

Research Article

Separation and Determination of Prochlorperazine Maleate and Pyridoxine Hydrochloride in Tablet Formulation by RP-HPLC

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Received: August 01, 2014; **Accepted:** August 20, 2014; **Published:** August 23, 2014

Abstract

A reverse phase HPLC method is developed for the simultaneous analysis of Prochlorperazine Maleate (PCM) and Pyridoxine hydrochloride (PDH) in pharmaceutical preparations. HPLC was carried out on a C18 column using methanol: water (40:60 v/v, pH 7) as a mobile phase at 1 mL/min flow rate and the effluent was monitored at 272 nm. The retention time for PCM was found to be 6.28 min and for PDH was found to be 3.48 min. The linearity was obtained in the concentration range of 5-25 µg/ml respectively for PCM and PDH. The method was successfully applied in the analysis of tablet dosage form because no chromatographic interferences from formulation recipients were found. In this study, a HPLC method was successfully applied for the quantitative assay of PCM and PDH in tablets which is simple, rapid, no interferences from formulation recipients and does not require any separation step for each drug. The method was validated as per the ICH guidelines. This method is precise, accurate and easy to analysis PCM and PDH in tablets.

Keywords: HPLC; Accuracy; Precision; ICH guidelines

Introduction

Prochlorperazine Maleate (PCM) is a well known phenothiazine analog, chemically it is [2-chloro-10-(3, 4'-methylpiperazin-1-yl)pylpropyl] phenothiazine; Maleate]. PCM (Figure 1) is widely used for the treatment of nausea, vomiting, migraine, anxiety and sometimes schizophrenia [1]. Pyridoxine hydrochloride (PDH) is chemically, 5-hydroxy-6-methyl-3, 4-pyridinedimethanol hydrochloride [2] (Figure 2). The term B-group vitamins usually refers to Thiamine, Riboflavin, Pyridoxine, Nicotinic acid, Pantothenic acid, Biotin, Cyanocobalamin and Folic acid. Vitamins are reported to reduce the damage by free radicals and prevent degenerative disease [3]. Pyridoxine hydrochloride is known as vitamin B6 and extensively used as nutritional component. Vitamins B6 and B2 are involved in the metabolism of homocysteine, vitamin B6 serves as a cofactor for cystathionine β-synthase and cystathioninylase, which convert homocysteine to cystathionine and then to cysteine. Vitamin B6 exists in seven forms: pyridoxine (PN), pyridoxine 5'-phosphate (PNP), pyridoxal (PL), pyridoxal 5'-phosphate (PLP), pyridoxamine (PM), pyridoxamine 5'-phosphate (PMP), and the catabolite,

4-pyridoxic acid. The Pyridoxine form is a water-soluble vitamin. It was discovered in 1934 by P. Gyorgy [4]. The management of vomiting has been always a problem for a physician and the search for a safe and effective alternative is still on. The combination of the PCM (5 mg) and PDH (25 mg) is used for getting relief from vomiting and nausea symptoms and used as an anti-emetic.

Both the drugs are official in IP, BP and USP. Various analytical procedures are available for estimation of PCM and PDH in single as well as in combination. Literature survey divulges that HPLC [5-7], GC-MS [8], LC-MS [9,10], UV [11,12], Volta metric [13] methods were described for the estimation of PCM. PDH can be evaluated by Perchloric acid titration [14] and colorimetric method [15]. The literature survey reveals that HPLC [16-20], MLC [21,22] and UV [23-27], methods have been reported for estimation of pyridoxine hydrochloride with other drugs. The UV spectrophotometric method has been developed recently for simultaneous estimation of PCM and PDH in tablet formulation [28].

So an attempt was being made to developed simple, accurate,

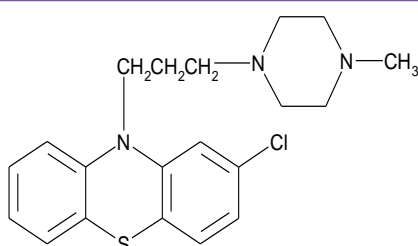


Figure 1: Structure of Prochlorpromazine.

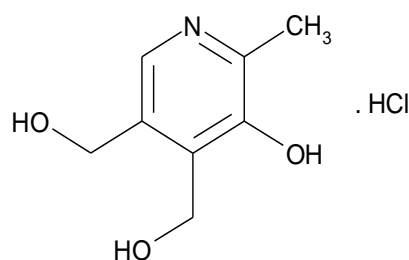


Figure 2: Structure of Pyridoxine Hydrochloride.

precise, economical and reproducible chromatographic method for simultaneous estimation of PCM and PDH in tablet dosage form. The developed method was validated in accordance with ICH guideline [29,30] and successfully employed in the assay of PCM and PDH in combined tablet dosage form.

