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Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Meclizine Hydrochloride and Pyridoxine Hydrochloride in Tablet Dosage Form

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ABSTRACT:

Two simple, rapid, precise and accurate UV-spectrophotometric methods have been developed for determination of Meclizine Hydrochloride (MEH) and Pyridoxine Hydrochloride (PYH) by Simultaneous Equation Method (SEM) and Q-Analysis Method in combined tablet dosage form. The simultaneous equation method is based on measurement of absorbances at 220 nm and 295 nm and Q-Analysis is based on measurement of absorbances at isobestic wavelength i.e. 257 nm and wavelength of maximum absorption of one of the two components i.e. 295 nm for simultaneous estimation of Meclizine Hydrochloride and Pyridoxine Hydrochloride. The proposed methods obeyed Beer's law in the concentration range of 5-30 µg/ml for MEH and 10-60 µg/ml for PYH. The proposed methods were validated and can be applied successfully for routine quality control analysis of MEH and PYH in bulk and pharmaceutical formulation.

Key words: Simultaneous Equation Method, Q-Analysis, λmax, Validation, Meclizine Hydrochloride, Pyridoxine Hydrochloride.

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INTRODUCTION:

Meclizine Hydrochloride, 1-[(4-Chlorophenyl)- Phenylmethyl] -4- [(3-methylphenyl) methyl] piperazine hydrochloride, is a antihistamines, antiemetics and antiallergic agent.[1] The mechanism of action of Meclizine is related to its central anticholinergic actions. It diminishes vestibular stimulations and depresses labyrinthine function. An action on the medullary chemoreceptive trigger zone may also involved in the antiemetic effect.[2] MEH having molecular formula C25H28Cl2N2 and molecular weight 427.4092 g/mol. It is official in IP, BP and USP[3] (Fig. No. 1).

Pyridoxine Hydrochloride, 5-hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride, is a Vitamin (It is used in the treatment and prevention of pyridoxine deficiency states).[4] Vitamin B6 , principally in its biologically active coenzyme form pyridoxal 5'-phosphate, is involved in a wide range of biochemical reactions, including the metabolism of amino acids and glycogen, the synthesis of nucleic acids, hemoglobin, sphingomyelin and other sphingolipids, and the synthesis of the neurotransmitters serotonin, dopamine, norepinephrine and gamma-aminobutyric acid (GABA).[5] PYH having Molecular formula C8H12ClNO3 and molecular weight 205.638 g/mol. It is official in IP, BP and USP[6] (Fig. No. 2).

On the basis of literature survey few analytical techniques like RP-HPLC, HPTLC, HPLC, Spectroscopic methods have been reported for the determination of MEH and its combination with other drugs in pharmaceutical preparations.[7] Estimation of PYH in combination with other drugs by spectrophotometric methods and HPLC has been reported.[8] But there are no methods reported in any pharmacopoeia for simultaneous estimation of Meclizine Hydrochloride and Pyridoxine Hydrochloride in tablet dosage form.

MATERIALS AND METHODS

Instruments:

(a) Spectrophotometer- Double beam UV–Visible spectrophotometer with 1 cm matched quartz cell Model UV-1601 Shimadzu.

(b) Electronic Balance- Shimadzu.

Reagents and Chemicals:

Meclizine Hydrochloride and Pyridoxine Hydrochloride reference standard were kindly provided by Yarrow Chem. Products Mumbai. Methanol of analytical grade was used as a solvent. All chemicals and reagents used were of analytical reagent grade.

Marketed Preparation:

The brand name of marketed combined tablet formulation is PNV containing Meclizine Hydrochloride 25 mg and Pyridoxine Hydrochloride 50 mg manufactured by Yash Pharma. Lab. Pvt. Ltd.

[I] Simultaneous Equation Method (SEM)

1. Preparation of standard stock solution:

(a) Meclizine Hydrochloride*:-* Standard stock solution of Meclizine Hydrochloride was prepared by dissolving 10 mg of Meclizine Hydrochloride in 100 ml of Methanol to produce a concentration of 100 µg/ml.

(b) Pyridoxine Hydrochloride*:-* Standard stock solution of Pyridoxine Hydrochloride was prepared by dissolving 10 mg of Pyridoxine Hydrochloride in 100 ml of methanol to produce a concentration of 100 µg/ml.

