

Research Article



Separation and Simultaneous Quantitation of Meclizine Hydrochloride and Pyridoxine Hydrochloride in their Solid and Semi-Solid Preparations using Validated HPLC method

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ABSTRACT

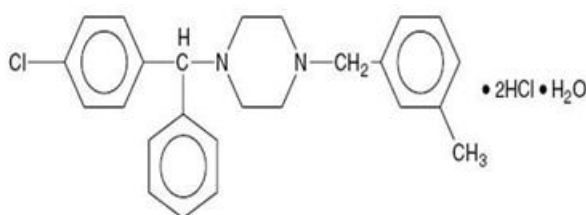
The purpose of this work was to develop a rapid, sensitive and validated HPLC (High Performance Liquid Chromatography) method for separation and analysis of pyridoxine hydrochloride and meclizine hydrochloride in their solid and semi-solid preparations. The two compounds were separated on a reversed-phase C18 silica column (250 x 4.6 mm) using a mobile phase containing dodecyl sulphate sodium salt dissolved in a mixture of water, acetonitrile, methanol, glacial acetic acid and tetrahydrofuran. The pH was adjusted to pH= 3.8 by the addition of hexane sulphonic acid sodium salt ion-pair reagent. The samples were detected using a UV detector at 253 nm. The validation study of the method included the effect of the flow rate, ratio of the components of the mobile phase and the pH of the mobile phase on the efficiency of separation. The linearity, accuracy, precision, specificity and robustness of the developed method showed acceptable values. The method was applied to the analysis of the samples of the two compounds under the study. The developed method is suitable for quality control (QC) of meclizine hydrochloride and pyridoxine hydrochloride in their pharmaceutical preparations (tablets and suppositories).

Keywords: HPLC, meclizine, pyridoxine, validation method.

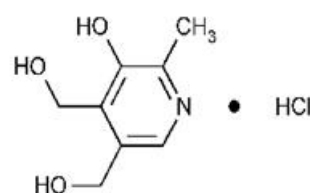
INTRODUCTION

Meclizine hydrochloride (MEC) is 1-[(RS 4 chlorophenyl) phenylmethyl] -4-(3methyl benzyl)piperazine dihydro chloride. (Figure 1) a piperazine derivative and antihistamine with anti muscarinic and central sedative properties, mainly used for its antiemetic action and in the prevention and treatment of nausea and vomiting associated with a variety of conditions. Pyridoxine hydrochloride (PYR) is (5-hydroxy-6-methyl pyridine-3,4-diyl)-dimethanol hydrochloride. (Figure 1), it is used in the treatment of sideroblastic anaemias, while it is readily absorbed from the gastrointestinal tract following oral administration it is converted to the active forms, pyridoxal phosphate. It is involved in amino acid as well as carbohydrate and fat metabolism. It is used against a variety of disorders including the treatment of depression.¹⁻³

Meclizine Hydrochloride and Pyridoxine Hydrochloride are combined in Preglazine tablets, used for the treatment of nausea and vomiting in pregnancy and for the prophylaxis and treatment of nausea, vomiting and dizziness associated with motion sickness.



Meclizine hydrochloride



Pyridoxine hydrochloride

Figure 1: Meclizine hydrochloride and Pyridoxine hydrochloride

A number of rapid chromatographic procedures for analytical quality control of pharmaceutical preparations containing PYR alone or with other antihistamine drugs is proposed; An LC method.⁴ For the analysis of PYR and MEC,⁵ another for the analysis of water soluble vitamins including PYR using C8 columns; and separation of water soluble vitamins including PYR by RP-LC was reported.⁶ These methods are applicable to pharmaceutical formulations only.⁷ reported a method to determine the acceptable residue level for MEC on pharmaceutical manufacturing equipment surfaces after cleaning with a recovery of 89.5% and other methods reported by various workers for the estimation of MEC has limited applications.^{4-6, 8-10}

