Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 12(1):30-43

Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Simultaneous Determination of Meclizine Hydrochloride in Its Mixtures with Pyridoxine Hydrochloride, Caffeine or Nicotinic Acid Using HPLC and TLC-Densitometric Methods

Sonia T Hassib[1], Ramzia I El-Bagary[2], Elham A Taha[3] and Ghada H Barakat[4*]

[1]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., Cairo; 11562, Egypt*

[2]*Pharmaceutical Chemistry Department, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University, Cairo, 11835, Egypt*

[3]*Analytical Chemistry Department, National Organization for Drug Control and Research, 51 Wezzart Elzeraa St., Giza, 35521, Egypt*

[4]*Pharmaceutical Chemistry Department, National Organization for Drug Control and Research, 51 Wezzart Elzeraa, St., 35521, Giza, Egypt*

ABSTRACT

Substandard /counterfeit drugs represent an expanding problem throughout developing countries with considerable consequences for global public health. This work aimed to provide two simple chromatographic methods for separation and simultaneous determination of meclizine HCl in its mixtures with pyridoxine HCl, caffeine or nicotinic acid. RP-HPLC method was developed using Inertsil C8 column at ambient temperature with flow rate of (1 min mL^{-1}) and UV detection at 230 nm, the mobile phase was composed of 0.1M NaH2PO4 (pH 3):acetonitrile:methanol (40:55:5 v/v/v). Cinnarizine was used as an internal standard. TLC method was developed using a mixture of [glacial acetic acid: dichloromethane: methanol (1.5:5:18.5 v/v/v)] as developing system. The spots were scanned at 230, 292, 273, 262 nm for meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid, respectively. The RP-HPLC method was linear over concentration range of (37.5-200.0), (75.0-400.0), (30.0-160.0) and (75.0-400.0 µg mL^{-1}) for meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid, respectively. The TLC method was linear over the range of (1.25-15), (1-20), (0.4-8) and (1-20 µg spot^{-1}) for meclizine HCl, pyridoxine HCl, caffeine and

nicotinic acid, respectively. The method provides sufficient selectivity and accuracy to be applied for routine analysis and quality control in laboratories of the cited drugs.

Keywords: Meclizine HCl; Pyridoxine HCl; Caffeine; Nicotinic acid; Chromatographic methods

---

INTRODUCTION

Meclizine HCl is a sedating antihistamine with antimuscarinic properties, it is used in the prevention and treatment of nausea and vomiting associated with motion sickness, vertigo and vestibular disorder. Pyridoxine HCl, helps in the treatment of nausea and vomiting (hyperemesis), also it stimulates the appetite that might be depressed. Caffeine is central nervous system stimulant. Nicotinic acid is a vasodilator agent which is found to be effective in the treatment of vertigo due to Ménière's syndrome and to cerebral arteriosclerosis [1,2].

On literature survey, there are several spectrophotometric and chromatographic methods for simultaneous determination of meclizine HCl and pyridoxine HCl [3-16]. Moreover there are several methods for simultaneous determination of meclizine HCl and caffeine [17-20] and for simultaneous determination of meclizine and nicotinic acid [21-23]. Spectrophotometric method for simultaneous determination of meclizine HCl, pyridoxine HCl and caffeine was published [24]. Also, simultaneous determination of meclizine HCl and pyridoxine HCl and nicotinic acid was reported [25].

Up to our knowledge, there is no method for simultaneous determination of meclizine HCl in its quaternary mixtures with pyridoxine HCl, caffeine and nicotinic acid**.** The aim of the present work was to develop and validate simple chromatographic methods for separation and determination of meclizine HCl in its mixtures. These methods can be used for quality control and routine analysis of the cited drugs separately or in combinations. Also the proposed methods can be used for counterfeit drug detection.

The problem of counterfeit medicines was first addressed at the international level in 1985 at the Conference of Experts on the Rational Use of Drugs in Nairobi. The meeting recommended that WHO, together with other international and nongovernmental organizations, should study the feasibility of setting up a clearing house to collect data and to inform governments about the nature and extent of counterfeiting. The factors facilitating the occurrence of counterfeit drugs vary from country to country. However, the most common factors are considered to be: lack of legislation prohibiting counterfeiting of drugs; weak penal sanctions; weak national drug regulatory authorities; weak enforcement of drug laws; shortage of drugs; lack of control of drugs for export; trade involving several intermediaries and free trade zones; and corruption and conflict of interest [26].

EXPERIMENTAL STUDY

Materials and Reagents

(a) Methanol and acetonitrile HPLC grade (Scharlau Co., Spain), NaH_2PO_4 (QualiKems Co., India), glacial acetic acid (Loba Chemie Co., India) and dichloromethane (Fisher Scientific Co., U.K.) were used.

