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Research Article

A New Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Pyridoxine Hydrochloride and Meclizine Hydrochloride in Pharmaceutical Solid Dosage Forms

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A simple, specific, accurate, precise stability indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous determination of pyridoxine hydrochloride (PYH) and meclizine hydrochloride (MEH). An isocratic separation of PYH and MEH were achieved on C 18, 250 × 4.6 mm ID, 5 μm particle size columns at column oven temperature 37°C with a flow rate of 0.5 mL min^{-1} and using a diode array detector to monitor the detection at 254 nm. The mobile phase consisted of buffer : acetonitrile : trifluoroacetic acid at a ratio of 30 : 70 : 0.1 (v/v). The retention times of PYH and MEH was found to be 5.25 and 10.14 min, respectively. Suitability, specificity, linearity, accuracy, precision, stability, and sensitivity of this method for the quantitative determination of the drugs were proved by validation in accordance with the requirements laid down by International Conference on Harmonization (ICH) Q2 (R1) guidelines. The proposed method is reliable and robust and can be used as quality control tool for the estimation of these drugs in combined pharmaceutical solid dosage forms.

1. Introduction

Pyridoxine hydrochloride (PYH) is chemically 3, 4-pyridinediacetonitrile, 5-hydroxy-6-methyl, hydrochloride (Figure 1). It is a water-soluble vitamin and involved principally in amino acid, carbohydrate, and fat metabolism [1]. It is also required for the formation of hemoglobin [2]. Meclizine hydrochloride (MEH) (Figure 2) is often used as "meclozine" which is chemically 1-[(4-Chlorophenyl)(phenyl)methyl]-4-(3-methylbenzyl)piperazine, dihydrochloride monohydrate, a first-generation antihistamine of the piperazine class. Meclizine is effective in inhibiting the symptoms of motion sickness, such as nausea, vomiting, and dizziness. PYH is official in IP [3], BP [4, 5], and USP [6], and MEH is also official in BP [4, 5] and USP [6]. The pharmacopeias describe potentiometric, spectrophotometrics and HPLC method for the determination of PYH and MEH individually from the bulk and tablet dosage form. No reversed-phase high-performance liquid chromatography (RP-HPLC) method is reported in pharmacopeias for the simultaneous estimation of PYH and MEH from their combined formulation, though there are various methods for the determination of PYH and MEH by spectrophotometric [7–12], voltammetric [13], HPLC [14–16], electrophoresis [17], GLC [18], HPTLC [19], and TLC [20] methods in different pharmaceutical dosages forms. The present work describes the development and validation of stability indicating RP-HPLC method, which can quantify PYH and MEH simultaneously in pharmaceutical solid dosage form. The confirmation of the applicability of this developed method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) [21].

2. Experimental

2.1. Reagents and Chemicals. Pure standards of PYH and MEH were obtained from Jianxi Sentai Pharmaceutical Co. Ltd., China, and M/S FDC Ltd., India, having purities of 99.23% and 99.10%, respectively. The formulated film-coated tablets (pyrimac tablet) were prepared from Advanced Chemical Industries (ACI) Limited, Narayanganj,

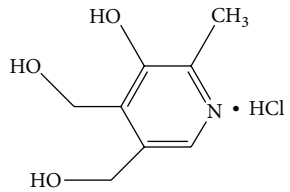

Figure 1: Chemical structure of pyridoxine hydrochloride.

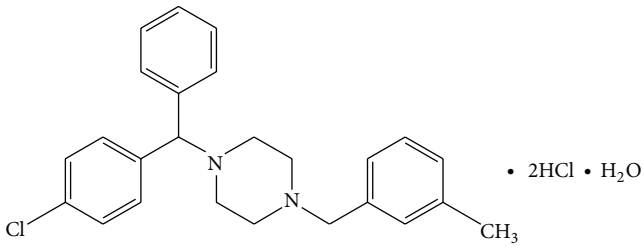

Figure 2: Chemical structure of meclizine hydrochloride.