2. Study of wavelengths of peak absorption of the drugs:

The aliquot portion of stock standard solutions of MEH and PYH were diluted appropriately with methanol to obtain concentration 25 µg/ml and 50 µg/ml respectively. The solutions were scanned in the range of 200-400 nm in 1 cm cell against blank. The λ max was determined on double beam UV – Visible Spectrophotometer using methanol as blank. The λmax was found to be 220 nm and 295 nm respectively. The Overlain UV absorbance spectrum of MEH and PYH is shown in **Fig. No. 3**.

3. Preparation of calibration curve or study of Beer-Lambert's Law:

The aliquot portion of stock standard solutions of MEH and PYH were diluted appropriately with methanol as solvent to get a series of concentration between 5-30 µg/ml of MEH and 10-60 µg/ml of PYH. The absorbance of each solution was measured at 220 nm and 295 nm in 1 cm cell against methanol as blank. The graphs plotted as concentration Vs absorbance at selected wavelengths for MEH and PYH are shown in **Fig. No. 4 and 5** respectively.

4. Determination of absorptivity values A (1%, 1cm) of drugs at selected wavelengths:

Aliquot portions of MEH from stock solution were transferred to five 10 ml volumetric flasks; volume was adjusted to mark to obtain the concentration of 25 µg/ml. Similarly, aliquot portions from PYH stock solution were transferred to 10 ml volumetric flasks; volume was adjusted to mark to obtain concentration of 50 µg/ml. Absorbance of these solutions was recorded at two wavelengths 220 nm and 295 nm. A (1%, 1cm) values of drugs were calculated using following formula-

A (1%, 1cm) = Absorbance / Concentration (g / 100 ml)

Results of A (1%, 1cm) of drugs are given in **Table No.1**.

Concentration Cx and Cy of MEH and PYH respectively in g/100 ml in the sample solution can be obtained by:

Simultaneous Equation Method-

$$Cx = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \quad \text{---------- (I)}$$

$$Cy = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \quad \text{---------- (II)}$$

Where,

A_1 and A_2 are the absorbance of the sample solution measured at 220 nm and 295 nm respectively.
ax_1 and ax_2 are absorptivity of MEH at 220 nm and 295 nm respectively.
ay_1 and ay_2 are absorptivity of PYH at 220 nm and 295 nm respectively.

5. Simultaneous estimation of drugs in standard laboratory mixture:

Accurately weighed 10 mg MEH and 10 mg PYH were transferred to 100 ml volumetric flask individually containing 40 ml methanol, shake manually for 10 minute and the volume was adjusted to the mark with the same solvent. Appropriate aliquot portion of these solutions were mixed to get the concentration 25 µg/ml MEH and 50 µg/ml of PYH. Absorbance was measured at 220 nm and 295 nm against methanol as blank. Amount of each drug was estimated using (I) and (II) equation and results are given in **Table No. 2**.

6. Simultaneous estimation of drugs in tablets:

Twenty tablets each containing 25 mg of MEH and 50 mg of PYH were weighed and average weight was calculated. The tablets were crushed to fine powder. The powder equivalent to 25 mg of MEH and 50 mg of PYH was transferred to 100 ml volumetric flask containing 70 ml of methanol followed by sonication for 10 min and then the volume was made upto 100 ml with methanol. The solution was diluted further with methanol to obtain 25 µg/ml of MEH and 50 µg/ml of PYH. The solution was filtered through a Whatman filter paper (No. 41). The absorbances were recorded. The concentrations of two drugs in sample were determined using equation No. (I) and (II). The results are given in **Table No. 3**.

Brand Name: PNV (Composition- Meclizine Hydrochloride: 25 mg, Pyridoxine Hydrochloride: 50 mg).

Average Weight: 250 mg.

[II] Q-Analysis Method (Graphical Absorbance Ratio / Q-Absorbance Ratio Method)

1. Determination of isobestic point (isoabsorptive point) and selection of suitable wavelength:

In the quantitative assay of two components by absorbance ratio method, absorbances were measured at two wavelength, one being the isobestic wavelength and the other being wavelength of maximum absorption of one of the two components. From the stock solutions MEH (25 µg/ml) and PYH (50 µg/ml) were prepared and scanned in the UV range. From overlain spectra of MEH and PYH **(Fig. No. 3)**, absorbances were measured at the selected wavelength i.e. at isobestic wavelength (257 nm) and at the wavelength of maximum absorption (295 nm). The two wavelengths i.e. 257 nm as isobestic point and 295 nm as wavelength of maximum absorption of one of the two components were selected for estimation of drugs simultaneously.

