20 mL) for 72 h. Before making up the final volumes, acid and alkali treated samples were neutralized by adding 5 mL of 5 N hydrochloric acid and 5 N sodium hydroxide interchangeably (Figure 3).

2.5.3. Temperature Stress Studies. In general, rate of a reaction increases with rise of temperature; hence, drugs are susceptible to degradation at higher temperature [24].

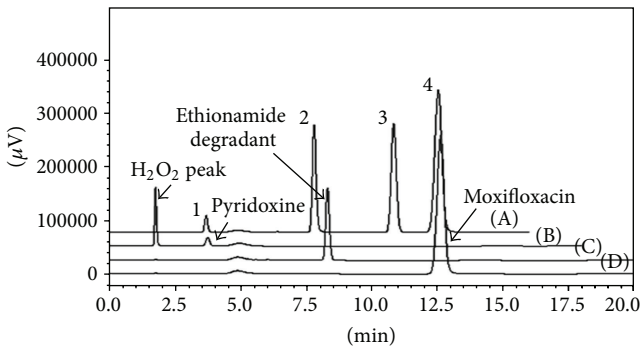

Figure 2: HPLC chromatograms comparison representing the effect of oxidative degradation on the developed stability indicating method. (A) Untreated standard solution containing (1) pyridoxine, (2) I.S. (ciprofloxacin), (3) ethionamide, and (4) moxifloxacin (30% H_2O_2 for 72 hours) and moxifloxacin. (B) Pyridoxine (30% H_2O_2 for 72 hours). (C) Ethionamide (3% H_2O_2 for 2 hours). (D) Moxifloxacin (30% H_2O_2 for 72 hours).

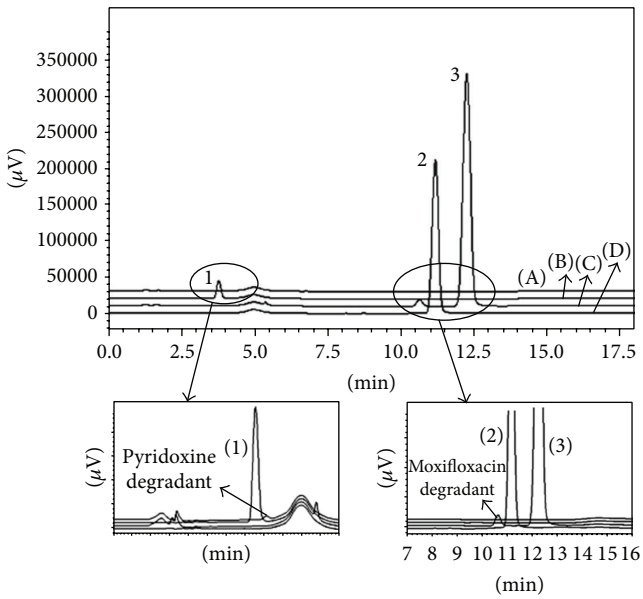

Figure 3: HPLC chromatograms comparison representing (1) pyridoxine, (2) ethionamide, and (3) moxifloxacin presenting effect of acid (HCl) degradation on the developed stability indicating method: (A) HPLC chromatograms of ethionamide (5 N HCl for 72 hours). (B) Pyridoxine (untreated). (C) HPLC chromatograms of moxifloxacin (5 N HCl for 72 hours). (D) Ethionamide (untreated).