(b) A list of the investigated drugs supplied by their producing companies and their certified potency is as follows:

Meclizine HCl (99.64%; Delta Pharma Co., Cairo, Egypt), pyridoxine HCl (100.23%; European Co., Cairo, Egypt), caffeine (99.97%; Lmco Co., Cairo, Egypt), nicotinic acid (100.37%; Eva Co., Cairo, Egypt) and cinnarizine (99.95%; Alex Co., Cairo, Egypt). The chemical structures and chemical names of the cited drugs are presented in Figure 1.

(c) Navoproxin tablet (B.N:590), 25 mg meclizine HCl/50 mg of pyridoxine HCl (Delta Pharma Co.).

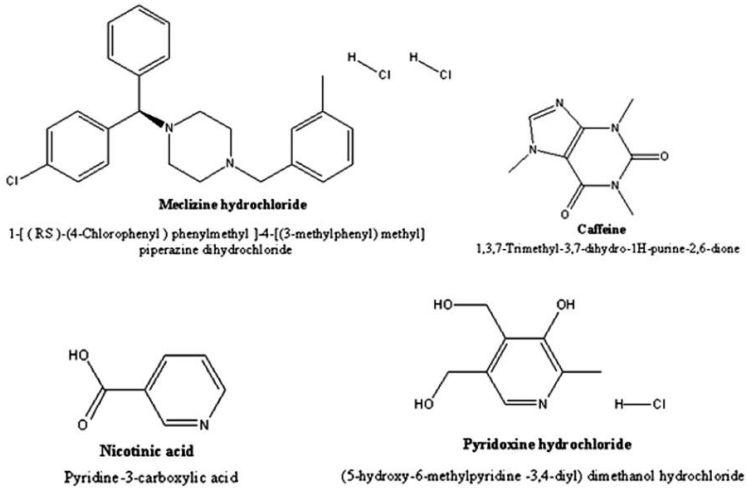

Figure 1. Chemical structures and chemical names of meclizine hydrochloride, caffeine, nicotinic acid and pyridoxine hydrochloride [1]

Instrumentation

(a) A Chromatographic system consisting of Agilant HPLC1100 series, interface equipped with degasser JP54427629 (Japan), quaternary pump DE43635580 (Germany), manual injector DE11405617 equipped with (20 µL) injector loop, column thermostat DE43651317 (Germany) and UV-visible detector DE60400332 (Germany). Agilent syringe, LC 250 µL (USA) and Inertsil C_8 column (250 x 4.6 mm) 5 µm [SN: ODI13798] were used.

(b) Shimadzu-Dual wavelength lamp flying CS9301 densitometer, Japan. TLC plates (20 x 20 cm) with 0.5 mm thickness aluminum sheet silica gel F_{254}, (E. Merck) and Hamilton syringe 50 µL capacity were used.

Preparation of Working Solutions and Internal Standard

HPLC method: Solutions of meclizine HCl (0.25 mg mL^{-1}), pyridoxine HCl (0.5 mg mL^{-1}), caffeine (0.2 mg mL^{-1}) and nicotinic acid (0.5 mg mL^{-1}) in mobile phase were prepared.

Cinnarizine internal standard working solution (0.1 mg mL^{-1}) in mobile phase was prepared.

TLC method: Solutions of meclizine HCl (2.5 mg mL^{-1}), pyridoxine HCl (5 mg mL^{-1}), caffeine (2 mg mL^{-1}) and nicotinic acid (5 mg mL^{-1}) in [methanol: H_2O (50: 50, *v/v*), solvent A] were prepared.

Preparation of Laboratory Prepared Mixture Solution

HPLC method: Mixture composed of meclizine HCl (0.25 mg mL^{-1}), pyridoxine HCl (0.5 mg mL^{-1}), caffeine (0.2 mg mL^{-1}) and nicotinic acid (0.5 mg mL^{-1}) in mobile phase was prepared.

TLC method: Mixture composed of meclizine HCl (2.5 mg mL^{-1}) and pyridoxine HCl (5 mg mL^{-1}) in solvent A was prepared.

Mixture composed of meclizine HCl (2.5 mg mL^{-1}) and caffeine (2 mg mL^{-1}) in solvent A was prepared.

Mixture composed of meclizine HCl (2.5 mg mL^{-1}) and nicotinic acid (5 mg mL^{-1}) in solvent A was prepared.

