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Establishment and validation of Two Smart UV-Spectrophotometric and a Novel Spectrodensitometric Methods for Simultaneous Determination of Metoclopramide Hydrochloride/ Pyridoxine Binary Mixture in Raw Material and in Syrup

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ABSTRACT

Currently the anti-emetic drug metoclopramide hydrochloride (MCP-HCl) is co-formulated with pyridoxine (VB6). Three simple, economic and fast spectroscopic methods for determination of both drugs, simultaneously and without previous separation were developed. The first method is a ratio derivative spectrophotometry. The peaks amplitudes of first derivative spectra of MCP-HCl and VB6 were measured at 318.5 nm and 327.5 nm, respectively. The second one is an isosbestic point spectrophotometry. The peaks amplitudes of MCP-HCl and VB6 spectra were measured at 321.5 and 299.5 nm, respectively. The third method is spectrodensitomerty. The mobile phase was composed of benzene, methanol, glacial acetic acid, and acetone (10: 8: 0.5: 0.5; by volume). It gave typical chromatogram for MCP-HCl and VB6 at R_fs 0.2 ± 0.02 & 0.51 ± 0.01, respectively and the UV scanning was carried out at 245 nm. Complete validation processes of the established methods were performed according to ICH guidelines and USP requirements. All methods were linear within wide concentrations ranges with high correlation coefficients. The proposed methods showed high accuracy, precision and selectivity results. Relative standard deviation values for all the key parameters were less than 2.00%.

Keywords: Metoclopramide-hydrochloride; pyridoxine; spectroscopy; assay; validation.

INTRODUCTION

MCP-HCl is an anti emetic and a pro-kinetic agent. It is one of many active ingredients of pharmaceutical preparations concerned with gastroenterology, surgery, gynaecology, and cardiology [1]. Moreover, MCP-HCl is widely used in the treatment of drug induced nausea and vomiting, including anti-cancer drugs [2]. MCP-HCl, [monohydrate of 4-amino-5-chloro-*N*-(2-diethylaminoethyl)-2-methoxy benzamide hydrochloride] [1], figure (1), is freely soluble in water and alcohol, sparingly soluble in methylene chloride and practically insoluble in ether [3]. USP monograph states that MCP- HCl should not be exposed to light [4]; it states an incompatibility with strong alkalis and strong oxidizing agents as well. Aubert *et al* investigated the effect of light on it and characterized the photolysis products [5]. Pyridoxine hydrochloride [5-Hydroxy-6-methyl-3, 4-pyridine di methanol] hydrochloride is reasonably stable to light and air [1]. It is freely soluble in water, slightly soluble in alcohol, sparingly sol in acetone and insoluble in ether. Acidic aqueous solutions are stable and may be heated for 30 min at 120° without decomposition [3].

There are many analytical methods employed for the determination of MCP-HCl [6: 10] and VB6 [11: 15]. But there is only one analytical assay is reported in the literature to describe an analytical assay for determination of this drugs mixture. El-Enany has determined of this drugs mixture via a fluorometric method [16]. Three simple, fast and economically effective methods for simultaneous determination of

both drugs, without preliminary separation, have been described in this paper. As well complete validation process was done to ensure selectivity, accuracy, and precision of the established methods. These methods can be used in routinely quality control analysis of this binary mixture in industry. Two highly economic, accurate and precise UV-spectrophotometric methods were established.

MATERIALS AND METHOD

***Instrumentation*:** Dual beam UV-VIS Spectrophotometer (1601 Shimadzu) with two matched 1cm 3quartz cells, Bundled UV-PC personal spectroscopy software version (3.7) were used to process the absorption spectra of the concerned drugs in methods A and B. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 $nm.min^{-1}$. A UV short wavelength 254 nm lamp (USA) was used for spots detection in method C. A Camag Linomat 5 auto-sampler with Camag micro-syringe (100 µL); CAMAG, Muttenz, Switzerland, was used for spotting. A Camag Linomat scanner 3 densitometer model 3 S/N 130319 equipped with wincat software; CAMAG, Muttenz, Switzerland, was used for densitometric evaluation.

Powders and Solvents: MCP-HCl and PCM USP grade powders were purchased by Professional Compounding Centers of America, Canada (PCCA). Anausan syrup (5 mg MCP-HCl and 25 mg VB6 / 5 mL), batch no 10166, Ramida, October City, Egypt was purchased from Egyptian market. The mixture of benzene, methanol, glacial acetic acid, acetone of analytical grade (ADWIC) in ratio (10: 8: 0.5: 0.5; by volume) was used as a mobile phase for method C. Whereas thin layer chromatography (TLC) plates pre-coated with silica gel60 F_{245} 0.25 mm thickness (E. Merk, Darmstadt, Germany) were used as a stationary phase.