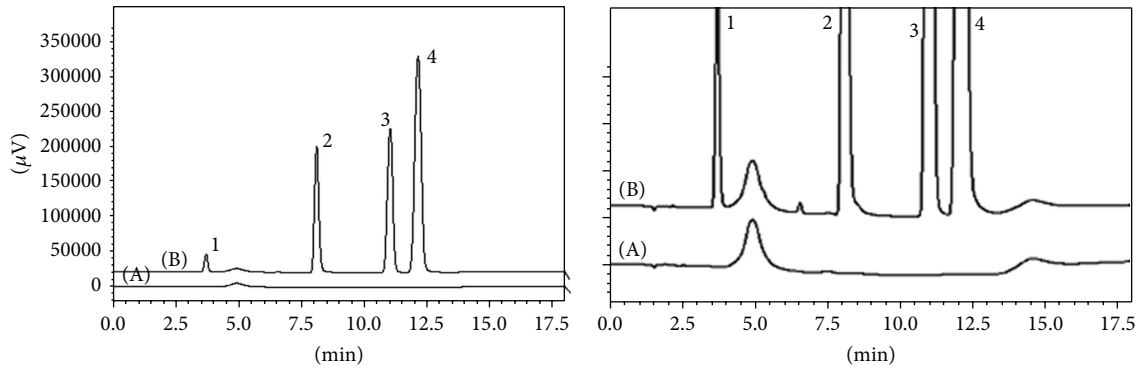

Figure 4: HPLC chromatogram comparison of blank (A) and a standard solution (B) having peak (1) pyridoxine, peak (2) ciprofloxacin (I.S.), peak (3) ethionamide, and peak (4) moxifloxacin—showing no interference of blank with any peak of interest.

For thermal degradation studies, solutions of PYR, ETH, and MOX were prepared in diluent and kept on oil bath at 90°C for 2 h [25]. The solutions were cooled to room temperature and diluted to volume with mobile phase (A) and analyzed immediately to observe the sign of degradation. The active pharmaceutical ingredients were also exposed to dry heat of 100 ± 5°C in a convection oven for 72 h and observed for degradation [25].

2.5.4. Photostability Studies. The solutions of PYR, ETH, and MOX were prepared in a similar manner as mentioned above and exposed to light in order to study the effects of irradiation on the stability of these compounds. For photostability testing, the test samples were placed in a UV light cabinet for 6 h. After being removed from the light cabinet, all solutions were prepared for analysis as previously described [24, 25].

3. Results and Discussion

3.1. HPLC Method Development and Optimization. A Hibar 150-4.6, Purospher STAR, RP-18e, 5 μ (Merck, Germany) column maintained at room temperature, was used for the method development and validation of PYR, ETH, and MOX.

In the course of method development, 0.1 M sodium acetate was used initially (pH 5.4) as mobile phase A while 100% acetonitrile was used as mobile phase B, at a flow rate of 1.5 mL/min using 20 μL of injection volume. With this composition of mobile phase, tailing in moxifloxacin peak was observed. The amount of pyridoxine was very low in the formulation as compared to the other two components. Therefore, it was necessary to either adjust the dilution of the standard and samples or select a wavelength, to help in determining the three peaks of interest at a same attenuation/scale of HPLC chromatogram. As the lowest concentration in the assay preparation is of pyridoxine, therefore, to attain consistent quantitative determination of pyridoxine, it was taken under consideration that its detection must be reliable. UV spectrum of pyridoxine showed that 223 nm was the peak maxima of the compound and 324 nm is the second peak maxima. A wavelength of 320 nm was selected for detection of analytes, because most of the interferences and solvent reduce at far UV region and the same wavelength happened to be the 2nd peak maxima of pyridoxine. At the selected wavelength, the detector response (absorbance) was reduced for rest of the two components; therefore, no further adjustment in the final concentration of the three drugs was required. By considering the pH dependent retention and zwitterion nature of MOX [26, 27], sodium citrate was tried sequentially in the concentrations of 0.02 M (pH 5.4) and 0.03 M (pH 5.0). The later concentration is found to be successful in reducing the tailing effect. Finally to make the method cost effective, acetonitrile was replaced by methanol; this intervention further improved the resolution between the peaks of ETH and MOX with a provision of adding ciprofloxacin as internal standard [28].

Under the described experimental conditions, all the peaks were well defined and free from tailing (Figure 4). The effects of small deliberate changes in the mobile phase composition, pH, wavelength, and flow rate were evaluated as a part of testing for method robustness.