Sample Preparation

HPLC method: Ten Navoproxin tablets were ground and mixed well, an accurately weighed amount equivalent to one tablet was transferred into a 100-mL volumetric flask. Accurate weights of caffeine (20 mg) and nicotinic acid (50 mg) were transferred to the same volumetric flask. 50 mL of mobile phase was added and the solution was sonicated for 5 min. The volume was completed to mark with mobile phase, to reach a laboratory prepared mixture simulated to their drug products [pregnidoxin 25 mg (25 mg meclizine HCl/20 mg caffeine), [27], other drug product contains 25 mg meclizine HCl/50 mg nicotinic acid), [28]]. The solution was filtered on a dry funnel and filter paper; the first ten mL of the filtrate were discarded.

TLC method: Ten Navoproxin tablets were ground and mixed well, accurately weighed amount equivalent to five tablets were transferred into 50-mL volumetric flask. Twenty mL of solvent A were added, and then the solution was sonicated for 5 min. The volume was completed to mark with solvent A, then the solution was filtered, the first 5 mL of filtrate were discarded.

Accurate weights of caffeine (100 mg) and nicotinic acid (250 mg) were transferred separately into two 50-mL volumetric flasks. Weights of meclizine HCl (125 mg) were added to both flasks, to reach a laboratory prepared mixtures equivalent to their drug products, [pregnidoxin 25 mg (25 mg meclizine HCl/ 20 mg caffeine, other drug product contains 25 mg meclizine HCl / 50 mg nicotinic acid].

Procedures

HPLC method

Chromatographic conditions: A mixture of 0.1N NaH_2PO_4 (pH 3 adjusted with orthophosphoric acid): acetonitrile: methanol (40: 55: 5, *v/v/v*) was prepared. The mobile phase was filtered through 0.45 μm nylon membrane filter. The column was equilibrated with the mobile phase until steady baseline is obtained and column pressure is stabilized.

General procedure and linearity: Different aliquots (1.5-8 mL) of standard working solutions were transferred into four series of 10-mL volumetric flasks, one mL of cinnarizine working solution (0.1 mg mL^{-1}) was added to each flask, the volume was completed to the mark with mobile phase, to reach final concentrations equivalent to [meclizine (37.5-200 µg mL^{-1}), pyridoxine and nicotinic acid (75-400 µg mL^{-1}) and caffeine (30-160 µg mL^{-1})]. Twenty µL aliquot of each solution was injected and chromatograms were recorded using the following parameters [flow rate was (1 min mL^{-1}), wavelength of UV detector was 230 nm].

Ratio of peak area for each compound to peak area of cinnarizine was plotted versus its corresponding concentrations in (µg mL^{-1}) and the regression equation for each compound was then computed.

Simultaneous determination of the cited drugs in laboratory prepared mixture using the proposed HPLC method: Different aliquots (1.7-7 mL) from a laboratory prepared mixture [meclizine HCl (0.25 mg mL^{-1}), pyridoxine HCl (0.5 mg mL^{-1}), caffeine (0.2 mg mL^{-1}) and nicotinic acid (0.5 mg mL^{-1})] were accurately transferred into a series of 10-mL volumetric flasks. One mL of cinnarizine working solution was added to each flask, and the volumes were completed to the mark with the mobile phase. Twenty µL aliquot of each solution was injected in triplicate using the chromatographic conditions (Table 1).

Determination of the cited drugs in their drug products using the proposed HPLC method: The chromatographic procedure was applied to laboratory prepared mixture equivalent to their drug products and the same procedure was repeated by applying the standard addition technique. The recovered concentrations of labeled and added drug were calculated using their regression equations (Table 1).

TLC-densitometric method: The chosen developing system was a mixture of [glacial acetic acid: dichloromethane: methanol (1.5: 5: 18.5, *v/v/v*)].

General procedure and linearity: Aliquots of working solutions equivalent to meclizine HCl (1.25-15 mg), pyridoxine HCl (1-20 mg), caffeine (0.4-8 mg) and nicotinic acid (1-20 mg) were accurately transferred into a four series of 10-mL volumetric flasks.

The volumes were completed with the solvent A.

Ten µL aliquot of each solution was applied to TLC plate (20 × 20 cm) using 50 µL hamilton syringe. Spots were spaced 2 cm apart and 1.5 cm from the bottom edge of the plate.

The plate was placed in a chromatographic tank, previously saturated with the selected developing system, the spots were detected under UV lamp F_{254} and scanned at 230 nm, 292 nm, 273 nm, 262 nm for meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid, respectively.

The peak area of each compound was plotted versus its corresponding concentration in (µg $spot^{-1}$). The regression equation for each compound was then computed.