Material and Methods

Standard and chemical reagents

The standard drug Prochlorperazine Maleate (99.90%) and Pyridoxine Hydrochloride (99.86%) were obtained from Mehata Pharmaceutical Industries, Mumbai, India. Demonized Distilled Water (DIW) was obtained from Loba Chemie Mumbai, India. HPLC grade methanol (99.80%) and acetonitrile (99.80%) were procured from Merck Ltd., India. Tri ethylamine (TEA, 99.14%) and ortho phosphoric acid (OPA, 99.37%) were procured from Fisher scientific, Mumbai, India.

Chromatographic conditions

Liquid chromatography was performed on Younglin (S.K) isocratic System (JASCO Corporation, Japan). The system built with UV 730 D as UV-VIS detector and C18 (Cosmosil) (4.6 × 250 mm, 5µm) column with a 20 µL manual sample injector. The HPLC system was equipped with Autochro -3000 software for data processing.

All compounds were eluted off the column with a mobile phase consisting of methanol: water (40:60 v/v, pH 7) at a flow rate of 1.0 mL/min in isocratic mode. The mobile phase was filtered through a 0.45 µm nylon filter and then ultrasonicated for 30 min. The injection volume was 20 µl and the eluent was detected at 272 nm, which was selected as wavelength for further analysis at ambient temperature. The retention time of PCM and PDH was 6.2833 and 3.4833 min, respectively, and the total run was 10 min.

The method was validated in accordance with the International Conference on Harmonization guidelines for validation of analytical procedures [29,30].

Specificity and selectivity

These parameters were determined by comparing the chromatograms of the PCM and PDH standard, tablet drug Emidoxyn forte and mobile phase as a solvent.

Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results, which are directly proportional to the concentration (amount) of analyze in the sample [29,30]. The linearity was tested for PCM and PDH in the concentration range value of 5-25 µg/mL.

Recovery studies

To check the degree of accuracy of the method, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150%. Known amounts of standard PCM and PDH were added to the pre-analyzed samples and were subjected to the proposed HPLC method.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The repeatability was

calculated as the relative standard deviation with three replications and three different concentrations during the same day. Intermediate precision was studied by comparing the assays on two different days.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyze in a sample which can be detected but not necessarily quantitated as an exact value. Limit of detection can be calculated using the following equation as per ICH guidelines [29,30].

$$LOD = 3.3 \times N/S$$

$$N = \sqrt{\sum (X - \bar{X})^2}$$

$$n-1$$

Where, N is the standard deviation of the peak area of the drug, S is the slope of the corresponding calibration curve, X is peak area, \bar{X} is average of the peak area and n is number of values.

Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyze in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Limit of quantification can be calculated using the following equation as per ICH guidelines [29,30].

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation of the peak area of the drug and S is the slope of the corresponding calibration curve.

Robustness

The robustness of analytical method is the measure of its capacity, to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Experiments are performed by changing conditions such as wavelength, flow rate and ratio of mobile phase. The method must be robust enough to withstand slight changes and allow routine analysis of sample.

Analysis of tablet formulation

A sample solution was prepared by taking accurately weighed uncoated twenty tablets (Emidoxyn forte) and finely powdered. A precisely weighed portion of the powder equivalent to 0.5 mg of PCM and 2.5 mg of PDH were extracted with the mobile phase. The extract was transferred to a 100 ml volumetric flask and volume made up to the mark with the mobile phase. The solution was filtered through 0.45 µm nylon filter to remove particulate matter, if any. Then sample solution was ultrasonicated for 15 min. The tablet extract was appropriately diluted with mobile phase to obtain at concentrations 5-25 µg/mL. The amount of drug present in the sample solution was calculated by using the calibration curve. The chromatogram was holding up to 10 min. The chromatogram obtained is shown in Figure 3 and the area obtained in each chromatogram of five replicates was correlated with regression equation and the amount of PCM and PDH was found to be 101.20% and 99.40% respectively, which was within the limit of label claim of Emidoxyn Forte tablet.

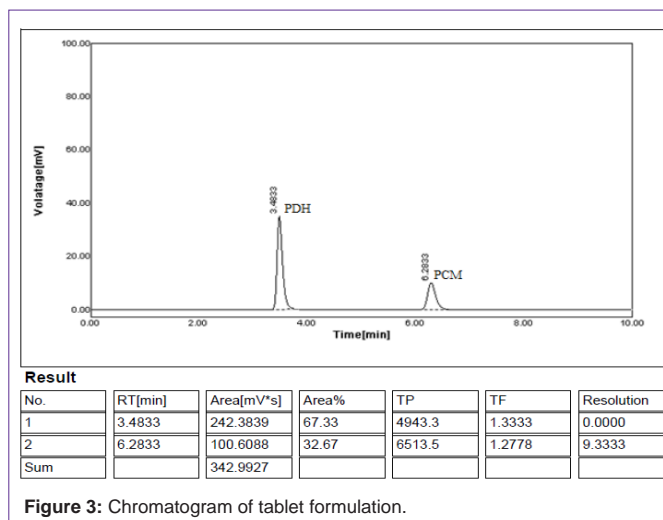


Figure 3: Chromatogram of tablet formulation.