3.2. Validation of the Method. The analytical method was validated for linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery, and robustness [21, 29, 30].

3.2.1. Linearity. Linearity evaluates the analytical procedure's ability (within a given range) to obtain a response that is directly proportional to the concentration (amount) of analyte in sample. For a linear method, the test results are directly or by well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range [31]. For linearity determination, seven different concentrations of standard (ETH, PYR, and MOX) were prepared as mentioned in Table 1.

Linearity was established by least square linear regression analysis [32, 33]. The constructed calibration curves were found linear over the concentration ranges of 2.5–17.5 μg/mL for PYR, 25–175 μg/mL for ETH, and 40–280 μg/mL for

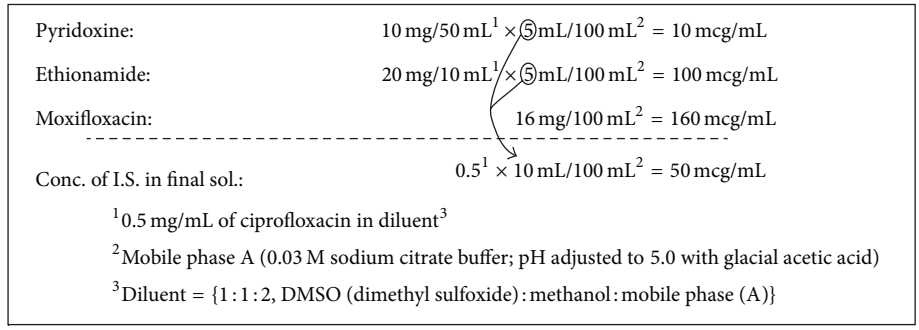

Figure 5: Preparation of stock and working solution of APIs of different concentration required for assay determination of fixed dose combination.

Table 1: Preparation of seven different concentrations of pyridoxine, ethionamide, and moxifloxacin standards for linearity determination.

(a)

Stock standard solution preparation	
Pyridoxine:	12.5 mg/25 mL* = 500 mcg/mL
Ethionamide:	125 mg/25 mL* = 5000 mcg/mL
Moxifloxacin:	200 mg/25 mL* = 8000 mcg/mL
Final concentration of APIs in assay	
Pyridoxine	10 mcg/mL
Ethionamide	100 mcg/mL
Moxifloxacin	160 mcg/mL

*Diluent = {1 : 1 : 2, DMSO : methanol : mobile phase (A)}.

(b)

Concentration	Pyridoxine		Ethionamide		Moxifloxacin		mL of I.S.†
(%)	Conc. (mcg/mL)	mL of stock Sol.	Conc. (mcg/mL)	mL of stock Sol.	Conc. (mcg/mL)	mL of stock Sol.	
175	17.5	3.5	175	3.5	280	3.5	10
150	15	3	150	3	240	3	10
125	12.5	2.5	125	2.5	200	2.5	10
100	10	2	100	2	160	2	10
75	7.5	1.5	75	1.5	120	1.5	10
50	5	1	50	1	80	1	10
25	2.5	0.5	25	0.5	40	0.5	10
Total vol. required	—	14	—	14	—	14	70

†0.5 mg/mL of ciprofloxacin (I.S.) in diluent.

MOX. Peak area ratios of active pharmaceutical ingredients were plotted versus their respective concentrations and regression analysis was performed; the correlation coefficients ($n = 6$) were found to be more than or equal to 0.999 for all drugs with % RSD values ranging from 0.04 to 0.3% across the concentration range studied. Typically, the regression equations were

$$\begin{aligned} y &= 0.0108x - 0.0004\,(R = 0.999950) \quad \text{for PYR,} \\ y &= 0.0136x - 0.0079\,(R = 0.999990) \quad \text{for ETH,} \\ y &= 0.0156x - 0.019\,(R = 0.999997) \quad \text{for MOX.} \end{aligned} \tag{1}$$

3.2.2. LOQ and LOD. The limit of quantitation (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy [34]. In this study, the limits of quantification of test drugs were found reproducible and were quantified above the baseline noise following 10 replicate injections. The resultant % RSD for these studies was 0.5%, 0.4%, and 1.0% for PYR, ETH, and MOX, respectively. The limit of quantification that produced the requisite precision and accuracy was 1.28 μg/mL for PYR, 2.56 μg/mL for ETH, and 3.65 μg/mL for MOX, respectively.