Standard solutions: Stock standard solution of MCP-HCl (3mg/mL) and of VB6 (2mg/mL) were prepared in distilled water for method A and B. A stock standard solution of drugs mixture was prepared in methanol to have concentration of MCP-HCl: 2 mg/ml and VB6: 10 mg/ml for method C.

Spectral characteristics of MCP-HCl and VB6: Standard solutions of MCP-HCl (35 µg/mL) and VB6 (140 µg/mL) in water were prepared. The zero order spectra of the prepared solutions were recorded in the range of 200-400 nm.

Development of the derivative ratio spectrophotometric method: The spectrum of MCP-HCl standard solution was recorded and divided by stored absorption spectrum of VB6 (50 µg/mL). Then the first order spectrum of the resultant spectrum was recorded, using $\Delta\lambda$ = 4 and scaling factor = 10 and the peaks amplitudes of the spectra were measured at λ_{max} = 305 & 318.5 nm. The spectrum of VB6 standard solution was recorded and divided by stored absorption spectrum of MCP-HCl (20 µg/mL). Then the first order spectra of the resultant spectra were recorded, using $\Delta\lambda$ = 16 and scaling factor = 10, and the peaks amplitudes of the spectra were measured at λ_{max} = 321.5 & 303 nm.

Development of the isosbestic point spectrophotometric method: The first derivative spectrum of MCP-HCl standard solution was recorded, using $\Delta\lambda$ = 16 and scaling factor = 10, and the peaks amplitudes of the spectra were measured at λ_{max} = 321.5 nm. The zero order spectrum of VB6 standard solution was recorded and the peaks amplitudes of the spectra were measured at the isosbestic point, λ= 229.5 nm.

Development of Spectrodensitometric method: 5 µl of stock standard solution of MCP-HCl/ VB6 mixture in methanol (2 & 10 mg/ml, respectively) was spotted. The plate was develop in a chromatographic tank previously saturated, for 30 min., with the mobile phase; benzene, methanol, glacial acetic acid, acetone (10: 8: 0.5: 0.5; by volume) by ascending chromatography through a distance of 15 cm at room temperature. The plate was dry in air then spots were visualized under UV lamp at 245 and 270 nm and scanned under the following conditions:

Source of radiation: Deuterium lamp,
Scan mode: Absorption mode,
Slit dimension: 3 mm x 0.45 mm,
Result output: chromatogram and integrated peak area,

Scanning speed: 20 mm/ s and
Wavelength: 245 nm.

Robustness and Ruggedness of method C: Simply, the procedures were repeated as under linearity but with some changes in chromatographic condition and by hand of different analyst. The plate was develop in a chromatographic tank previously saturated, for 20 min., with the mobile phase; benzene, methanol, glacial acetic acid, acetone (30: 27: 1.5: 2; by volume); excess 3ml of methanol and 0.5 mL acetone acid; by ascending chromatography through a distance of 10 cm at room temperature.

RESULTS AND DISCUSSION

Method A development: The absorption spectra of MCP-HCl and VB6 showed major overlapping as shown in figure (3). The ratio derivative method was suggested to solve this overlapping and enable determination of each drug selectively. For determination of MCP-HCl and VB6in its mixture with VB6, the spectrum of MCP-HCl/ VB6 mixture was divided, one time, by VB6 spectrum. Afterward, the resulting ratio spectrum was derivatized in first order, figure (4). The peak amplitude at 318.5 nm was corresponding to MCP-HCl concentration. Next, the spectrum of MCP-HCl/ VB6 mixture was divided by spectrum of MCP-HCl then recorded in first order. Measuring peak amplitude of the resulting first derivative ratio spectrum at 327.5 nm was corresponding to the concentration of VB6 in the mixture, this time, figure (5). The selection of the divisor concentration was significant; hence different concentrations of MCP-HCl and VB6 were tried as divisors separately. It was found that minimum noise and better selectivity were obtained upon using 50 µg/mL of VB6 spectrum and 20 µg /mL of MCP-HCl spectrum as a divisor.

Method B development: In method B, isosbestic point method was used for the determination of total mixture concentration. The point at which the absorption spectra of MCP=HCl, VB6 and their mixture showed crossing, isosbestic point, was at **λ=** 299.5 nm. Since MCP-HCl can be determined separately by derivatizing of its spectrum at first order and measuring the peak amplitude at **λ=** 321.5 nm whereas first derivative spectrum of VB6 give zero value at the same wavelength, figure (6). Therefore VB6 concentration can be obtained by derivative of MCP-HCl concentration from total concentration of the mixture.