There is a number of liquid chromatographic methods reported in the literature for the individual assays of PYR, MEC and buclizine (BUC).¹³⁻¹⁷ The reason for choosing these analytes is that MEC and PYR are commercially available as co-formulation. A spectro photometric method for the simultaneous analysis of MEC and PYR in bulk drug and pharmaceutical formulations was reported.^{18, 19}

The simultaneous determination of both drugs in mixtures using spectrophotometric methods,^{20,21} and HPLC methods using a reverse-phase, C18 column with methanol, potassium and sodium di hydrogen phosphate as the mobile phase have been reported.²²

Pharmacopoeial HPLC methods reported for each drug are unsuitable for their simultaneous determination in Preglazine tablets (generic product from a local company "MPI"), because of an overlap between the corresponding chromatographic peaks.

We designed an HPLC method for the determination of both components in standard solutions of Meclizine hydrochloride and pyridoxine hydrochloride and in Preglazine tablets and suppository. The aim of this study was to develop a valid, rapid, and sensitive analytical procedure using high performance liquid chromatography (HPLC) for the separation and concentration determination of both of Meclizine hydrochloride and pyridoxine hydrochloride in solid and semisolid dosages forms.

MATERIALS AND METHODS

Materials

Pharmaceutical grade meclizine hydrochloride and pyridoxine hydrochloride, and Preglazine tablets (50 mg pyridoxine hydrochloride, 25 mg meclizine hydrochloride) and Suppository (62.5 mg pyridoxine hydrochloride, 30 mg meclizine hydrochloride) were supplied by the Syrian Pharmaceutical Manufacturing Company (Damascus, Syria).

The HPLC system comprised a Jasco UV-970 intelligent UV/VIS system, equipped with a PU-980 pump, UV spectrophotometric detector set at 254 nm and a D6-980-50 degasser. A Jasco HPLC UV-970 UV/VIS spectrophotometer was used to assess the maximum absorption wavelength for both drugs.

Standard solutions

Approximately 50 mg pyridoxine hydrochloride and 25 mg Meclizine hydrochloride were accurately weighed and dissolved in 100mL mobile phase.

Meclizine and Pyridoxine were obtained from a local private pharmaceutical factory (Damascus, Syria).

Sample solutions

The finished pharmaceutical samples (tablets and suppositories) were also obtained from a local private pharmaceutical factory. Twenty Preglazine tablets were finely powdered and mixed well. A quantity equivalent to 50 mg pyridoxine hydrochloride and 25 mg meclizine hydrochloride was accurately weighed and transferred to a 100mL volumetric flask. Mobile phase was added and the solution was shaken for 15 min. The volume was made up to 100mL with mobile phase.

Methods

Chromatographic procedure

A Symmetry column (Thermo Scientific), 250 ×4.6 mm, packed with BDS Hypersil C18, particle size 5m, was used. The mobile phase consisted of acetonitrile, methanol and water (4:2:1.5). The pH (pH=3.8) was adjusted by adding glacial acetic acid. The Flow rate was maintained at 1.5mL/min. The injection volume was 20μL. The relative standard deviation of the peak areas for six replicate injections was not greater than 2%. Samples were injected into the column, chromatograms were recorded at 254 nm, and the areas for standard and sample solutions were tabulated.²³

Preparation of solutions for validation study

Standard solutions for linearity study

Five sequential concentrations were prepared containing 50%, 75%, 100%, 125% and 150% respectively of the standard solution concentration. They were consecutively added to a 100mL flask, the flask was filled to mark with the mobile phase, and the mixture mixed.

Solutions for accuracy study

Tablet excipients (i.e. Avicel and magnesium stearate) and suppository excipients (i.e. Mass BP) were individually spiked to the standard solutions to obtain analysis samples. Nine samples were divided into three groups containing respectively 70%, 100% and 130% respectively of corresponding standard solution.