Bangladesh. Each tablet contains combined 50 mg pyridoxine hydrochloride and 25 mg meclizine hydrochloride (based on 100% potency) as active ingredients, as well as microcrystalline cellulose (avicel PH 102), sodium starch glycolate (SSG), Colloidal silicon dioxide (aerosil-200), croscarmellose sodium, Allura red lake, and magnesium stearate as excipients in addition to Opadry II 85G54426 pink as coating material.

Acetonitrile HPLC grade (Scharlau), anhydrous sodium dihydrogen phosphate (Scharlau), phosphoric acid AR grade, (Merck) and trifluoroacetic acid (Fisher Scientific) were used for analytical purposes. Ultrapure water was used to prepare the mobile phase. Ultrapure water was prepared by using Labconco WaterPro PS purification system.

2.2. Instrument and Chromatographic Condition. Chromatographic separation was achieved by using Shimzadu prominence LC-20AD high-performance liquid chromatography, equipped with degasser PGU-20A 5, variable wavelength programmable diode array detector SPD-M20A, autosampler SIL-20 AC HT, and column oven CTO-10 A5 VP. ProntoSIL C 18, 250 × 4.6 mm ID, 5 μm particle size was used as the stationary phase. The column temperature was kept at 37°C, and the mobile phase flow rate was maintained at 0.5 mL min^{-1}. The detection was monitored at 254 nm. The injection volume was 20 μL, and the run time was 13 min for each injection. Other instruments such as pH meter (Jenway 3510), electronic weighing balance (Mettler-Toledo), and ultrasonic bath (Clifton) were also used.

Dionex ultimate 3000 series HPLC and Hichrom C18, 250 × 4.6 mm ID, 5 μm particle size column were used for ruggedness study.

2.2.1. Mobile Phase. A mixture of buffer, acetonitrile, and trifluoroacetic acid (TFA) at a ratio of 30 : 70 : 0.1 (v/v) was prepared. The resulting solution was sonicated for 5 min using ultrasonic bath, and finally the mixture was filtered using 0.2 μm membrane filter.

2.2.2. Preparation of Buffer for Mobile Phase. 12 g of anhydrous sodium dihydrogen phosphate was dissolved in 900 mL of ultrapure water. Then the pH was adjusted to 3.0 with orthophosphoric acid and volumed up to 1000 mL with ultrapure water and sonicated for 5 min using ultrasonic bath then filtered through 0.2 μm membrane filter.

2.2.3. Diluting Solution. A mixture of buffer, acetonitrile, and trifluoroacetic acid (TFA) at a ratio of 35 : 65 : 0.1 (v/v) was used as the diluents.

2.2.4. Standard Preparation (at Nominal Concentration). PYH and MEH working standards were accurately weighed and were transferred into a clean and dry 100 mL standard volumetric flask and dissolved to prepare 0.50 mg mL^{-1} and 0.25 mg mL^{-1} concentrations of PYH and MEH stock solution, respectively, with the diluting solution and finally volumed up to the mark. The solution was sonicated for 5 min using ultrasonic bath and then filtered through 0.2 μm disk filter.

2.2.5. Sample Preparation. Twenty tablets (pyrimac tablet) were crushed and then powdered finely. To prepare assay sample solution, powdered sample equivalent to 50 mg pyridoxine hydrochloride and 25 mg meclizine hydrochloride was weighed accurately and taken into 100 mL volumetric flask. About 40 mL of diluting solution was added and shaken thoroughly to extract the drug from the excipients and then sonicated for 5 min to complete dissolution of drug. The solution was allowed to cool at room temperature and then volume up to the mark. The solution was filtered through Whatman filter paper (no. 42) and then finally filtered through 0.2 μm disk filter.

2.3. Method Validation Parameters

2.3.1. System Suitability. To assess system suitability of the method, the repeatability, theoretical plates, tailing factor, and retention time of six replicate injections of standard PYH and MEH of concentrations 0.50 mg mL^{-1} and 0.25 mg mL^{-1}, respectively, were used, and the percent relative standard deviation (%RSD) values were calculated in each case.