Table 1. Validation report of meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid using the proposed HPLC method

Parameters	Meclizine HCl		Pyridoxine HCl		Caffeine		Nicotinic acid	
Linearity and range (µg mL^{-1})	37.50-200.00		75.00-400.00		30.00-160.00		75.00-400.00	
Accuracy (Mean ± R.S.D.)	100.34 ± 0.69		100.35 ± 0.72		100.04 ± 0.83		100.00 ± 0.75	
Limit of detection (µg mL^{-1})	4.28		11.63		4.25		7.23	
Limit of quantitation (µg mL^{-1})	12.96		35.25		12.88		21.9	
Regression equation	Y=0.0638 X+0.0392		Y=0.028 X+0.1655		Y=0.0553 X + 0.079		Y=0.0415 X+0.5158	
Regression coefficient	0.9995		0.9991		0.9992		0.9996	
Standard deviation of slope	0.708×10^{-3}		0.422×10^{-3}		0.761×10^{-3}		0.389×10^{-3}	
Confidence limit of the slope	$0.0638 \pm 1.6 \times 10^{-3}$		$0.028 \pm 0.955 \times 10^{-3}$		$0.0553 \pm 1.721 \times 10^{-3}$		$0.0415 \pm 0.88 \times 10^{-3}$	
Standard deviation of intercept	0.0827		0.0987		0.0712		0.0909	
Confidence limit of the intercept	0.0392 ± 0.187		0.1655 ± 0.223		0.079 ± 0.161		0.5158 ± 0.206	
Standard error of estimation	0.0988		0.1179		0.085		0.1086	
Precision	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**
	62.5	100.92 ± 0.52	125	100.41 ± 0.57	50	100.34 ± 0.55	125	100.32 ± 0.51
	112.5	100.6 ± 0.50	225	100.81 ± 0.48	90	100.15 ± 0.42	225	100.54 ± 0.48
Repeatability	175	99.28 ± 0.52	350	99.81 ± 0.51	140	100.38 ± 0.56	350	99.15 ± 0.52
	Conc*	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**
	62.5	100.92 ± 0.29	125	100.7 ± 0.28	50	100.34 ± 0.21	125	100.77 ± 0.22
Intermediate precision	112.5	100.42 ± 0.36	225	101.02 ± 0.31	90	100.41 ± 0.23	225	100.72 ± 0.32
	175	99.04 ± 0.78	350	99.57 ± 0.76	140	100.03 ± 0.83	350	99.21 ± 0.76
Drug product or lab prepared mixture equivalent to their drug products	99.74 ± 0.87		100.30 ± 0.50		100.16 ± 0.77		99.52 ± 0.89	
Drug added	98.95 ± 0.40		98.77 ± 0.29		99.07± 0.25		99.75 ± 0.28	

*Concentration (µg mL^{-1})

Determination of caffeine or nicotinic acid in laboratory prepared mixtures containing meclizine HCl simulated to their drug products using TLC-densitometric method: The procedure was applied as previously mentioned under "general procedure and linearity". The recovered concentrations of each compound were calculated using their regression equations, (Table 2).

Table 2. Validation report of meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid using the proposed TLC method

Parameters	**Meclizine HCl**		**Pyridoxine HCl**		**Caffeine**		**Nicotinic acid**	
Linearity and range (µg spot^{-1})	1.25-15.00		1.00-20.00		0.40-8.00		1.00-20.00	
Accuracy (Mean ± R.S.D.)	99.77 ± 0.85		99.80 ± 0.35		99.33 ± 0.82		99.87 ± 0.51	
Limit of detection (µg spot^{-1})	0.26		0.11		0.05		0.13	
Limit of quantitation (µg spot^{-1})	0.78		0.34		0.15		0.4	
Regression equation	Y=334.95 x+486.04		Y=735.59 X+1442.2		Y=1316.7 X+797.17		Y=564.65 X+1311.7	
Regression coefficient	0.9981		0.9978		0.9983		0.998	
Standard deviation of slope	6.01		17.18		27.31		12.62	
Confidence limit of the slope	334.95 ± 13.86		735.59 ± 39.62		1316.7 ± 62.98		564.65 ± 29.1	
Standard deviation of intercept	51.34		192.96		122.74		141.78	
Confidence limit of the intercept	486.04 ± 118.39		1442.2 ± 444.97		797.17 ± 283.04		1311.7 ± 326.94	
Standard error of estimation	78.58		287.52		182.89		211.26	
Precision	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**
	4	99.17 ± 0.52	8	100.04 ± 0.26	3.2	100.42 ± 0.18	8	99.50 ± 0.57
	6.5	98.72 ± 0.55	13	100.16 ± 0.35	5.2	98.33 ± 0.29	13	99.95 ± 0.31
Repeatability	8	99.75 ± 0.50	16	99.81 ± 0.19	6.4	98.70 ± 0.32	16	100.42 ± 0.31
	Conc*	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**	**Conc***	**Mean ± R.S.D.**
	4..00	99.08 ± 0.38	8	99.25 ± 0.79	3.2	100.21 ± 0.48	8	100.63 ± 0.57
	6.5	99.13 ± 0.47	13	99.51 ± 0.51	5.2	99.17 ± 0.49	13	100.05 ± 0.39
Intermediate precision	8	99.58 ± 0.52	16	99.44 ± 0.50	6.4	98.96 ± 0.33	16	100.13 ± 0.50
Drug product or Lab prepared mixture equivalent to their drug products	100.06 ± 0.53		99.49 ± 0.55		99.91 ± 0.57		100.04 ± 0.58	
Drug added	99.57 ± 1.02		99.00 ± 0.39					