2. Preparation of calibration curve or study of Beer-Lambert's Law:

The aliquot portion of stock standard solutions of MEH and PYH were diluted appropriately with methanol as solvent to get a series of concentration between 5-30 µg/ml of MEH and 10-60 µg/ml of PYH. The absorbance of each solution was measured at 257 nm (isobestic wavelength) and 295 nm (wavelength of maximum absorption) in 1 cm cell against methanol as blank. The graphs plotted as concentration Vs absorbance at selected wavelengths for MEH, PYH and mixture. Both the drugs obeyed linearity individually and combination within the concentration range of 5-30 µg/ml of MEH and 10-60 µg/ml of PYH solutions.

3. Determination of absorptivity values A (1%, 1cm) of drugs at selected wavelengths:

Aliquot portions of MEH and PYH standard stock solution were diluted with methnol to obtain concentration 25 µg/ml of MEH and 50 µg/ml of PYH. The absorbance of each resulting solution was measured at 257 nm (isobestic wavelength) and 295 nm (wavelength of max. absorption) in 1 cm cell using methanol as blank. A (1%, 1cm) values of drugs were calculated using following formula-

A (1%, 1cm) = Absorbance / Concentration (g / 100 ml)

Results of A (1%, 1cm) of drugs are given in **Table No. 1**.

4. Simultaneous estimation of drugs in standard laboratory mixture:

Accurately weighed 10 mg MEH and 10 mg PYH were transferred to 100 ml volumetric flask individually containing 40 ml methanol, shake manually for 10 minute and the volume was adjusted to the mark with the same solvent. Appropriate aliquot portion of these solutions were mixed to get the concentration 25 µg/ml

MEH and 50 µg/ml of PYH. Absorbance was measured at 257 nm (isobestic wavelength) and 295 nm (wavelength of maximum absorption) against methanol as blank. The concentration of each component was calculated by mathematical treatment of the following mentioned equation No. (III) and (IV) and results are given in **Table No. 2**.

For MEH, $C_x = Q_m - Q_x / Q_y - Q_x . A_1 / ax_1$ ----------**(III)**

For PYH, $C_y = Q_m - Q_y / Q_x - Q_y . A_1 / ay_1$ ----------**(IV)**

Where,
Cx=Concentration of MEH and **Cy**=Concentration of PYH,
A1=Absorbance of sample at isobestic wavelength (257 nm),
ax1 and ay1=Absorptivity of pure MEH and PYH respectively at isobestic wavelength (257 nm),
Qx=Absorptivity of MEH at wavelength of max. absorption (295 nm) **/** Absorptivity of MEH at isobestic wavelength (257 nm),
Qy=Absorptivity of PYH at wavelength of max. absorption (295 nm) **/** Absorptivity of PYH at isobestic wavelength (257 nm),
Qm=Absorptivity of sample solution at wavelength of max. absorption (295 nm) **/** Absorptivity of sample solution at isobestic wavelength (257 nm).

5. Simultaneous estimation of drugs in tablets:

Twenty tablets each containing 25 mg of MEH and 50 mg of PYH were weighed and average weight was calculated. The tablets were crushed to fine powder. The powder equivalent to 25 mg of MEH and 50 mg of PYH was transferred to 100 ml volumetric flask containing 70 ml of methanol followed by sonication for 10 min and then the volume was made upto 100 ml with methanol. The solution was diluted further with methanol to obtain 25 µg/ml of MEH and 50 µg/ml of PYH. The solution was filtered through a Whatman filter paper (No. 41). The absorbances were recorded at 257 nm (isobestic wavelength) and 295 nm (wavelength of max. absorption). The concentration of each component was calculated by using equation No. (III) and (IV) and results are given in **Table No. 3**.

Brand Name: PNV (Composition- Meclizine Hydrochloride: 25 mg, Pyridoxine Hydrochloride: 50 mg).

Average Weight: 250 mg.

Recovery Studies:

In recovery study to the preanalysed sample solutions (25 µg/ml MEH and 50 µg/ml of PYH) a known amount of standard solutions of pure drugs (MEH and PYH) was added at different level. The % recovery was calculated by using formula,

% Recovery = (A / B + C) X 100

Where,
A = Total amount of drug estimated,
B = Amount of drug found on preanalysed basis and
C = Amount of pure drug added.

Results of recovery studies for developed methods are shown in **Table No. 4.**

Validation of Developed Methods:

Validation of developed methods were done as per ICH guidelines. Methods were validated for various parameters such as accuracy, precision, linearity, repeatability and ruggedness.

