

Spectrophotometric methods for analysis of different dosage forms containing pyridoxine hydrochloride

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ABSTRACT

Two accurate, precise, sensitive and selective spectrophotometric methods have been developed and validated for determination of pyridoxine hydrochloride (PYH), cyclizine hydrochloride (CYH) and meclizine hydrochloride (MEH) either in their binary mixtures or in synthetic ternary mixtures. In modified area under the curve method (Method I), PYH has been determined by measuring amplitude value of the plateau at 283 nm (obtained after dividing the ternary mixture by standard spectrum of 20 µg/mL of PYH at which no interference from CYH and MEH) while area under the curve spectrophotometric method (AUC) has been used for calculating CYH and MEH concentrations in the ternary mixture by measuring the area under the curve in the range of 215-228 and 230-243 nm in the obtained division spectrum and after subtraction of the constant value at 283 nm. Method II is mean centering of ratio spectra spectrophotometric method (MCR) at which the mean centered ratio spectra in two successive steps have been used for measuring the amplitudes of the mean centered second ratio spectra amplitudes at 228.8, 262.0 and 270.8 nm for PYH, CYH and MEH, respectively. The proposed methods were successfully applied for determination of the cited drugs in their pharmaceutical formulations. Also they were statistically compared with the reported methods using student's-t and F-tests and there was no significant difference between them regarding both accuracy and precision.

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1. Introduction

Pyridoxine hydrochloride, 5-hydroxy-6-methyl-3,4 pyridine dimethanol hydrochloride (Figure 1) [1]. It is a water-soluble vitamin which is involved mainly in amino acid, carbohydrate and fat metabolism. Pyridoxine has also been used in the treatment of many disorders including depression and symptoms associated with the premenstrual syndrome [2]. Cyclizine hydrochloride is chemically designated as 1-diphenyl-4-methylpiperazine (Figure 1) [1]. It is a piperazine derivative and sedating antihistamine with anti-muscarinic activity, it is used as an antiemetic in the management of nausea and vomiting including motion sickness, post-operative nausea and vomiting [2]. Meclizine hydrochloride is chemically known as 1-[(4-chlorophenyl)phenyl methyl]-4-[3-methylphenyl)methyl]piperazine (Figure 1) [1]. It is a piperazine derivative with anti-muscarinic and moderate sedative properties. It is also used in the prevention and treatment of nausea and vomiting associated with a variety of conditions including motion sickness [2].

Reviewing the literature in hand, different methods have been published for determination of PYH and CYH in their binary mixtures such as second derivative of ratio spectra

(²DD) spectrophotometric method, partial least squares (PLS-1) spectrophotometric method and a RP-HPLC methods [3]. While the binary mixture a long with caffeine were analyzed by a RP-HPLC method [4].

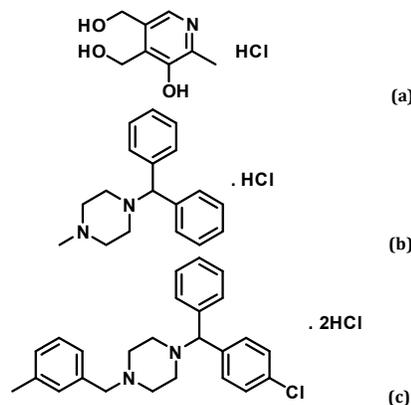


Figure 1. Chemical structure of pyridoxine hydrochloride (a), cyclizine hydrochloride (b) and meclizine hydrochloride (c).

On the other hand PYH and MEH binary mixture was resolved by different spectrophotometric methods such as simultaneous equation method (using the absorbance at 231 and 220 nm) and formation of ion pair complex with FeCl_3 spectrophotometric method [5]. Also different RP-HPLC methods were reported for determination of PYH and MEH mixture [6-8], this mixture was also analyzed using a TLC-densitometric method [8]. The ternary mixture containing PYH, MEH and caffeine was analyzed by the extension of Vierordt's spectrophotometric method [9]. Moreover, the ternary mixture of PYH, MEH and buclizine was determined either in pharmaceutical formulation or in human serum by a HPLC method [10].

From the previous literature review, no reported method has been developed for determination of the three components. So, the goal of the present work is to develop and validate two spectrophotometric methods for determination of ternary mixture containing PYH, CYH and MEH which will be time and money saving that is important factors in quality control drug analysis. The developed methods are selective, accurate, precise, and could be successfully applied to their pharmaceutical formulations.