*Concentration (µg spot^{-1})

Determination of meclizine HCl and pyridoxine HCl in their drug product using TLC-densitometric method: The procedure was applied as previously mentioned under "general procedure and linearity". The same procedure was repeated by applying the standard addition technique. The recovered concentrations of labeled & added of each compound were calculated using their regression equations, (Table 2).

RESULTS AND DISCUSSION

HPLC Method

During the optimization cycle, different proportions of mobile phase [acetonitrile: methanol: phosphate buffer] were tried in an isocratic or/and gradient mode. The influence of organic modifier on the separation efficiency was investigated at constant pH, increasing the proportion of organic modifier led to incomplete separation of drugs, internal standard. Increasing the aqueous proportion led to prolonged retention times. Various column were tried such as Hypersil C_{18} (250 × 4.6mm) 5 µm, Nucleosil C_8 (250 × 4.6mm) 5 µm and the separation was obtained by using Inertsil C_8 (250 × 4.6mm) 5 µm [SN: ODI13798].

The use of internal standard is recommended to compensate injection errors, minor fluctuations effect of the retention time and to improve the quantitative analysis. Several compounds were tried as an internal standard such as ondansetron HCl, tropisetron HCl and cinnarizine. Cinnarizine was chosen, as it showed good separation from all compounds, also it gave high response at the UV detector used (230 nm).

pH of the mobile phase was found to be critical in achieving the separation of components, increasing pH led to prolonged retention time of meclizine HCl and loss of peak symmetry of pyridoxine HCl. The best resolution pattern was attained at (pH 3).

Mobile phase was composed of 0.1 N NaH_2PO_4 (pH 3 with orthophosphoric acid): acetonitrile: methanol (40: 55: 5, *v/v/v*), which gave satisfactory separation and peak symmetry of all compounds. The method was able to separate and to determine the cited drugs in one run and the total run time is not exceed 6 min, retention times were 2.1 ± 0.01 min, 2.59 ± 0.01 min, 2.9 ± 0.01 min, 4.79 ± 0.02 and 5.69 ± 0.02 min for pyridoxine HCl, nicotinic acid, caffeine, cinnarizine and meclizine HCl, respectively as shown in Figure 2.

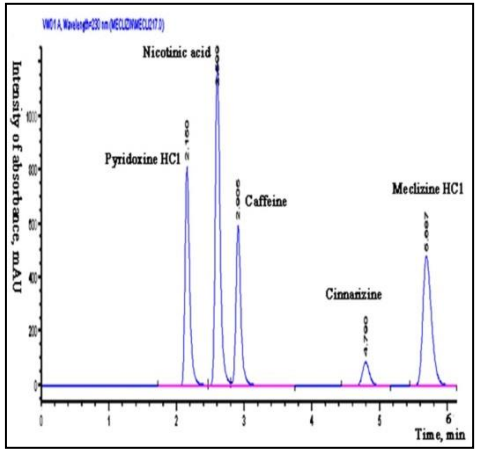

Figure 2. HPLC chromatogram of 100 µg mL^{-1} of meclizine HCl (t_R=5.69 min), 200 µg mL^{-1} of pyridoxine HCl (t_R=2.15), 200 µg mL^{-1} of nicotinic acid (t_R=2.599), caffeine 80 µg mL^{-1} (t_R=2.9) and cinnarizine (10 µg mL^{-1}) as an internal standard (t_R=4.79)

System suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method, (Table 3).