Solutions for precision study

Tablet samples were analyzed. Nine samples were prepared and divided into three groups containing respectively 70%, 100% and 130% respectively of standard solution concentration.

Solutions for selectivity study

A drug-free sample was prepared from the excipients (Avicel and magnesium stearate) and another free sample was prepared from the excipients (Mass BP). Three samples containing 100% of standard solution concentration were also analyzed.

Solutions for robustness study

Three tablet samples containing 100% of standard solution concentration were analyzed.

RESULTS AND DISCUSSION

Chromatographic parameters

Various trials were carried out to select the optimum chromatographic parameters for suitable resolution between the two active components, excipients and degradation products.

Wavelength selection

Maximum absorption wavelengths for pyridoxine hydrochloride and meclizine hydrochloride were at

290nm and 220nm, respectively. A wavelength of 254 nm was chosen as the optimum wavelength for detection of both components. UV spectra of solutions containing 1mg=100mL of each of pyridoxine hydrochloride and meclizine hydrochloride in methanol were scanned between 200 and 300 nm.

Selection of mobile phase and experimental conditions

The individual mobile phases used for pyridoxine hydrochloride comprised glacial acetic acid, hexane sulphonic acid sodium salt and methanol, with detection wavelength at 280nm. For Meclizine hydrochloride it comprised heptane sulphonic acid sodium salt and acetonitrile, with pH=4 and detection wavelength at 230nm. These mobile phases were unsuitable for the simultaneous determination of both components because of an overlap between the HPLC peaks of the two components. Therefore the mobile phase was manipulated to obtain a combination that could separate both components and other drug excipients without interference. Water, methanol and acetonitrile of a ratio (1.5:2:4) were used. The results were not satisfactory and an overlap between the peaks of the two components was observed.

The introduction of hexane sulphonic acid sodium salt ion-pair reagent resulted in good resolution between the peaks of the two components, but the meclizine hydrochloride peak was very broad. Using higher molecular weight ion-pair reagent (dodecyl sulphate sodium salt) gave better resolution. Increasing the ratio of methanol caused splitting in the meclizine hydrochloride peak. The pH of the mobile phase was 3.8. A combination of dodecyl sulphate sodium salt dissolved in water, acetonitrile and methanol as the mobile phase was used in conjunction with a C18 BDS hypersil column (250 x 4.6 mm) that gave good resolution with minimal tailing factors. The chromatograms showed acceptable resolution between the two components. However, the tailing factor for meclizine hydrochloride was relatively high (approx.2) the addition of tetrahydrofuran to the mobile phase reduced the tailing factor for meclizine hydrochloride peak to 1. (Figure 2) shows the separation of meclizine hydrochloride and pyridoxine hydrochloride peaks with no interference from drug excipients or degradation products.

Selection of flow rate

Increasing the flow rate from 1 ml/min to 1.5 ml/min showed a similar decrease in the retention time. Sufficient flow rate of 1.5 ml/min was chosen to avoid overlap between peaks and the loss of its acceptable resolutions value.

Validation of the method

Validation was based on the requirements of ICH guidelines for validation of analytical procedures and US Pharmacopoeia [24-25]. Parameters like specificity, linearity, LOQ, LOD, accuracy, precision, and robustness were determined.

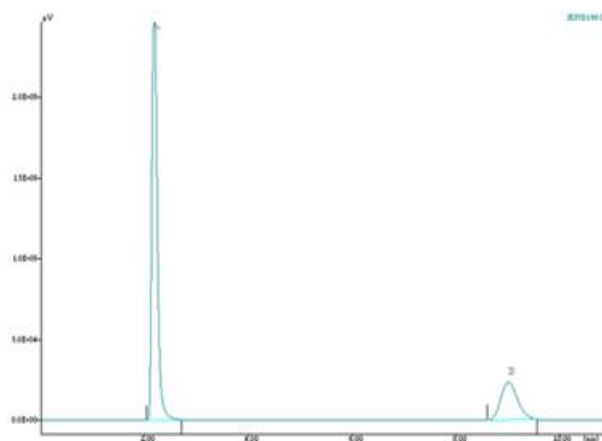


Figure 2: HPLC chromatogram of a mixture of pyridoxine hydrochloride (P) and meclizine hydrochloride (M) using a mobile phase of dodecyl sulphate sodium salt dissolved in water, acetonitrile, methanol, glacial acetic acid, and tetrahydrofuran.