2.3.2. Linearity. The linearity of the method was determined at five different concentration levels (80%, 90%, 100%, 110%, and 120%) ranging from 0.395 to 0.592 mg mL^{-1} of PYH and 0.203–0.304 mg mL^{-1}of MEH, respectively. The linearity was evaluated by peak area versus concentration, which was calculated by the least-square regression analysis, and the respective regression equation was computed.

2.3.3. Specificity. The specificity of the developed RP-HPLC method for the determination of PYH and MEH in bulk

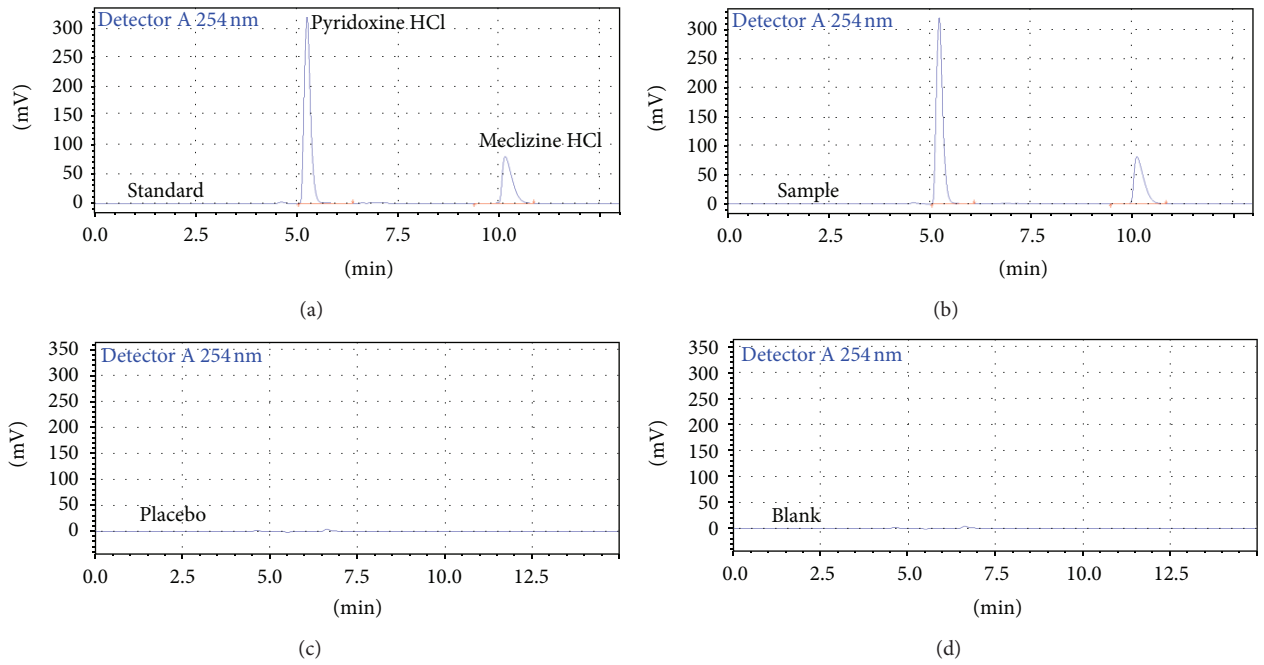

Figure 3: RP-HPLC chromatogram of blank, placebo, tablet sample, and standard of pyridoxine HCl and meclizine HCl.

drug and pharmaceutical preparation (pyrimac tablet) was investigated by chromatographic analysis of the following.

Noninterference of Placebo. To check the noninterference of placebo, placebo solution was prepared in the same way of the sample solution in the presence of all inactive ingredients of the pyrimac tablet formulation but without PYH and MEH.