1. Accuracy: Accuracy of the proposed methods were ascertained on the basis of recovery studies performed by the standard addition method. The results of recovery studies for both the methods are shown in **Table No. 4**.

2. Precision: Precision of the developed methods were determined by intra-day and inter-day precision. Intra-day precision was determined by analyzing the 20, 25, 30 µg/ml of MEH and 40, 50, 60 µg/ml of PYH drug solution for three times in the same day. Inter-day precision was determined by analyzing the same concentration at three different days. The results of precision of proposed methods are shown in **Table No. 5**.

3. Linearity and Range: The study of linearity and range was performed as per ICH guidelines. MEH and PYH was found to be linear at a concentration range of 5-30 µg/ml and 10-60 µg/ml respectively with R^2 = 0.99 at selected wavelengths for both the methods.

4. Repeatability: Repeatability of the developed methods were determined by analyzing MEH (25 µg/ml) and PYH (50 µg/ml) of drug solutions for five times and results are given in **Table No. 6**.

5. Ruggedness: Ruggedness of the proposed methods were determined by analysis of aliquots from homogenous slot by two analyst using same operational

and environmental conditions. Results are given in **Table No. 7**.

RESULTS AND DISCUSSION

The simultaneous equation method was carried out at 220 nm and 295 nm i.e. λmax of MEH and PYH respectively. Whereas Q-analysis was performed by using isobestic wavelength (257 nm) and wavelength of maximum absorption of one of the two components (295 nm). In both the methods drugs obeyed Beer's law in the concentration range of 5-30 µg/ml of MEH and 10-60 µg/ml of PYH. Standard calibration curves for MEH and PYH were linear with correlation coefficients R^2 =0.99 at all the selected wavelengths. The results for simultaneous estimation of standard laboratory mixture and marketed tablets by proposed methods are shown in **Table No. 2 and 3** respectively. Validation of proposed methods were done as per ICH Validation parameters like Accuracy, Precision, Linearity, Repeatability and Ruggedness. The method showed accuracy in the range of 96-100 % . Results of Validation parameters are shown in **Table No. from 4-7**.

CONCLUSION

A simple, accurate, precise and rapid UV-spectrophotometric methods were developed for simultaneous estimation of MEH and PYH in combined tablet dosage form. The methods were validated as per ICH guidelines. The methods developed for simultaneous estimation of MEH and PYH are rapid, precise and accurate. The results of validation tests were found to be satisfactory and therefore, these methods can be used for quality control analysis of MEH and PYH in bulk and pharmaceutical formulations. The proposed methods use inexpensive reagents, solvents and instruments that are available in laboratories. Hence, these methods can be conveniently adopted for the routine quality control laboratories.

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TABLES AND FIGURES

Table 1: Absorptivity values of MEH and PYH

Sr. No.	Drugs	Absorptivity values for Method-[I] SEM		Absorptivity values for Method-[II] Q-Analysis	
		220 nm	295 nm	257 nm	295 nm
1	MEH	150.8	99.84	39.2	32
2	PYH	52.72	153.04	24	85

Table 2: Estimation of MEH and PYH in Standard Laboratory Mixture

Sr. No.	Methods	Drugs	Conc. of std. (µg/ml)	Amount found (µg/ml)	% Amount found	%RSD (n=5)
1	Method –[I] SEM	MEH	25	24.78	99.12	0.73
		PYH	50	49.30	98.6	0.95
2	Method –[II] Q-Analysis	MEH	25	24.61	98.44	0.82
		PYH	50	49.59	99.1	0.59

Table 3: Estimation of MEH and PYH in Tablets

Sr. No.	Methods	Drugs	Conc. of tablet (µg/ml)	Mean amount found (mg/tab)	% Amount found (mean)	% RSD (n=5)
1	Method –[I] SEM	MEH	25	24.80	99.2	0.51
		PYH	50	48.63	97.26	0.98
2	Method –[II] Q-Analysis	MEH	25	24.19	96.76	0.99
		PYH	50	48.95	97.9	0.75

Table 4: Results of Recovery Studies

Sr. No.	Methods	Pre-analysed samples (µg/ml)		Pure drug added (µg/ml)		Drug recovered (µg/ml)		% Recovery		% RSD (n=5)	
		MEH	**PYH**	**MEH**	**PYH**	**MEH**	**PYH**	**MEH**	**PYH**	**MEH**	**PYH**
		25	50	20	40	19.96	39.43	99.8	98.57	0.77	0.85
1	Method –[I] SEM	25	50	25	50	24.11	49.30	96.44	98.60	1.40	1.19
		25	50	30	60	29.66	58.15	97.54	96.92	0.96	1.10
		25	50	20	40	19.95	39.94	99.76	99.93	1.28	0.64
		25	50	25	50	24.49	49.27	97.96	98.56	1.59	0.36
2	Method –[II] Q-Analysis	25	50	30	60	29.08	59.79	96.94	99.65	0.81	1.44