TLC method

Several developing systems with different ratios of their components were tried such as:

Ammonia: methanol: dichloromethane (0.5: 5:19.5, *v/v/v*), methanol: toluene: dichloromethane (2: 5: 18, *v/v/v*),

toluene: dichloromethane (5: 20, *v/v*), ammonia: methanol: n-butanol (0.5: 5: 19.5, *v/v/v*),

dimethylamine: methanol (3: 22, *v/v*), glacial acetic acid: methanol (3: 22, *v/v*) and (1.5: 23.5, *v/v*),

glacial acetic acid: dichloromethane: methanol (1, 1.5, 2, 3: 5: to 25, *v/v/v*).

The chosen developing system was a mixture of [glacial acetic acid: dichloromethane: methanol (1.5: 5: 18.5, *v/v/v*)] as it showed good separation of meclizine HCl from pyridoxine HCl or caffeine or nicotinic acid in its binary mixtures. (R_f values were 0.88, 0.65, 0.73, 0.72) for meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid, respectively as shown in Figure 3.

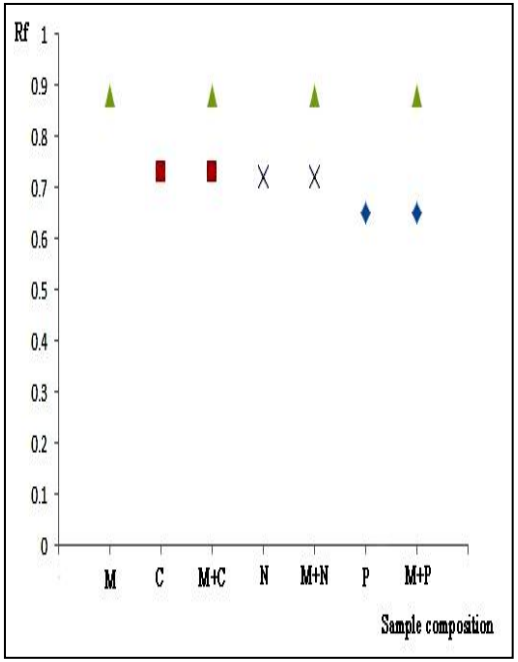

Figure 3. TLC chromatogram of meclizine HCl (M), pyridoxine HCl (P), caffeine (C) and nicotinic acid (N) at (R_f 0.88, 0.65, 0.73, 0.72), respectively

Table 3. System suitability tests of the proposed HPLC method for the determination of meclizine HCl in all its binary mixtures with pyridoxine HCl or caffeine or nicotinic acid.

Parameter	Obtained values	
Resolution (R)	Pyridoxine HCl	3.75
	Nicotinic acid	2.51
	Caffeine	12.27
	Cinnarizine	4.29
	Meclizine HCl	
Tailing factor	Pyridoxine HCl	1.4
	Nicotinic acid	1.34
	Caffeine	1.3
	Cinnarizine	1.19
	Meclizine HCl	1.31
Selectivity (α)	Pyridoxine HCl	1.27
	Nicotinic acid	1.15
	Caffeine	1.78
	Cinnarizine	1.21
	Meclizine HCl	
Capacity factor (K')	Pyridoxine HCl	3.3
	Nicotinic acid	4.2
	Caffeine	4.81
	Cinnarizine	8.58
	Meclizine HCl	10.37
Number of theoretical plates (N)	Pyridoxine HCl	5548
	Nicotinic acid	7061
	Caffeine	9443
	Cinnarizine	10502
	Meclizine HCl	9703

The acidity of the developing system was critical for appearance of meclizine HCl. Upon increasing mL of glacial acetic acid (3 mL/25 mL of developing system), pyridoxine HCl gave deformed spot.

The spots were scanned at 230, 292, 273, 262 nm for meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid, respectively as shown in Figure 4.

