Research Article

A Validated RP-HPLC Method for the Estimation of Pizotifen in Pharmaceutical Dosage Form

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A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for rapid assay of Pizotifen in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL/min was employed on Chromosil C18 (250 mm × 4.6 mm, 5 μm) column at ambient temperature. The mobile phase consists of methanol:acetonitrile in the ratio of 10:90 v/v. The UV detection wavelength was 230 nm, and 20 μL sample was injected. The retention time for Pizotifen was 2.019 min. The percent RSD for accuracy of the method was found to be 0.2603%. The method was validated as per the ICH guidelines. The method can be successfully applied for routine analysis of Pizotifen in the rapid and reliable determination of Pizotifen in pharmaceutical dosage form.

1. Introduction

Pizotifen (4-(1-methyl-4-piperidylidene)-9,10-dihydro-4H-benzo-[4,5]cyclohepta[1,2]-thiophene); Figure 1(a)) is a benzocycloheptene-based drug used for the prevention of vascular headache including migraine and cluster headache. Alternatively used instead of propanolol, topiramate, valproic acid, and amitriptyline. Pizotifen is reasonably effective, but its use is limited by side effects, principally drowsiness and weight gain, and it is usually not the first-choice medicine for preventing migraines, instead it is used as an alternative when other drugs have failed to be effective [1–3]. Pizotifen is also used as antidepressant or for the treatment of anxiety or social phobia. Animal studies also suggest that pizotifen could be used in the treatment of serotonin syndrome or MDMA overdose in a similar manner to the closely related antihistamine/antiserotonin drug cyproheptadine [4–6]. Pizotifen is contraindicated in gastric outlet obstruction, pregnancy, angle-closure glaucoma, and difficulty in urination [6–8].

Abounassif et al. [9] reported the stability-indicating methods and validated through a study of UV-degraded

solutions contained in quartz cells. RP-HPLC liquid chromatographic method for quantification of Pizotifen malate in pharmaceutical solid dosage forms was reported by Rahman et al. [10]. Here we with reporting precise and accurate RP-HPLC method developed and validated for rapid assay of Pizotifen in pharmaceutical dosage forms.

2. Materials and Methods

2.1. Chemicals and Reagents. The reference sample of Pizotifen (API) was obtained from V.V MED, Hyderabad. The Formulation was procured from the local market. acetonitrile, methanol, ammonium dihydrogen phosphate, triethylamine, and orthophosphoric acid used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

2.2. Instruments. Peak HPLC containing LC 20 AT pump, variable wavelength programmable UV/VIS detector, and Rheodyne injector were employed for the investigation. The chromatographic analysis was performed on a Chromosil C18 column (250 mm × 4.6 mm, 5 μm). Degassing of

FIGURE 1: (a) Structure of Pizotifen. (b) RP-HPLC Chromatogram of standard solution (c) On x-axis: concentration of sample solution, on y-axis: peak area response.

the mobile phase was done by using a Loba ultrasonic bath sonicator.

2.3. Chromatographic Conditions. Proper selection of the stationary phase depends upon the nature of the sample, molecular weight, and solubility. Pizotifen, the selected drug was analyzed by reverse phase columns. Among C8 and C18, Chromosil C18 column (250 mm × 4.6 mm, 5 μm) was selected. Nonpolar compound is very attractive with reverse phase columns. Various combinations of methanol, acetonitrile, orthophosphoric acid ammonium dihydrogen phosphate, and Triethylamine were tested. Mixture of acetonitrile: methanol (90 : 10 v/v) was selected as mobile phase and the pH was adjusted to 5.8 using orthophosphoric acid. Composition of mobile phase on the retention time of Pizotifen was thoroughly investigated. The concentrations of the acetonitrile: methanol (90:10 v/v) were optimized to give symmetric peak with short runtime. UV detection wavelength was 230 nm, flow rate was 1.0 mL/min, injection volume was 20 μL, with ambient temperature, runtime was 6 min, and retention time was 2.019 min; the resulting HPLC chromatogram is shown in Figure 1(b).

2.4. Preparation of Standard Solution. For analysis 100 ppm standard solution was prepared in mobile phase. Required concentrations were obtained from 100 ppm standard solution by proper dilution. pH of the mobile phase was adjusted to 5.8 with orthophosphoric acid and filtered through 0.45 μ nylon filter.

2.5. Preparation of Sample Solution. The formulation tablets of Pizotifen (Migralin—0.725 mg) were crushed to give finely powdered material. With powder we prepared 70 ppm solution in mobile phase and then filtered it through Ultipor N₆₆ Nylon 6,6 membrane sample filter paper.

2.6. Method Development. For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and choice of stationary and mobile phase. The following studies were conducted for this purpose.

2.7. Wavelength Detection. The spectrum of diluted solutions of Pizotifen in mobile phase was recorded separately on

TABLE 1: Specificity study.

Name of the solution	Retention time in min
Blank	No peaks
Pizotifen	2.01

TABLE 2: Linearity results.

Level	Concentration of Pizotifen in ppm	Mean peak area
Level-1	5.0	30333.1
Level-2	10.0	61990.7
Level-3	15.0	88759.2
Level-4	20.0	121043.1
Level-5	25.0	148510.7
Level-6	30.0	186164.2
		6119.996
Slope intercept correlation coefficient		966.4
		0.998

UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the Pizotifen showed that a wavelength was found to be 230 nm. RP-HPLC chromatogram of standard solution is given in Figure 1(b).