Degradation Studies. Degradation studies were carried out under acid hydrolysis, alkali hydrolysis, oxidation, and reduction. One formulated full tablet was taken as a sample for degradation study. For acid hydrolysis 5 mL of 0.1 N HCl was added to sample, kept for 8 hours (at 25 ± 2°C), then neutralized with 0.1 N NaOH and volumed up to mark with diluent. For alkali hydrolysis 5 mL of 0.1 N NaOH was added to sample, kept for 8 hours (at 25 ± 2°C), then neutralized with 0.1 N HCl and made to volume with diluent. A 10% H_2O_2 solution and 10% sodium bisulphate solution were used for oxidative and reductive study, consecutively. The final solution was injected for assay analysis, and the presence of interfering peak(s) eluted at or near the retention time of PYH and MEH was also checked. All determinations were conducted in triplicate. The Peak purity tool was used to check the purity of the test solution.

2.3.4. Accuracy. Accuracy was carried out for drug-matrix solutions. Accuracy parameter was determined by the recovery test, which consisted of adding known amounts of PYH and MEH in to the placebo sample solutions. This test was conducted by three different concentrations (80, 100, and 120%) of test sample in three replicate sample preparations, and the percent recoveries (mean ± %RSD of three replicates) of PYH and MEH in drug-matrix form were calculated.

Table 1: Chromatographic characteristics of system suitability study.

Parameters	Value (Mean ± %RSD)*	
	PYH	MEH
Peak area	3485193 ± 0.08	1347970 ± 0.08
Tailing factors	1.29 ± 1.06	1.53 ± 0.45
Retention time	5.25 ± 0.02	10.14 ± 0.04
Theoretical plates	5198 ± 0.10	8127 ± 0.08

*Mean and %RSD of six replicate.

Table 2: Parameters of regression analysis.

Parameters	PYH	MEH
Linearity range (mg mL^{-1})	0.395–0.592	0.203–0.304
Correlation coefficient	0.999	1.000
% Y intercept	1.61	0.17

The accuracy was also evaluated by linear regression analysis and computed.

2.3.5. Precision. Precision of the method was studied by analysis of three replicates of standard solution in three different concentrations (80, 100, and 120%). It was demonstrated by repeatability (intraday precision) and intermediate precision (interday precision) of standard solutions. The results were expressed as %RSD of the measurements.

2.3.6. Stability of Solution. The solution stability is tested by allowing the prepared drug matrix tested sample to stand exposed to room light and ambient room temperature for

Table 3: Results showing different degradative outcomes.

Parameters	Pyrimac tablet (mg tab^{-1})		Assay at forced condition (mg tab^{-1})*		Percent of degradation (%)	
	PYH	MEH	PYH	MEH	PYH	MEH
Normal	50.00	25.00				
Acidic			49.39 ± 0.10	23.84 ± 0.09	1.22	4.64
Alkaline			49.40 ± 0.08	23.75 ± 0.11	1.20	5.00
Oxidation			48.11 ± 0.06	23.85 ± 0.07	3.78	4.60
Reduction			49.68 ± 0.12	23.90 ± 0.15	0.64	4.40

*Mean of three replicate, tab: Tablet.

Table 4: Accuracy studies of PYH and MEH in drug-matrix solutions.

	Amount added (mg mL^{-1})	Peak area	Amount recovered (mg mL^{-1})	% Recovery	% Recovery (mean ± %RSD)	Over all (mean ± %RSD)
PYH	0.395	2804505	0.392	99.24	100.85 ± 1.38	100.32 ± 1.06
	0.395	2805301	0.402	101.65		
	0.395	2804670	0.402	101.65		
	0.494	3481689	0.497	100.71	100.57 ± 0.33	
	0.494	3487433	0.495	100.20		
	0.494	3485039	0.498	100.81		
	0.592	4181905	0.595	100.51	99.55 ± 1.02	
	0.592	4181507	0.590	99.66		
	0.592	4183455	0.583	98.48		
MEH	0.203	1084674	0.207	101.88	101.76 ± 0.17	100.81 ± 1.01
	0.203	1084350	0.206	101.64		
	0.203	1085947	0.207	101.88		
	0.252	1345809	0.253	100.38	100.00 ± 0.33	
	0.252	1342589	0.252	99.81		
	0.252	1348349	0.252	99.81		
	0.304	1624589	0.308	101.41	100.63 ± 1.21	
	0.304	1623470	0.308	101.25		
	0.304	1625347	0.302	99.22		

three consecutive days. The sample is to be assayed daily and compared to freshly prepared standard solutions.