2. Experimental

2.1. Instruments

A double beam UV-Visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC with 1 cm path length, quartz cell is used and connected to IBM compatible computer. The spectrophotometer is operated by using UVPC personal spectroscopy software version 3.7. Matlab® version 2007b used for the proposed MCR method.

2.2. Samples

2.2.1. Pure samples

Pyridoxine HCl and cyclizine HCl were kindly supplied by AMOUN Pharmaceutical Company (El Obour city, Cairo, Egypt). Their purity was found to be 99.75 and 99.40%, respectively, according to manufacturer certificates. While meclizine HCl was supplied by SIGMA Pharmaceutical Company (Zone 1, Moubarak Industrial City, Quesna, Menoufia, Egypt). Its purity was 99.65% according to manufacturer certificate.

2.2.2. Marketed samples

Emetrex® tablets (Batch No. 20766) manufactured by AMOUN Pharmaceutical Company labeled to contain 30 mg of PYH and 50 mg of CYH per tablet. Dizerest B6® tablets (Batch No. 30248) manufactured by SIGMA Pharmaceutical Company labeled to contain 50 mg PYH and 25 mg of MEH.

2.3. Chemicals and solvents

All chemicals and solvents used throughout this work were of analytical grade, and the solvents were of analytical grade and were used without further purification, methanol (Sigma-Aldrich, Chemie GmbH, Germany) and hydrochloric acid (El-Nasr pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).

2.4. Solutions

Stock standard solutions of PYH, CYH and MEH were prepared in methanol in the concentration of 1 mg/mL. Working standard solutions of PYH, CYH and MEH (0.1 mg/mL) were prepared by suitable dilutions of their respective stock solutions using 0.05 N HCl solution.

2.5. Laboratory prepared mixtures

Different mixtures containing different ratios of PYH, CYH and MEH were prepared using their respective working solutions (0.1 mg/mL), including the ratio of their marketed formulations and using 0.05 N HCl as a solvent.

2.6. Procedure

2.6.1. Spectral characteristics of PYH, CYH and MEH

Zero order absorption spectra of 5 $\mu\text{g/mL}$ each of PYH, CYH and MEH were recorded from 200 to 400 nm using 0.05N HCl as a solvent.

2.6.2. Construction of calibration curves

2.6.2.1. Modified area under curve method (Method I)

Accurate aliquots equivalent to 50-400, 40-220 and 30-200 μg of PYH, CYH and MEH, respectively, were transferred separately from their respective working standard solutions (100 $\mu\text{g/mL}$) into three separate series of 10 mL volumetric flasks. The volume was completed to the mark with 0.05 N HCl solution to obtain the final concentration ranges of each one. The absorption spectra of the prepared solutions were measured in the range of 200-400 nm. For determination of PYH, the obtained spectra were divided by standard spectrum of 20 $\mu\text{g/mL}$ of PYH and then the amplitude value of the plateau at 283 nm were recorded where no interference from CYH and MEH was found. Then the calibration graph was constructed relating the amplitudes values to the corresponding concentrations of PYH. For CYH and MEH the area under curve method (AUC) was applied. Where the area under the curve in the range of 215-228 (λ_1 - λ_2) and 230-243 nm (λ_3 - λ_4) were recorded for the prepared solutions of pure CYH and MEH. The absorptivity (Y) value was then calculated for each component where $Y = \frac{\text{recorded area under the curve of the component}}{\text{concentration of the component in } \mu\text{g/mL}}$. The concentrations of the components in the prepared solutions were determined by using Cramer's rule and matrices according to the following equations:

$$A_1 = Y_{x1}C_x + Y_{z1}C_z (\lambda_1-\lambda_2) \quad (1)$$

$$A_2 = Y_{x2}C_x + Y_{z2}C_z (\lambda_3-\lambda_4) \quad (2)$$

A_1, A_2 are the area under the curve in the range of 215-228 and 230-243 nm, respectively. Y_{x1}, Y_{x2} are the absorptivity values of CYH at (λ_1 - λ_2) and (λ_3 - λ_4), respectively. Y_{z1}, Y_{z2} are the absorptivity values of MEH at (λ_1 - λ_2) and (λ_3 - λ_4), respectively. C_x and C_z are the concentration of CYH and MEH, respectively.