The limit of detection (LOD) of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The LOD

Table 2: Data representing accuracy and precision studies for three consecutive days.

APIs	Pyridoxine					Ethionamide					Moxifloxacin				
Actual concentration (μg/mL)	5	7.5	10	12.5	15	50	75	100	125	150	80	120	160	200	240
Measured concentration	5.03	7.49	10.05	12.49	15.10	49.93	74.93	100.31	124.86	150.21	78.54	122.88	159.05	199.33	240.25
St. deviation	0.01	0.02	0.01	0.01	0.01	0.20	0.25	0.39	0.26	0.34	0.27	0.27	0.10	0.11	0.51
Precision (%)	0.24	0.26	0.10	0.07	0.07	0.40	0.34	0.39	0.21	0.23	0.34	0.22	0.06	0.06	0.21
Accuracy (%)	100.64	99.84	100.50	99.92	100.67	99.86	99.91	100.31	99.89	100.14	98.18	102.40	99.40	99.66	100.10
Number of samples	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

Table 3: Impact of deliberate changes on system suitability of the developed analytical method to determine the robustness of the developed analytical method.

Parameters	Selected	Difference	Theoretical plates (N) of PYR	Tailing factor (T) of I.S.	Tailing factor (T) of MOX	Resolution between ETH and MOX (R_S)
			NLT 1500	0.9–2.2	0.9–2.2	NLT 2.0
Wavelength (nm)	318	−2 nm	4005	1.162	1.003	2.713
	320	0	4405	1.177	1.003	2.683
	322	+2 nm	4040	1.152	1.003	2.690
Injection volume (μL)	18	−20%	5314	1.253	1.041	2.420
	20	0	4405	1.177	1.003	2.683
	22	+20%	5302	1.261	1.051	2.427
Flow rate (mL/min)	0.8	−0.2 mL/min	6348	1.266	1.016	2.308
	1.0	0	4405	1.177	1.003	2.683
	1.2	+0.2 mL/min	4656	1.232	1.038	2.320
Analytical column	1	—	4987	1.828	1.664	4.109
	2	—	4405	1.177	1.003	2.683
	3	—	5425	1.266	1.041	2.431
Organic medium (%)	10.78, 39.2	−2%	4191	1.148	0.993	3.291
	11, 40	0	4405	1.177	1.003	2.683
	11.22, 40.8	+2%	4073	1.159	1.008	2.097
Buffer conc. (mole)	0.027	−10%	4346	1.184	1.007	2.676
	0.03	0	4405	1.177	1.003	2.683
	0.033	+10%	4395	1.164	0.999	2.942
pH of buffer	4.8	−0.2 units	4239	1.158	0.995	3.136
	5.0	0	4405	1.177	1.003	2.683
	5.2	+0.2 units	4395	1.193	1.004	2.469

Table 4: Mobile phase gradient programming of HPLC run using "LC solution" software.

Time (min)	Unit	Command	Value
0.05	Pumps	B. conc.	40
10.00	Pumps	B. conc.	40
10.05	Pumps	B. conc.	11
16.00	Controller	Stop	—

is a parameter of limit tests, that is, tests that only determine if the analyte concentration is above or below a specification limit [31]. The LOD was determined on the basis of signal-to-noise ratios. The limits of detections were 0.125 μg/mL for PYR, 0.25 μg/mL for ETH, and 0.35 μg/mL for MOX.