Results and Discussion

Analytical method development

In order to achieve the good separation and quantification different chromatographic parameters like mobile phase, flow rate, wavelength was optimized individually for PCM and PDH then optimized for in combination. The mobile phase chosen for analytical method validation was methanol: water (40:60 v/v, PH 7), presented a mobile phase holdup time of 6.2833 and 3.4833 min for PCM and PDH respectively giving good separation, and well defined symmetrical peaks with more number of theoretical plates.

The flow rate was optimized with 0.5, 1.0, 1.5 and 2 mL/min. At 0.5 mL/min, there was no peak appeared in the chromatogram with 3 replications. This is attributed to the insufficient flow rate to elute PCM and PDH through the column. However, a significant difference was observed among all the rest flow rates. Based on the results obtained, 1 mL/min showed the best results in terms of peak area and retention time. Detector response for quantification of PCM and PDH was studied and the best wavelength was found to be 272 nm showing highest sensitivity.

Analytical method validation

Linearity

Linearity was established by least square linear regression analysis. Standard solution of the PCM and PDH of different concentration range (5-25 µg/mL) were used for this purpose. Each measurement was carried out in five replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves. Linear regression of absorbance on concentration gave the equation $y = 5.139x - 1.293$ (for PCM) and $y = 12.23x - 6.974$ (for PDH) with a correlation coefficient (r^2) 0.998 for PCM and 0.996 for PDH. Result of linearity study has been shown in Table 1.

Table 1: Linearity data for PCM and PDH.

Linearity (n=5)	PCM	PDH
Range	5-25µg/mL	5-25µg/mL
Mean 'r ² ' value	0.998	0.996
Regression equation	$y = 5.139x - 1.293$	$y = 12.23x - 6.974$

Table 2: Results of recovery study by standard addition procedure.

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	Percent recovery ± SD*	RSD%
PCM	5.0	2.5	7.495	99.93±0.26	0.2698
	5.0	5.0	9.979	99.79±0.19	0.1897
	5.0	10.0	15.171	101.14±0.85	0.8613
PDH	5.0	2.5	7.513	100.17±0.33	0.3698
	5.0	5.0	9.894	98.94±0.68	0.6189
	5.0	10.0	14.896	99.30±0.47	0.4727

Recovery studies

To check the degree of accuracy of the method, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150%. Known amounts of standard PCM and PDH were added to the pre-analyzed samples and were subjected to the proposed HPLC method. The standard addition method was presented good recoveries and agreement with the standards of method validation [29,30] as shown in Table 2.

Precision

The precision of the method was evaluated based on the results of the analysis of three samples with three replications for each one at day 1 and the results from intermediate precision from other three samples at day 2. The values were compared with the standards [29,30] thus all values demonstrated good results as shown in Table 3.

Limits of quantification (LOQ) and detection (LOD)

The LOD and LOQ were calculated according to the ICH guidelines of method validation [29,30]. LOD for PCM and PDH was found to be 0.0236 µg/ml and 0.0856 µg/ml respectively. The LOQ of proposed method was found to be 0.4125 µg/mL and 0.3458 µg/ml respectively for PCM and PDH.

Selectivity

Comparison of the chromatograms obtained from the mobile phase (blank), PCM, PDH standards and the tablet revealed no significant interference, using same chromatographic conditions for all samples as indicated in Figures 3-5 and referring to the method selectivity and specificity for the analyze concerned as there is no interference of formulation recipients during the analysis.

Robustness

Results of robustness study were shown in Table 4. The observations in all robustness parameters were examined and found within limit. The system suitability parameter were unaffected by

Table 3: Precision of method development on PRG and ACE analysis.

Drug	Repeatability				Intermediate Precision		
	conc. µg/mL	Rt ±SD	Peak area (mV/S) ±SD'	RSD %	Rt ±SD	Peak area (mV/S) ±SD'	RSD %
PCM	5	6.283±0.014	23.589 ±0.259	1.006	6.279±0.145	23.699 ±0.332	0.369
	10	6.280±0.096	49.781±0.412	0.799	6.282±0.698	49.559±0.673	0.623
	15	6.291±0.042	78.911±0.095	0.045	6.281±0.541	78.542±0.442	0.856
PDH	5	3.486±0.093	60.856±0.652	0.612	3.483±0.445	60.845±0.965	0.824
	10	3.480±0.454	107.246±0.712	0.645	3.489±0.895	107.689±0.248	0.586
	15	3.489±0.627	173.23±0.822	0.944	3.479±0.364	173.478±0.723	0.773

*Average of three determinations.