2.6.2.2. Mean centering of ratio spectra spectrophotometric method (MCR) (Method II)

Accurately measured aliquots equivalent to 50-400, 10-220 and 20-200 μg of PYH, CYH and MEH, respectively, were separately transferred from their working standard solution (100 $\mu\text{g/mL}$) into three separate sets of 10 mL volumetric flasks. The volume was then completed with 0.05 N HCl. The absorption spectra of the prepared solutions were measured in the range of 200-400 nm.

In order to determine PYH, 20 $\mu\text{g/mL}$ of CYH was used as a divisor where the stored spectra of PYH were divided by it to obtain the first ratio spectra then these spectra were mean centered. These vectors were then divided by the mean centered ratio of $\alpha_{\text{MEH}}/\alpha_{\text{CYH}}$ and the mean centering of the second ratio spectra were then obtained [11]. Also the recorded spectra of CYH were divided by the spectrum of PYH

(20 µg/mL then the obtained ratio spectra were mean centered, these vectors (mean centered ratio spectra) were divided by the mean centered (MC) ratio of ($\alpha_{MEH}/\alpha_{PYH}$) to obtain the second ratio spectra which were then mean centered. By the same way, the recorded spectra of MEH were divided by the standard spectrum of 20 µg/mL of PYH and the obtained ratio spectra were mean centered. These vectors were divided by the mean centered ratio (MCR) of ($\alpha_{CYH}/\alpha_{PYH}$) and the second ratio spectra were then mean centered.

The mean centered values of the second ratio spectra at 228.8, 26.02 and 270.8 nm for PYH, CYH and MEH, respectively, were recorded and then plotted against the corresponding concentration of each component. Their calibration curves were constructed and regression equations were computed.

2.6.3. Analysis of laboratory prepared mixtures

The instructions given under construction of calibration curves for each method were followed but using the scanned spectra of different laboratory prepared mixtures and the previously computed regression equations were used for calculating PYH, CYH and MEH concentrations.

2.6.4. Application to pharmaceutical formulation (Emetrex® and Dizerest B6® tablets)

Ten tablets each of Emetrex® and Dizerest B6® were separately grinded, mixed and accurately weighted. An amount of Emetrex® powder equivalent to 50 mg of CYH (containing 30 mg PYH) and weighed an amount equivalent to 50 mg of PYH (containing 25 mg MEH) was taken from Dizerest B6® powder.

The accurately weighed powders were separately moved to two 50 mL volumetric flasks. 50 mL methanol was added and the prepared solutions were sonicated for 15 min then cooled well and filtered to be used as stock solutions (containing either 1 µg/mL PYH or CYH). Working standard solutions (0.1 mg/mL) were prepared by suitable dilutions of the previously prepared sample stock solutions using 0.05 N HCl solution.

Different dilutions were prepared and then the previously mentioned procedure for each method was carried out on Emetrex® and Dizerest B6® prepared samples. Concentrations of PYH, CYH and MEH were then calculated from the previously computed regression equations and the percentage recoveries were then calculated.

2.6.5. Application of standard addition technique

When performing the standard addition technique, different known concentrations of pure standard PYH, CYH and MEH (80-120%) were accurately added to the prepared pharmaceutical formulation samples before proceeding in the previously mentioned methods.

3. Result and discussion

The combination of PYH with CYH or MEH has been used for safely treatment and /or prevention of nausea and vomiting during pregnancy [12]. Till now there is no reported method for determination of PYH, CYH and MYH in their ternary mixture. So this work seeks to develop and validate highly selective and precise methods of analysis for accurate determination of the three drugs together and in their combined dosage forms.

The absorption spectra of PYH, CYH and MEH showed overlap which makes determination of each of them in the mixture more difficult, Figure 2. Derivative and derivative ratio spectrophotometric methods were tried but due to severe overlap of the spectra of the three drugs, these methods

failed to resolve their spectral overlap. By applying the modified area under curve method and mean centering of ratio spectra method to the spectral data of the mixture, PYH, CYH and MEH concentrations could be determined without any interference.