2.3.7. Sensitivity. For sensitivity study the limit of detection (LOD) and limit of quantitation (LOQ) were estimated by determination of signal-noise ratio. The LOD ($\alpha = 3.3$) and LOQ ($\alpha = 10$) of the proposed method were calculated using the following equation:

$$A = \alpha \times C \times \frac{N}{S}, \quad (1)$$

where A is LOD or LOQ, C is the concentration in ppm, and N/S is the signal-noise ratio.

2.3.8. Ruggedness. Ruggedness of the current method was determined by analyzing six assay sample solutions of pyrimac tablet by different instrument, column, and two analysts in the same laboratory to check the reproducibility of the test result.

2.3.9. Robustness. To determine the robustness of the current method, the effect of flow rate was studied at 0.48 and 0.52 mL min^{-1} instead of 0.5 mL min^{-1}. The effect of column temperature was studied at 35 and 39°C instead of 37°C. The effect of mobile phase composition was assessed at (buffer : ACN = 31.3 : 68.7, v/v) and (buffer : ACN = 28.7 : 71.3, v/v) instead of (buffer : ACN = 30 : 70, v/v). The effect of wavelength change was studied at 252 nm and 256 nm instead of at 254 nm.

3. Results and Discussion

3.1. Method Validation

3.1.1. System Suitability. The results (Mean ± %RSD of six replicates) of the chromatographic parameters (Table 1) indicate the good performance of the system.

3.1.2. Linearity. The peak area was dynamic-linear in the concentration ranges of 0.395–0.592 mg mL^{-1} for PYH and

Table 5: Intra-day and inter-day precision of the method.

	Sample Conc. (%)	Peak area (day 1)	Peak Area (Mean ± %RSD)	Peak area (day 2)	Peak Area (Mean ± %RSD)	Overall (Mean ± %RSD)
PYH	80	2802395	2803478 ± 0.04	2804067	2804395 ± 0.01	2803937 ± 0.03
		2803486		2804588		
		2804553		2804530		
	100	3484270	3485861 ± 0.05	3485121	3486142 ± 0.04	3486001 ± 0.04
		3487551		3487734		
		3485761		3485570		
	120	4181755	4182542 ± 0.04	4180232	4181749 ± 0.04	4182145 ± 0.04
		4181438		4181345		
		4184432		4183670		
MEH	80	1084378	1084599 ± 0.02	1084421	1084530 ± 0.01	1084565 ± 0.02
		1084566		1084666		
		1084854		1084502		
	100	1346372	1346558 ± 0.06	1345207	1345681 ± 0.04	1346120 ± 0.06
		1345756		1345391		
		1347547		1346445		
	120	1625634	1624784 ± 0.08	1623245	1623586 ± 0.04	1624185 ± 0.07
		1623340		1623167		
		1625378		1624345		

Table 6: Stability of analytical sample solution.

Day	Room Temperature (°C)	% Recovery*	
		PYH	MEH
1	25 ± 2	100.94	100.46
2	25 ± 2	100.17	99.65
3	25 ± 2	96.67	99.00

*Mean of three replicates.

0.203–0.304 mg mL^{-1} for MEH, respectively. Highly significant correlation coefficient (R^2) demonstrated the linearity of the method (Table 2).

3.1.3. Specificity. The chromatograms of blank, placebo, test sample, and standard were used to justify the specificity of target analyte. The method was specific since excipients in the formulation did not interfere in the estimation of PYH and MEH (Figure 3).