Table 5: Results of Precision Studies (Intra-day and Inter-day)

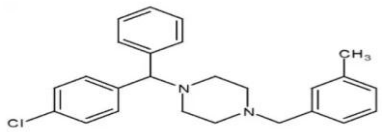
Sr. No.	Methods	Drugs	Conc. (µg/ml)	Intra-day amount found (n=5)		Inter-day amount found (n=5)	
				Mean	**% RSD**	**Mean**	**% RSD**
		MEH	20	19.86	1.30	19.97	0.49
			25	24.95	0.45	24.29	0.33
			30	28.91	0.93	29.81	0.50
	Method –[I]	PYH	40	39.62	0.53	39.51	0.63
1	SEM		50	48.24	1.22	49.95	0.82
			60	59.33	0.76	58.38	0.74
		MEH	20	19.72	0.60	19.96	0.50
			25	24.83	0.80	24.95	1.26
			30	29.94	0.13	28.93	0.76
2	Method –[II]	PYH	40	39.86	0.17	39.96	0.10
	Q-Analysis		50	49.92	0.22	48.92	1.04
			60	58.80	0.55	59.86	0.35

Table 6: Results of Repeatability Studies

Sr. No.	Methods	Drugs	Conc. (µg/ml)	Mean conc. found (µg/ml)	% RSD (n=5)
1	Method – [I] SEM	MEH	25	24.90	0.46
		PYH	50	43.88	0.89
2	Method – [II] Q-Analysis	MEH	25	24.6	0.65
		PYH	50	44.01	0.94

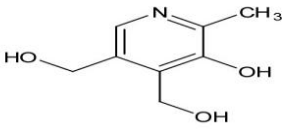
Table 7: Results of Ruggedness studies

Sr. No.	Methods		Amount found (%)		% RSD (n=3)	
			MEH	PYH	MEH	PYH
1	Method –[I] SEM	Analyst-I	99.05	98.12	1.21	0.74
		Analyst-II	97.91	98.31	0.56	0.80
2	Method –[II] Q-Analysis	Analyst-I	99.18	98.87	0.33	0.72
		Analyst-II	98.26	99.03	1.44	0.92



. HCl

Figure 1: Chemical Structure of Meclizine Hydrochloride



• HCl

Figure 2: Chemical Structure of Pyridoxine Hydrochloride

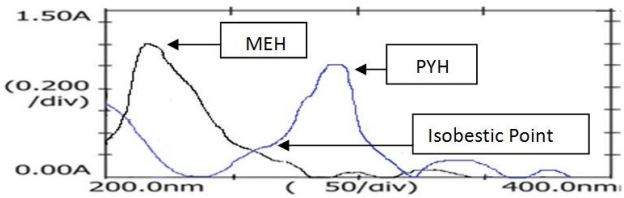


Figure. 3: Overlain spectra of MEH and PYH (25:50 µg/ml)

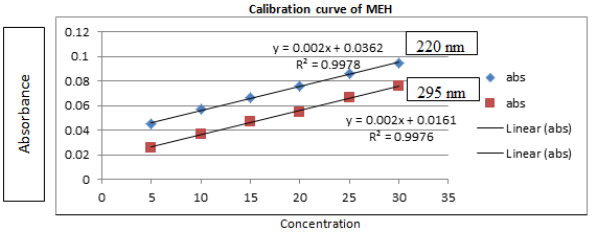


Figure 4: Plot of Beer-Lambert's study for MEH at 220 nm and 295 nm

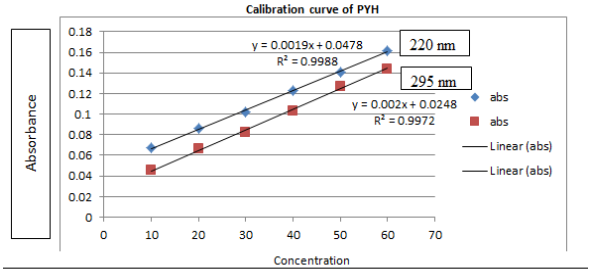


Figure 5: Plot of Beer- Lambert's study for PYH at 220 nm and 295 nm