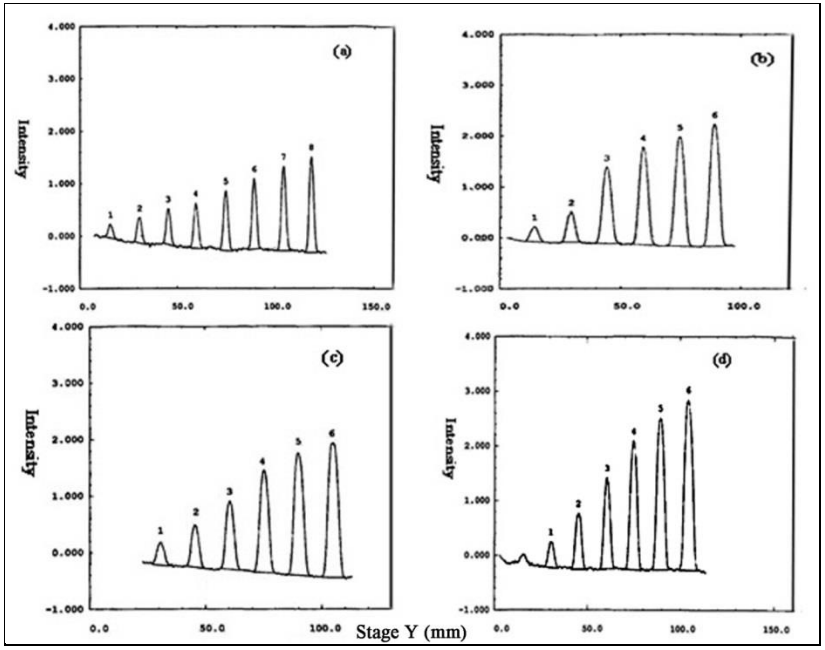

Figure 4. Densitometric scanning profile

(a) Different concentration (1.25-15 µg spot^{-1}) of meclizine HCl at 230 nm.

(b) Different concentration (0.4-8 µg spot^{-1}) of caffeine at 273 nm.

(c) Different concentration (1-20 µg spot^{-1}) of nicotinic acid at 262 nm.

(d) Different concentration (1-20 µg spot^{-1}) of pyridoxine HCl at 292 nm.

On literature survey, TLC method was developed for the determination of meclizine HCL and pyridoxine HCl by densitometric measurement at 237 nm and 283 nm, respectively. The developing system consists of hexane: 1-butanol: methanol: distilled water (1: 11: 1: 0.2, *v/v/v/v*) and methanol used as a solvent for both. For pyridoxine HCl, the relationship between the peak area and corresponding concentrations showed bad correlation coefficient upon applying the linear regression fit. The second order polynomial fit was found to be better than the linear fit [8].

Table 4. Statistical analysis of the results obtained by applying the proposed (HPLC and TLC) methods and official ones for the determination of meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid.

Parameters	Meclizine HCl			Pyridoxine HCl		
	Proposed HPLC method	**Proposed TLC method**	**Official HPLC method [30]**	**Proposed HPLC method**	**Proposed TLC method**	**Official HPLC method [30]**
Mean	100.34	99.77	99.85	100.35	99.8	100.14
S.D.	0.69	0.85	0.45	0.72	0.35	0.74
S.E.	0.28	0.38	0.2	0.29	0.16	0.33
n	6	5	5	6	5	5
Variance	0.48	0.72	0.2	0.52	0.12	0.55
Student t test	1.388 (2.262)*	0.187 (2.306)*		0.474 (2.262)*	0.929 (2.306)*	
F-value	2.4 (6.26)*	3.6 (6.39)*		1.058 (5.19)*	4.583 (6.39)*	
Parameters	**Caffeine**			**Nicotinic acid**		
	Proposed HPLC method	**Proposed TLC method**	**Official HPLC method [30]**	**Proposed HPLC method**	**Proposed TLC method**	**Official HPLC method [30]**
Mean	100.04	99.33	100.15	100	99.87	99.78
S.D.	0.83	0.81	0.55	0.75	0.51	0.7
S.E.	0.34	0.36	0.25	0.31	0.23	0.31
n	6	5	5	6	5	5
Variance	0.69	0.66	0.3	0.56	0.26	0.49
Student t test	0.258 (2.262)*	1.871 (2.306)*		0.501 (2.262)*	0.232 (2.306)*	
F-value	2.3 (6.26)*	2.2 (6.39)*		1.143 (6.26)*	1.885 (6.39)*	

*Tabulated values of t-test and F at confidence limit 95%

Method Validation

The methods were validated according to ICH guidelines [29]. Good linearity was verified by the high correlation coefficient. Accuracy of the results was calculated by% recovery of [six different concentrations (injected in triplicates) of each drug in laboratory prepared mixture, in case of HPLC method] or [five different concentrations of each drug in drug substance, in case of TLC method]. The repeatability and intermediate precision of the method were assessed by three determinations for each of the three concentrations on the same day and on three consecutive days, respectively. Limit of detection and limit of quantitation were calculated using the following formula.

LOD=3.3 σ/S,

LOQ=10 σ/S

Where σ is the standard deviation of the response and S is the slope of the calibration curve of the analyte.

Selectivity was assessed by separating four drugs with good resolution factor and without interference from each other.

In case of HPLC method: Changing flow rate from (1 mL min^{-1} to 1 ± 0.1 mL min^{-1}) or change pH (3 ± 0.3) showed no significant effect on the chromatograms. Good values of the resolution factor were obtained for all these variations, indicating good robustness of the proposed LC method.