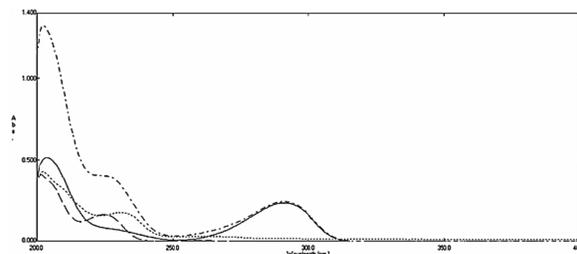


Figure 2. Zero order absorption spectra of 5 µg/mL each of pyridoxine hydrochloride (—), cyclizine hydrochloride (---), meclizine hydrochloride (....) and mixture containing 5 µg/mL of each (-.-) using 0.05 N HCl as a solvent.

3.1. Method development and optimization

As it is important to develop and validate a highly selective spectrophotometric methods to resolve the overlapping spectra of the studied drugs, so factors affecting method selectivity had to be studied and optimized to obtain the best results [13]. These factors include:

Effect of divisor and its concentration: The divisor concentration has a great effect on the method selectivity and analytical parameters such as correlation coefficients, slopes and intercepts of the calibration equations. So different concentrations of PYH, CYH and MEH were tested (5, 10, 15 and 20 µg/mL for each). It was observed that changing the concentration of the divisors had a great effect on the method selectivity therefore 20 µg/mL of each of PYH, CYH and MEH was used as divisor for all the developed methods. In the area under the curve method (AUC), selection of wavelength ranges is a critical step in method optimization as it affects selectivity of the method [14]. So different wavelength ranges were examined and it was found that wavelength ranges of 215-228 and 230-243 nm were the most suitable ranges regarding selectivity of CYH and MEH, respectively.

Effect of solvent: Different solvents were tried (methanol, ethanol, acetonitrile, water, 0.05 N HCl and 0.05 N NaOH), regarding sensitivity and selectivity, it was found that 0.05 N HCl was the best solvent for the suggested spectrophotometric methods.

3.2. Modified area under curve method (method I)

As shown in Figure 2, PYH has an extended spectrum over 280 nm at which no calibration from CYH and MEH. Hence, we tried to determine it in zero order spectra at 285 nm but bad results were obtained. In order to enhance selectivity of the method and for determination of PYH in the ternary mixture, peak amplitude was measured at 283 nm obtained after division by standard spectrum of 20 µg/mL PYH where no interference from CYH or MEH was observed, Figure 3. The peak amplitude was plotted versus the corresponding concentration of PYH in the range of 5-40 µg/mL and the calibration curve was constructed. The regression equation was found to be:

$$Y = 0.0493x + 0.0237 \quad r = 0.9998 \quad (3)$$

where Y is the peak amplitude at 283 nm, C is the concentration in µg/mL and r is the correlation coefficient.

Table 1. Assay results for the determination of PYH, CYH and MEH in synthetic mixtures using the proposed spectrophotometric methods.

Mixture no	Claimed taken ($\mu\text{g/mL}$)			Recovery (%) ^a					
	PYH	CYH	MEH	Method I			Method II		
				PYH	CYH	MEH	PYH	CYH	MEH
1	10.00	6.00	5.00	99.05	101.15	101.38	99.40	101.06	101.34
2	9.00	15.00	10.00	100.36	98.86	98.18	98.83	99.82	102.89
3	30.00	18.00	12.00	99.82	98.88	97.50	98.27	100.88	97.68
4	20.00	10.00	10.00	100.13	99.40	99.30	97.82	98.72	100.04
5	35.00	15.00	15.00	99.14	100.78	100.62	100.90	100.68	101.53
6	12.00	18.00	6.00	99.61	97.37	100.30	99.89	98.88	102.04
Mean \pm SD				99.69 \pm 0.481	99.40 \pm 1.276	99.55 \pm 1.372	99.19 \pm 1.034	100.00 \pm 0.938	100.92 \pm 1.666

^a Average of three determinations.

In order to determine CYH and MEH, the amplitude value of the plateau at 283 nm is subtracted from the division spectrum obtained after division of the ternary mixture by standard spectrum of 20 $\mu\text{g/mL}$ of PYH Figure 4, and then the area under the curve method was applied.