3.2.3. Accuracy and Precision. Table 2 presents the accuracy data, that was determined by interpolation of replicate ($n = 3$) peak area ratios of five accuracy standards of different concentration, from a calibration curve that had been prepared as described above.

The intra- and interday variability or precision data are summarized in Table 2 and were assessed by using standard solutions prepared to produce solutions of three different concentrations of each drug. Repeatability or intraday precision was investigated by injecting three replicates of each of the samples of five different concentrations. Interday precision was assessed by injecting the same five samples over three consecutive days.

3.2.4. Robustness. The proposed analytical method was assessed for robustness following ICH Guidelines and the Dutch Pharmacists [33]. Robustness is defined as the measure of the ability of an analytical method to remain unaffected by

small and deliberate variations in method parameters (e.g., pH, mobile-phase composition, temperature, and instrument settings) and provides an indication of its reliability during normal usage [31]. Robustness is usually performed by making minor changes in pH and concentration of buffer [34, 35], percentage of organic phase, flow rate, injection volume, wavelength, and analytical column of the same make. Therefore, in present study, standard solutions of active pharmaceutical ingredients were injected in duplicate under small variations of each parameter. The impacts of alterations on system suitability are mentioned in Table 3. The shifting in retention time due to deliberate small changes was assessed as inconsequent. The method proved to be quite stable.

3.2.5. Specificity. The results of stress testing and monitoring of standard solutions in the presence of impurities and degradation products revealed high degree of specificity of the proposed method for PYR, ETH, and MOX. There also found no interference from mobile phase (A) used as a diluting solvent for preparing final dilutions of standard and samples.

3.2.6. Stability Studies. According to ICH Guidelines, the stress samples of ETH, PYR, and MOX were studied in solid state while their solutions were observed for color changes. The color of test solutions remained unchanged throughout and the effects of stressors were studied on the stability of test compounds. PYR and MOX were found to be relatively stable following exposure to dry heat, oxidative, and acidic/alkaline hydrolytic conditions. Pyridoxine, on the other hand, degraded under UV light but the degradation product did not show any interference with the analytical run. The oxidative degradation under the influence of 30% H_2O_2 of ETH was very remarkable but PYR and MOX exhibited degradation of 35% and 1.3%, respectively. The generated degradant of these compounds did not interfere with the analytical run (Figure 2). After heat exposure, MOX was found to be considerably stable and showed degradation of only 4% while no degradation occurred under acidic/alkaline stress conditions. Similarly, PYR did not pose any notable degradation when exposed to 5 N HCl/5 N NaOH for 72 h, while ETH degraded almost completely on exposure to 5 N HCl for 72 h but showed only 53% degradation when exposed to 5 N NaOH for the same time period. The resulting degradant did not affect the elution of rest of the components (Figure 3).

The stability of stock solutions was determined by quantitation of each drug in solution in comparison to the response obtained for freshly prepared standard solutions. No significant changes (RSD $<$ 2%) were observed for the chromatographic responses for the stock solutions analyzed, relative to freshly prepared standards.

Stability testing was also conducted to study the impact of autosampler on 24 h stay of standard solutions at room temperature. The results were found satisfactory and standard solutions were found stable.

3.3. Assay. The validated method was applied to determine the content assay of ETH, PYR, and MOX in formulated fixed dose combination tablets. The content assay was found in the range of 98–102% for the test compounds for twenty tablets with the %RSD of 1.2% (ETH), 3.1% (PYR), and 1.8% (MOX). The assay results revealed that the method is selective for the analysis of ETH, PYR, and MOX without interference of any added excipients in formulation.

4. Conclusions

A simple, rapid, accurate, and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of PYR, ETH, and MOX in a fixed dose combination formulation. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) Guidelines revealed that the method is selective and stability indicating and has ability to separate these drugs from their degradation products and excipients selected for formulating tablet dosage forms. The method can be applied to the analysis of accelerated stability samples of these drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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