The statistical comparison between the results obtained by applying the proposed HPLC and TLC methods and those of official ones [30] for meclizine HCl, pyridoxine HCl, caffeine and nicotinic acid are summarized in (Table 4). Calculated t & F values were less than the tabulated ones. Therefore no significant difference between methods at 95% confidence level, with respect to accuracy and precision.

CONCLUSION

The developed HPLC and TLC methods were applied for the simultaneous determination of meclizine HCl in its mixtures with caffeine, nicotinic acid or pyridoxine HCl. The obtained results were found to be statistically agreed with those obtained from the official ones. The validity of the method was assessed according to ICH guidelines. Besides it can be used for quality control and routine analysis of the cited drugs separately or in combinations. Also the proposed method can be used for counterfeit drug detection.

REFERENCES

1. British Pharmacopoeia. *The Stationary Office, London*. **2019**.
2. A Brayfield, Martindale. *The Pharmaceutical Press, London*. **2017**.
3. ME Abdel Hamid; MH Barary; MA Korany; EM Hassan. *Scientia pharm.* **1985**, 53(2): 105-110.
4. T Al-Jallad; Z Al-Kurdi; A Badwan; AMY Jaber. *Pharm Pharmacol Commun.* **1999**, 5(8): 479-483.
5. A El-Gindy. *J Pharm Biomed Anal*. **2003**, 32(2): 277-286.
6. RI El-Bagary. *Bull Fac Pharm*. **2007**, 45(3): 145-154.
7. MS Arayne; N Sultana; FA Siddiqui; H Zuberi. *Pak J Pharm Sci.* **2007**, 20(2): 149-156.
8. MY Salem; M Nebsen. *Bull Fac Pharm.* **2007**, 45(3): 55-60.
9. MS Arayne; N Sultana; FA Siddiqui. *Chromatographia*. **2008**, 67(11-12): 941-945.
10. Md. S Nawaz. *Chromatogr. Res Int.* **2013**, 7.
11. N Al-Kafri; MA Al-Mardini. *Int J Pharm Sci Review Res.* **2013**, 21(1): 138-142.
12. SA Shinde; ZM Sayyed; BP Chaudhari; VJ Chaware; KR Biyani. *J Pharm Sci Bioscientific Res.* **2016**, 6(1): 137-143.

13. NM Habib; NS Abdelwahab; MM Abdelrahman; NW Ali. *European Journal of Chemistry*. **2016**, 7(1): 30-36.
14. NM Habib; MM Abdelrahman; NS Abdelwhab; NW Ali. *J AOAC Int.* **2017**, 100(2): 414-421.
15. MM Ibrahim; ES Elzanfaly; MB El-Zeiny; NK Ramadan; KM Kelani. *Spectrochim Acta A Mol Biomol Spectrosc.* **2017**, 178: 234-238.
16. A Saad; I Naguib; M Draz; HE Zaazaa; AS Lashien. *J AOAC Int*. **2017**, 101(2): 427-436.
17. F Onur; E Dinc. *Anal Lett.* **1995**, 28(14): 2521-2534.
18. SB Bari; SG Kaskhedikar. *Indian Drugs*. **1997**, 34(2): 85-88.
19. R Raman; VM Shinde. *Indian Drugs.* **2000**, 37(2): 90-94.
20. MB Ravalji; SA Shah; DR Shah; KL Daxina; RS Chauhan. *Asian J Res Chem.* **2011**, 4(8): 1249-1253.
21. SB Bari; SG Kashedikar. *Indian Drugs*. **1996**, 33(8): 411-414.
22. VM Shinde; R Raman. *Indian Drugs*. **1998**, 35(12): 748-753.
23. RM Sudheer; KP Krishna; KK Vinod. *Asian J Pharmaceut Res Health Care*. **2013**, 5(2): 73-80.
24. SC Sharma; SC Sharma; RC Saxena; SK Talwar. *J Pharm Biomed Anal*. **1989**, 7(3): 321-327.
25. F Onur; E Dinc. *Gazi Univ Eczacilik Fak Derg*. **1990**, 7(2): 77-90.
26. Department of Essential Drugs and Other Medicines. *World Health Organization, Geneva.* **1999.**
27. https://www.1mg.com/drugs/pregnidoxin-tablet-358038
28. tajpharma.com/Generics/Generics%20Index%20M/Meclizine.pdf
29. Commission of the European Communities. *Geneva*. **1996**.
30. United States Pharmacopoeia. **2019**.