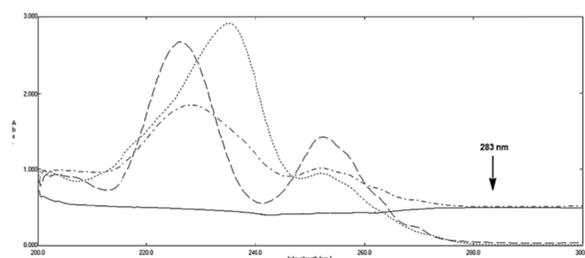


Figure 3. Division spectra of 10 $\mu\text{g/mL}$ each of pyridoxine hydrochloride (—) and 20 $\mu\text{g/mL}$ of cyclizine hydrochloride (---) and meclizine hydrochloride (....) and a mixture containing 10 $\mu\text{g/mL}$ of pyridoxine hydrochloride (---) using standard spectrum of 20 $\mu\text{g/mL}$ pyridoxine hydrochloride as a divisor.

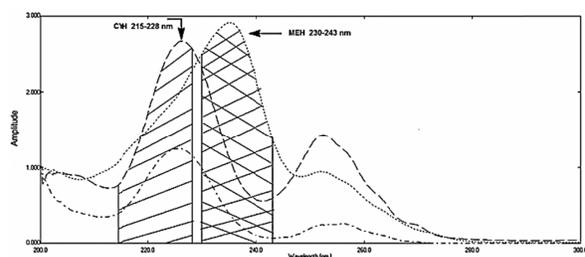


Figure 4. The selected area under curve for determination of cyclizine hydrochloride (---) and meclizine hydrochloride (....) in zero order absorption spectra and in a mixture (-.-.-) in the subtracted division spectra.

The area under curve of the absorption spectra in the wavelength ranges 215-228 nm ($\lambda_1-\lambda_2$) and 230-243 nm ($\lambda_3-\lambda_4$) of CYH in the concentration range of 4-22 $\mu\text{g/mL}$ was calculated. For MEH, area under curve of the absorption spectra in the wavelength ranges 215-228 nm ($\lambda_1-\lambda_2$) and 215-228 nm ($\lambda_3-\lambda_4$) in the concentration range of 3-20 $\mu\text{g/mL}$ was also calculated. The absorptivity 'Y' values of CYH and MEH were calculated at each wavelength range (Figure 4). The concentrations of CYH and MEH can be obtained by applying Cramer's rule and matrices in Equation (4) and (5). Concentration of the two drugs in mixed standard and the sample solution were calculated according to the following equations:

$$A_1 = 1.2308 C_{\text{CYH}} + 1.0802 C_{\text{MEH}} \text{ at } 215-228 \text{ nm } (\lambda_1-\lambda_2) \quad (4)$$

$$A_2 = 0.6094 C_{\text{CYH}} + 1.6324 C_{\text{MEH}} \text{ at } 230-243 \text{ nm } (\lambda_3-\lambda_4) \quad (5)$$

where C_{CYH} and C_{MEH} are the concentrations of CYH and MEH in $\mu\text{g/mL}$, respectively. 1.2308 and 0.6094 are the absorptivity (Y

value) of CYH at ($\lambda_1-\lambda_2$) and ($\lambda_3-\lambda_4$), respectively. 1.0802 and 1.6324 are absorptivity (Y value) of MEH at ($\lambda_1-\lambda_2$) and ($\lambda_3-\lambda_4$), respectively. A_1 and A_2 are the area under curve of sample solutions at the wavelength range ($\lambda_1-\lambda_2$) and ($\lambda_3-\lambda_4$), respectively.

The proposed area under curve method was successfully applied for determination of CYH and PYH in their laboratory prepared mixtures containing different ratios of them with mean percentage recoveries 99.40 \pm 1.276 and 99.55 \pm 1.372, respectively. Table 1 is confirming that each of the cited drugs could be successfully determined without interference from the other which indicated that the method is selective for determination of the studied drugs.