The samples submitted to acidic and alkaline condition showed significant alteration in the peak area, and also there was no detectable degradation peak(s). Similarly, during oxidative and reductive hydrolysis study, degradation peak(s) was not found. In every case the peak purity was 99.99%. The results acquired from peak purity tool confirmed that the active components' peak response was pure proving no other substances in the same retention time. Percent of degradation is mainly 3.78% for PYH during oxidation study as well as 4.64% for MEH in acidic condition (Table 3).

3.1.4. Accuracy. The results were expressed as percent recoveries of the particular components in the samples. The overall results of percent recoveries (mean ± %RSD) of drug-matrix solutions are indicating good accuracy of the proposed RP-HPLC method (Table 4). Correlation coefficient $R^2 = 0.999$ and 0.999 established excellent accuracy for the active ingredients PYH and MEH, respectively.

3.1.5. Precision. The values of %RSD for intraday and interday variation were found very well and within 2% limit, indicating that the current method is repeatable (Table 5).

3.1.6. Stability of Solution. In the stability study, the retention time remained unchanged till third day, but peak area of PYH and MEH deviated at third day by more than 2.0% from initial. This indicates that both solutions were stable for at least 48 hours, which was sufficient to complete the analytical procedure (Table 6).

3.1.7. Sensitivity. The LOD and LOQ by the proposed method were found for PYH 1.90 ppm and 5.74 ppm as well as for MYH 3.75 ppm and 11.35 ppm, respectively.

3.1.8. Ruggedness. The results (% of Recovery ± RSD) of six assay samples are indicating the ruggedness of the current method (Table 7).

3.1.9. Robustness. The effects of robustness study under different altered conditions of this proposed method are

Table 7: Ruggedness of the method.

Amount of PYH (mg tab^{-1})	Amount of MEH (mg tab^{-1})	Analyst 1, instrument 1, column 1		Analyst 2, instrument 2, column 2	
		Amount found PYH (mg tab^{-1}) (Mean ± %RSD)*	Amount found MEH (mg tab^{-1}) (Mean ± %RSD)*	Amount found PYH (mg tab^{-1}) (Mean ± %RSD)*	Amount found MEH (mg tab^{-1}) (Mean ± %RSD)*
50	25	50.53 ± 0.3	25.63 ± 0.3	50.97 ± 0.04	25.60 ± 0.50

*Mean of six replicates.

Table 8: Robustness of the method.

Parameters	Actual Variance	Amount added (mg mL^{-1})		% Recovery (Mean ± %RSD)*	
		PYH	MEH	PYH	MEH
Flow Rate	0.48 mL min^{-1}	0.5	0.25	100.55 ± 0.33	100.04 ± 0.04
	0.52 mL min^{-1}	0.5	0.25	100.12 ± 0.02	100.18 ± 0.07
Organic (%) in mobile phase	68.7	0.5	0.25	99.95 ± 0.22	100.15 ± 0.04
	71.3	0.5	0.25	99.75 ± 0.51	100.18 ± 0.07
Detector wavelength	252 nm	0.5	0.25	100.00 ± 0.15	99.81 ± 0.19
	256 nm	0.5	0.25	100.00 ± 0.11	99.53 ± 0.37
Column Temperature	39°C	0.5	0.25	100.17 ± 0.20	100.08 ± 0.11
	35°C	0.5	0.25	100.41 ± 0.46	100.07 ± 0.05

*Mean of three replicates.

satisfactory (Table 8). The mean recovery and %RSD of analyzed sample indicate that the current method is robust.

4. Conclusion

The developed RP-HPLC method for the simultaneous determination of pyridoxine hydrochloride and meclizine hydrochloride is simple, precise, accurate, reproducible and highly sensitive. The developed method was validated based on ICH guidelines [21]. Hence, this method can be routinely used for the simultaneous determination of pyridoxine hydrochloride and meclizine hydrochloride in pure and pharmaceutical formulations.

Conflict of Interests

The author wishes to confirm that there is no known conflict of interests associated with this paper. The author confirms that he/she has given due consideration to the protection of intellectual property associated with this work and that there is no impediment to publication, including the trademarks mentioned in my paper.

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