2.8. Validation of the Proposed Method. The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

2.9. Specificity. The specificity of the method was performed by comparing the chromatograms of blank, standard, and sample. It was found that there is no interference due to excipients in the tablet formulation and also that there is good correlation between the retention times of standard and sample. The specificity results are shown in Table 1.

2.10. Linearity. Linearity was performed by preparing mixed standard solutions of Pizotifen at different concentration levels including working concentration mentioned in experimental condition, that is, 25 ppm. Twenty microliters of each concentration was injected in duplicate into the HPLC system. The response was read at 230 nm, and the corresponding chromatograms were recorded. The regressions of the plots were computed by least square regression method. Linearity results are presented in Table 2 and linearity graph in Figure 1(c).

2.11. Precision. Precision of the method was performed as intraday precision and interday precision. To study the intraday precision, six-replicate standard solution of Pizotifen was injected. The percent relative standard deviation (% RSD) was calculated, and it was found to be 1.4, which is within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 3.

For interday precision six-replicate standard solution of Pizotifen was injected on the third day of sample preparation. The percent relative standard deviation (% RSD) was

TABLE 3: Results of Precision Study (Intraday).

Sample	Conc (PPM)	Injection no.	Peaks area	RSD (acceptance criteria 2.0)
Pizotifen	25 ppm	1	152526.6	1.40
		2	151615.4	
		3	152127.8	
		4	153481.7	
		5	150705.3	
		6	156830.7	

TABLE 4: Results of precision study (interday).

Sample	Conc (PPM)	Injection no.	Peaks area	RSD (acceptance criteria 2.0)
Pizotifen	25	1	146180.5	1.07
		2	142312.7	
		3	143153.8	
		4	145268.1	
		5	143858.6	
		6	145253.9	

calculated, and it was found to be 1.07, which is within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 4.

2.12. Accuracy. A known amount of standard drug was added to the fixed amount of preanalyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 25%, 50%, and 75% level. The percent recovery and % RSD were calculated, and results are presented in Table 5. Satisfactory recoveries ranging from 99.93 to 100.4 were obtained by the proposed method.

2.13. Robustness. Pizotifen at 6 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 6.

2.14. System Suitability. System suitability was studied under each validation parameter by injecting six replicates of the standard solution. The system suitability parameters are given in Table 7.

2.15. Limit of Detection and Limit of Quantification for Pizotifen. For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Pizotifen are given in Table 8.

3. Discussion on Results

Various combinations of orthophosphoric acid, triethylamine, ammonium dihydrogen phosphate, acetonitrile, and

TABLE 5: Mean percentage recovery.

Level	Amount of Pizotifen spiked (ppm)	Amount of Pizotifen recovered (ppm)	% recovery	Mean % recovery	% RSD	Mean % RSD		
25%	5	4.97	99.4	99.92994	0.53	0.2603		
	5	5.02	100.4					
	5	4.98	99.6					
50%	10	10.01	100.1					
	10	9.99	99.9					
	10	9.98	99.8				0.15	
75%	15	14.99	99.93					
	15	15.02	100.13				0.101	
	15	15.01	100.06					

TABLE 6: Robustness.

Condition	Mean area	% assay	% difference
Unaltered	144805.5	100.0	0.0
Flow rate at 0.8 mL/min	144720.3	99.94	0.06
Flow rate at 1.2 mL/min	143951.2	99.41	0.59
Mobile phase: Acetonitrile : Methanol			
92% 08%	144902.5	100.06	0.06
88% 12%	144763.4	99.97	0.03
pH of mobile phase at 6.0	144275.2	99.63	0.37
pH of mobile phase at 5.6	144358.7	99.69	0.31

TABLE 7: System suitability parameters.

Parameter	Tailing factor	Theoretical plates
Specificity study	1.34	4964.61
Linearity study	1.17	4590.18
Precision study	1.48	4875.34

TABLE 8: LOQ and LOD.

Parameter	Measured volume
Limit of quantification	65 ng/mL
Limit of detection	20 ng/mL

methanol were tested as mobile phase. The concentrations of acetonitrile and methanol were optimized to give symmetric peak with short runtime. A system suitability test was applied to representative chromatograms for various parameters. Ten-point graph was constructed covering a concentration range 5–30 ppm (three independent determinations were performed at each concentration). Linear relationship between the peak area signals of Pizotifen and the corresponding drug concentrations was observed. The calibration curve was obtained for a series of concentration in the range of 05–30 ppm and it was found to be linear. The data of regression analysis of the calibration curves are shown in Table 2. Low values of standard deviation denoted very good repeatability of the measurement. Thus, it was

shown that the equipment used for the study was appropriate and the developed analytical method was consistent. For the intermediate precision a study carried out indicated a RSD of 1.235.

The stability of Pizotifen in standard and sample solutions was determined at ambient temperature ($20 \pm 10^\circ\text{C}$). The solutions were checked in triplicate after three successive days of storage, and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time, the results did not decrease below 98%. This denotes that Pizotifen is stable for at least 2 days at ambient temperature. The system suitability parameter values are within the limits. The statistical evaluation of the proposed method revealed its good linearity, reproducibility, and its validation for different parameters.

4. Conclusion

A validated RP-HPLC method has been developed for the determination of Pizotifen in tablet dosage form. The proposed method is simple, rapid, accurate, precise, and specific. Its chromatographic runtime of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Pizotifen in pharmaceutical dosage forms. So it could be used for the rapid and reliable determination of Pizotifen in tablet formulations.

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