3.3. Mean centering of ratio spectra spectrophotometric method (MCR) (method II)

This method depends on the mean centering of ratio spectra, it eliminates the derivative steps and therefore signal-to-noise ratio is enhanced [15]. The mathematical explanation of the method was illustrated by Afkhami and Bahram [16,17] Linearity of the proposed method was proved and it was represented in the range of 5-40, 12-2 and 2-20 $\mu\text{g/mL}$ for PYH, CYH and MEH, respectively (Figure 5-7). Calibration curves relating the mean centered values at 228.2, 262.0 nm and 270.8 nm to the corresponding concentrations of PYH, CYH and MEH, respectively have been constructed from which the regression equation parameters found in Table 2 have been obtained. Specificity of the method has been validated by application on different synthetic mixtures containing different ratios of the three studied drugs where good percentage recoveries with low SD% values were obtained, Table 1. The following regression equations for the proposed method were calculated:

$$Y_1 = 130.95 C_1 + 109.02, r_1 = 0.9998 \text{ at } 228.8 \text{ nm for PYH} \quad (6)$$

$$Y_2 = 6.0460 C_2 + 0.3475, r_2 = 0.9999 \text{ at } 262.0 \text{ nm for CYH} \quad (7)$$

$$Y_3 = 14.394 C_3 - 1.4296, r_3 = 0.9999 \text{ at } 270.8 \text{ nm for MEH} \quad (8)$$

where Y_1 , Y_2 and Y_3 are the peak amplitudes at the selected wavelengths, C_1 , C_2 and C_3 are the concentrations in $\mu\text{g/mL}$ and r_1 , r_2 and r_3 are the correlation coefficients. Good linearity is confirmed by the high value of the correlation coefficient and the low value of intercept, Table 2.

3.4. Application to pharmaceutical formulations

After methods development and optimization, they had been applied for determination of PYH and CYH in Emetrex® tablets and for PYH and MEH in Dizerest B6® tablets. The obtained results were represented in the form of percentage recoveries and they were in the acceptable limit (90-110%). The results obtained proved that the methods are suitable for determination of PYH, CYH and MEH in their pharmaceutical formulations and there were no interference from additives;

Table 3. Also, results of standard addition technique; Table 3, confirmed the accuracy of the methods.

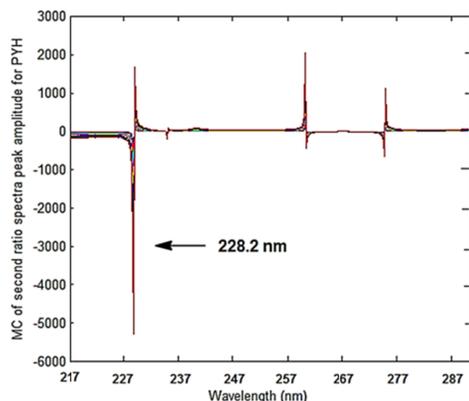


Figure 5. The mean centered first ratio absorption spectra of PYH in the range of 5-40 $\mu\text{g}/\text{mL}$ using 0.05 N as a solvent.

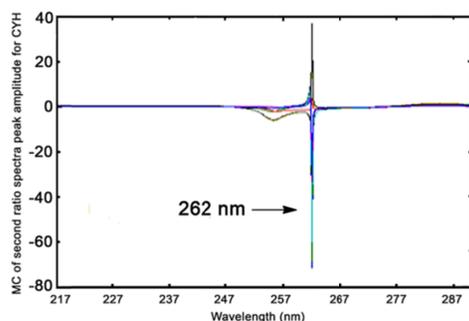


Figure 6. The mean centered first ratio absorption spectra of CYH in the range of 1-22 $\mu\text{g}/\text{mL}$ using 0.05 N as a solvent.

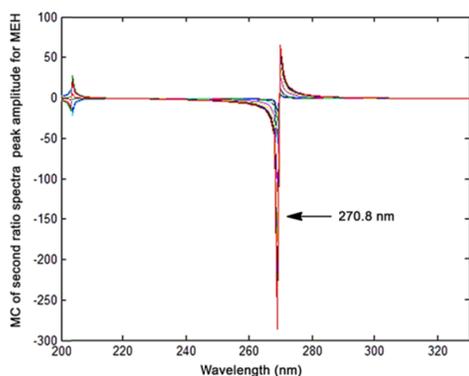


Figure 7. The mean centered first ratio absorption spectra of MEH in the range of 2-20 $\mu\text{g}/\text{mL}$ using 0.05 N HCl as a solvent.

3.5. Statistical analysis

Results of analysis of the studied components by the proposed methods were compared statistically with those obtained by reported HPLC methods for PYH and CYH [3] and for PYH and MYH [6]. The methods showed no significant difference between them using student's-t and F- ratio tests, Table 4.