Specificity

The specificity was evaluated by analyzing solutions containing the excipients present in tablet and suppository formulations. The system response was examined for the presence of interference or overlaps with the PYR and MEC responses.

Linearity and range

The calibration curves were constructed with five concentrations ranging from 50 to 150mg/mL for MEC and PYR. Analyses were performed in triplicate to determine the linearity of the assay. The regression lines were calculated by the method of least squares of peak area versus analyte concentrations.

Accuracy

The accuracy of the developed method was determined by measuring the drug recoveries by the standard addition method. Known amounts of each drug standard were added to pre-analyzed tablet and suppository sample solutions containing the excipients of tablets and suppository respectively to obtain concentrations corresponding to 70%, 100% and 130% of the label claim for each pharmaceutical formulation. The recovery of the added standard was determined in triplicate analyses and calculated as the percentage of the drug recovered from each formulation.

Precision

The precision of the method was determined by repeatability (intraday) and intermediate precision (interday) studies. The repeatability was evaluated by assaying nine samples of three standard concentrations (70%, 100%, and 130%) on the same day and under the same experimental conditions. The intermediate precision was evaluated by assaying solutions by another analyst. The precisions were expressed as RSD.

Robustness and ruggedness

The robustness of the developed method was examined by comparing results after making small and deliberate

variations of critical parameters such as flow rate (from 1.4 to 1.6 mL/min).

Table 1: Recovery characteristics of Meclizine pyridoxine in dosage formulation

Validation results		Meclizine Hydrochloride			Pyridoxine Hydrochloride	
Selectivity	Rec.	Tab.	Supp.	Tab.	Supp.	
		100.1	99.95	99.54	99.49	
Accuracy	Rec.	Tab.	Supp.	Tab.	Supp.	
		100.5	100.24	100.37	99.49	
Linearity		(r) : 0.9973		(r) : 0.999		
		Slope: 22758.9		Slope:30422.9		
		KF : 22023.4		KF : 30335.4		
		RF : 15532.5		RF : 22297.4		
Precision	repeatability	Recovery		100.04	100.7	
		SD		1.54	1.68	
		RSD		1.54	1.67	
		CI		1.49	1.62	
	Intermediate Precision	Recovery		100.74	99.86	
		SD		1.68	1.52	
		RSD		1.67	1.52	
		CI		1.62	1.47	
Robustness	Flow Rate	1.4 ml/m	Rec. RT	99.48	100.63	
				5.71	2.35	
		1.5 ml/m	Rec. RT	100.42	99.83	
				5.32	2.20	
		1.6 ml/m	Rec. RT	99.15	99.94	
				4.98	2.05	

KF: Response Factor; RF: Residual Factor; R: Correlation factor; RSD: Relative Standard Deviation; Rec: Recovery; RT: Retention Time; SD: Standard Deviation; CL: Confidence Limit

CONCLUSION

Our HPLC-method is specific, sensitive, rapid and easy to perform. The limit of quantification, small sample volume and short chromatographic time of this method make it advantageous for adaptation to routine assay requirements and enables simultaneous determination of PYR and MEC with good separation and resolution of the chromatographic peaks.

Obtained results are in a good agreement with the declared contents of dosage formulations. Results are accurate and precise and are confirmed by the statistical parameters. Reliability, rapidness, sensitivity, economical nature, good recovery and precision of this HPLC method give it advantage over the other reported methods.

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