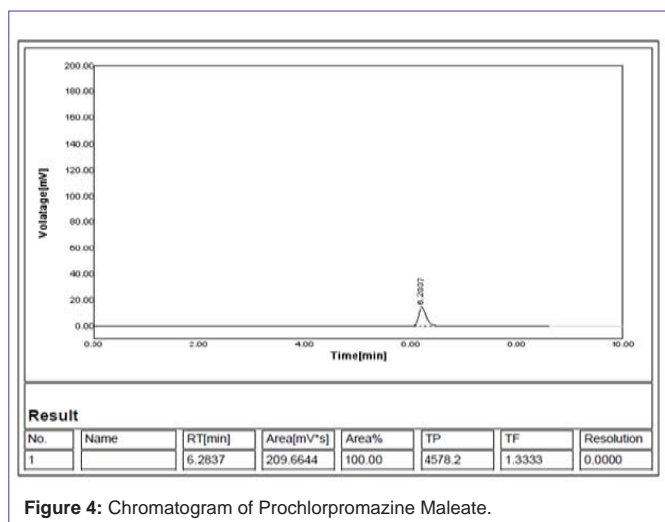


Figure 4: Chromatogram of Prochlorpromazine Maleate.

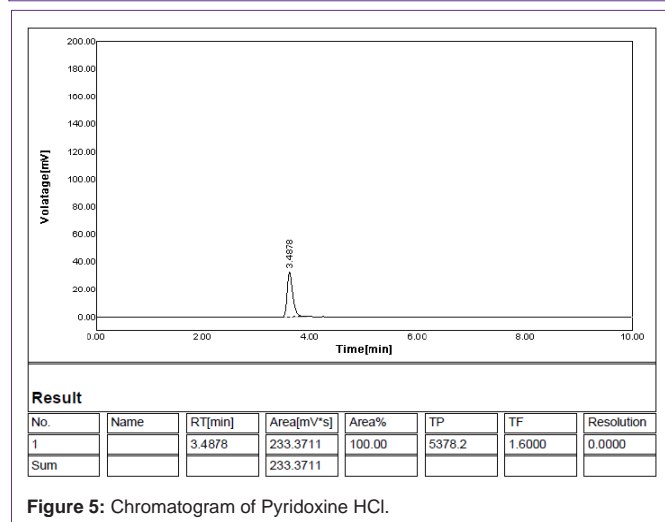


Figure 5: Chromatogram of Pyridoxine HCl.

small variations in wavelength, flow rate and mobile phase ratio.

Conclusion

The results show that the HPLC method presented here can be considered suitable for the analytical determination of PCM and PDH in tablet dosage form. The proposed method is being linear in the concentration range used, high selectivity and specificity, high precision and adequate accuracy at the concentrations studied. The proposed method uses a simple mobile phase compared to the multi-component mobile phase in many reported methods. The separation and determination were achieved at an ambient temperature. Thus, it offers the advantages of low column back pressure, good peak shape, improved column efficiency, higher theoretical plates and consistent retention time. The developed method suggested non interference of formulation recipients in the estimation. Hence it can be easily and conveniently adopted for routine analysis. The developed chromatographic method is more accurate, precise, reliable and specific for estimation of PCM and PDH as compare to reported spectrophotometric method.

Acknowledgement

Authors are heartily thankful to Mehata Pharmaceutical

Table 4: Robustness study of system suitability parameter.

RSD of Parameters	Drug	Observation								
		Wavelength (nm)			Change in FR (mL/min)			MP ratio		
		270	272	274	0.98	1.0	1.02	-10%	OMP	+10%
TP*	PCM	0.731	0.858	0.965	0.336	0.656	0.798	0.995	0.423	0.526
	PDH	1.215	0.556	1.102	0.512	0.556	0.238	0.816	0.664	0.369
TF*	PCM	0.0961	0.125	0.352	0.095	0.245	0.234	0.213	0.092	0.228
	PDH	0.1256	0.153	0.126	0.625	0.285	0.311	0.424	0.560	1.136
RT*	PCM	0.1221	0.220	0.187	0.331	0.230	0.360	0.562	0.341	0.354
	PDH	0.3329	0.369	0.695	0.755	0.156	0.289	0.589	0.663	0.644

* Indicates average of three determinations; TP: Theoretical Plate; TF: Tailing Factor; RT: Retention time;

MP: Mobile Phase; OMP: Optimized Mobile Phase

Industries, Mumbai, India for providing pure standard drug samples. The authors also express gratitude to the management and the Principal of MES's College of pharmacy Sonai, India for providing amenities to carry out this work.

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