3.6. Method Validation

Method validation was carried out according to International Conference on Harmonization (ICH) guidelines [18].

3.6.1. Linearity

The linearity of the developed methods was proved by analyzing different concentrations of standard solutions of PYH, CYH and MEH in triplicates. It was evaluated in the range 5-40, 4-22 and 3-20 $\mu\text{g}/\text{mL}$ for PYH, CYH and MEH, respectively (for method I) and 5-40, 1-22 and 2-20 $\mu\text{g}/\text{mL}$ for PYH, CYH and MEH, respectively (for method II). The evaluation parameters like slopes, intercepts and the correlation coefficients were calculated and are presented in Table 2.

3.6.2. Accuracy

The accuracy of these proposed methods was checked by their application for determination of different concentrations of pure samples of the studied drugs within their linearity ranges. The concentrations were obtained from the corresponding regression equations then the percentage recoveries were calculated as given in Table 2. In addition, application of standard addition technique was carried out on three different levels to access method accuracy. Results given in Table 3 proved accuracy of the proposed methods.

3.6.3. Precision

It was studied with respect to both repeatability and intermediate precision. Repeatability was calculated through analysis of three different concentrations of pure components in triplicates at the same day [19]. The used concentrations were 10, 15 and 20 $\mu\text{g}/\text{mL}$ (for PYH), 4, 8 and 10 $\mu\text{g}/\text{mL}$ (for CYH) and 4, 8 and 12 $\mu\text{g}/\text{mL}$ (for MEH). The experiment was repeated using the same concentrations three times on three consecutive days in order to determine the intermediate precision which showed that the developed methods are precise. Good results and acceptable percentage relative standard deviation % RSD was obtained, Table 2.

3.6.4. Specificity

Specificity of the methods was proved by their application to different mixtures containing different ratios of PYH, CYH and MYH. Satisfactory results were obtained and represented in Table 1, confirming that each of the cited drugs could be determined successfully without interference from the other.

3.6.5. Limits of detection and limits of quantitation (LOD and LOQ)

In order to determine detection and quantification limits, PYH, CYH and MEH concentrations in the lower part of the linear range of the calibration curve and the equations $\text{LOD} = 3.3 \times N/B$ and $\text{LOQ} = 10 \times N/B$ were used, where N is the standard deviation of the response and B is the slope of the corresponding calibration curve [20]. The low values indicate the high sensitivity of the methods, Table 2.

3.7. Statistical analysis

Table 2 shows results of statistical comparison of the results obtained by the proposed methods and the reported HPLC method [3,6].

The calculated t- and F-values are less than the theoretical ones indicating that there is no significant difference between the proposed methods and the reported ones with respect to accuracy and precision.

Table 2. Regression and analytical parameters of the proposed methods for determination of PYH, CYH and MEH.

Parameters	Method I			Method II		
	PYH	CYH	MEH	PYH	CYH	MEH
Calibration range ($\mu\text{g/mL}$)	5-40	4-22	3-20	5-40	1-22	2-20
Slope	-	-	-	130.59	6.0461	14.394
Intercept	-	-	-	109.02	0.3475	-1.4296
Correlation coefficient	-	-	-	0.9998	0.9999	0.9999
Accuracy	99.76	100.05	99.97	99.88	100.08	99.98
Repeatability (%RSD) ^a	0.69	0.29	1.16	0.51	1.05	1.15
Intermediate precision (%RSD) ^b	0.85	0.89	1.30	0.71	1.67	1.40
LOD ^c	1.06	1.10	0.80	1.10	0.23	0.53
LOQ ^d	3.20	3.30	2.40	3.00	0.70	1.60

^a The intra-day precision (n = 9), average of three different concentrations repeated three times within day.

^b The intra-day precision (n = 9), average of three different concentrations repeated three times in three successive days.

^c LOD = (SD of the response / slope) \times 3.3.

^d LOQ = (SD of the response / slope) \times 10.

Table 3. Determination of studied drugs in Emetrex® and Dizerest B6® tablets by the proposed spectrophotometric methods and the application of standard addition technique.

Method	Pharmaceutical formulation	Component	Taken ($\mu\text{g/mL}$)	Found ($\% \pm \text{SD}$) ^a	Standard addition				
					Added ($\mu\text{g/mL}$)	Found ($\mu\text{g/mL}$) ^b	Found (%)		
I	Emetrex® tablets claimed to contain 30 mg of PYH and 50 mg CYH (Batch No. 20766)	PYH	6.00	97.56 \pm 1.530	6.00	6.06	101.00		
					10.00	10.16	101.60		
					15.00	14.86	99.07		
		Mean \pm SD = 100.56 \pm 1.079					4.00	3.97	99.25
		CYH	10.00	105.24 \pm 0.080	10.00	10.00	100.00		
					12.00	12.24	102.00		
Mean \pm SD = 100.42 \pm 1.161									
I	Dizerest B6® tablets claimed to contain 50 mg of PYH and 25 mg MEH (Batch No. 30248)	PYH	10.00	104.32 \pm 1.642	5.00	5.04	100.80		
					10.00	9.82	98.20		
					15.00	14.63	97.53		
		Mean \pm SD = 98.84 \pm 1.411					3.00	3.02	100.67
		MEH	5.00	101.98 \pm 1.624	5.00	4.95	99.00		
					10.00	9.80	98.00		
Mean \pm SD = 99.22 \pm 1.014									
II	Emetrex® tablets claimed to contain 30 mg of PYH and 50 mg CYH (Batch No. 20766)	PYH	6.00	99.85 \pm 1.460	6.00	5.59	99.17		
					10.00	10.03	100.30		
					15.00	14.81	98.73		
		Mean \pm SD = 99.40 \pm 0.661					3.00	3.05	101.67
		CYH	10.00	103.73 \pm 0.731	10.00	10.12	101.21		
					12.00	12.09	100.75		
Mean \pm SD = 101.21 \pm 0.376									
II	Dizerest B6® tablets claimed to contain 50 mg of PYH and 25 mg MEH (Batch No. 30248)	PYH	10.00	104.83 \pm 1.464	5.00	5.12	102.40		
					10.00	10.17	101.70		
					15.00	14.90	99.33		
		Mean \pm SD = 101.14 \pm 1.314					3.00	3.03	101.00
		MEH	5.00	100.10 \pm 1.126	5.00	4.97	99.40		
					10.00	9.71	97.10		
Mean \pm SD = 99.17 \pm 1.600									

^a Average of six determination.

^b Average of three determination.

Table 4. Statistical comparison between the results obtained by the proposed spectrophotometric methods and the reported methods for the determination of pyridoxine hydrochloride, cyclizine hydrochloride and meclizine hydrochloride in pure powder form.

Component	Method I			Method II			Reported methods		
	PYH	CYH	MEH	PYH	CYH	MEH	PYH ^a	CYH ^a	MEH ^b
Mean	99.76	100.05	99.97	99.88	100.09	99.98	99.00	100.11	100.06
SD	1.663	1.199	0.999	1.122	0.975	0.969	1.306	1.137	0.819
Variance	2.766	1.438	0.998	1.259	0.951	0.939	1.705	1.293	0.671
N	7	7	7	7	7	7	7	7	7
t-Test (2.179) ^c	0.887	0.085	0.152	1.252	0.0836	0.136	-	-	-
F-Test (4.284) ^c	1.622	1.114	1.489	1.355	1.360	1.401	-	-	-

^a HPLC determination of PYH and CYH using acetonitrile/0.05 M KH₂PO₄ (50:50, v:v, pH = 4.0) as developing system and detection at 239 nm [3].

^b HPLC determination of PYH and MEH using phosphate buffer / acetonitrile / trifluoroacetic acid (30:70:0.1, v:v:v) as developing system and detection at 254 nm [6].

^c The values between parenthesis are corresponding to the theoretical values of t and F (p = 0.05).

4. Conclusion

Selective spectrophotometric methods have been established for determination of PYH, CYH and MEH in their ternary mixture for the first time. The developed methods are considered to be simple, selective and could be used for determination of the cited drugs (PYH, CYH and MEH) either in their laboratory prepared mixtures or in their pharmaceutical

formulations (Emetrex® tablets and Dizerest B6® tablets). The validity of the methods has been ascertained by the good results which obtained by applying the standard addition techniques confirming that it is valuable for application in quality control laboratories for determination of the studied drugs.

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