Method C development: To optimize the chromatographic conditions, different mobile phases were tried to separate the drug mixture and give well shaped band of the simultaneously. Starting from methanol: conc. ammonia (10: 0.15; v/v) as preliminary mobile phase, it didn't separate the drugs. Chloroform: methanol: conc. ammonia (10: 2: 0.15; v/v/v) could separate them giving good band for MCP-HCl but broad one for VB6. Increasing ratio of chloroform didn't improve the chromatogram leading to switching to other solvent of different components. Toluene methanol: ethyl acetate (5: 3: 7; v/v/v) gave good separated bands for the drugs but just next band of MCP-HCl to stating line. Increasing ratio of methanol to 10 and frequently to 20 increasing the polarity of the mobile phase and then distance traveled by MCP-HCl band helped a lot but gave bad formed spot. Adding drops of conc. ammonia adjusted the spot of MCP-HCl but tailed the spot of VB6. Later trying another quaternary components solvent; benzene, methanol, glacial acetic acid and acetone in ratio (7: 2: 0.5: 0.5; by volume) was promising, it gave separated but not very well formed bands of drugs. Little change in methanol volume adjusted the band shapes. The mobile phase consisted of benzene, methanol, glacial acetic acid and acetone in ratio (10: 8: 0.5: 0.5; by volume) gave perfect chromatogram of both drugs with reasonable R_fs. 0.2 ± 0.02 & 0.51 ± 0.01, respectively. Scanning of the developed band of each drug via densitometer gave higher sensitivity measurement for both drugs at 245 nm as shown in figure (7).

Methods validation:

Linearity: Upon analysis of a series of working standard solutions of each drug separately by the method A, it was found that a linear relationship between the peak amplitude and the corresponding concentration of MCP-HCl at 305 & 318.5 nm and VB6 at 327.5 & 303 nm. When they were analyzed by method B, a linear relationship between the peak amplitude and the corresponding concentration of MCP-HCl at 321.5 nm and VB6 at 299.5 nm was exist. As well, in method C, this linear relationship between the integrated area under the peak of the separated spot at the selected wavelength (245 nm) and the corresponding concentration of each drug was found. A summary of linearity parameters of the proposed methods is depicted in table (I).

Precision: Repeatability of the proposed methods, intra-day precision, was assessed by triplicate analysis of MCP-HCl and VB6 at three different concentrations. Whereas reproducibility of them, inter-day precision, was assessed by triplicate assaying of the same three concentrations over three consecutive days. In method A, upon measuring the precision for determination of MCP-HCl, it gave lower standard deviation and relative standard deviation of recovery percentages at 318.5 nm than those obtained at 305 nm, table (II). That made the determination of MCP-HCl at 318.5 nm preferable. Tables (III & IV) show the high precisions of methods C & D, respectively.

Accuracy: In method A, application of standard addition technique on MCP-HCl standard solutions gave acceptable standard deviation of recovery percentages at both wavelengths 305 and 318.5 nm but with better results at 318.5 nm, table (V). Whereas when standard addition technique was applied on the VB6 standard solutions, measuring the

amplitude of first derivative of ratio spectra gave acceptable standard deviation of recovery percentages at 327.5nm rather than those obtained at 303 nm, table (V). In method B & C, accuracy of the methods were proved by application of standard addition technique on MCP-HCl and VB6 standard solutions, separately, table (VI & VII).

Selectivity: Selectivity of the proposed methods (A, B & C) was tested by analysis of laboratory mixtures of the concerned drugs in different ratios and it was found to be highly selective, as figured out in table (VIII).

Applicability: Applicability of the suggested methods (A, B & C) were confirmed by analysis the drugs in anausan® syrup and they gave high recovery percent with acceptable RSD % of not more than 2%, table (IX).

Robustness and Ruggedness of method C: Robustness and raggedness of the method was performed by changing the composition of mobile phase; benzene, methanol, glacial acetic acid, acetone (10: 8: 0.5: 0.5; v/v/vlv). Chromatograms of four samples of laboratory mixture solutions, unfortunately, gave continuous one band for both drugs proving that the method was not tolerant for minor change in solvent ratios in the mobile phase.

CONCLUSION

The established first derivative ratio spectrophotometric assay can determine MCP-HCl and VB6, in binary mixture, simultaneously, at 318.5& 327.5 nm, respectively. Whereas the isosbestic point spectrophotometric assay can determine both drugs, simultaneously, at 321.5& 299.5 nm, respectively. As well the spectrodensitometric assay can determine the drugs when the system consisting benzene, methanol, glacial acetic acid and acetone in ratio (10: 8: 0.5: 0.5; by volume) was used as a mobile phase. The precision, accuracy and specificity of the methods were found to be perfect. Applicability of the methods on anausan® syrup gave excellent results for both drugs**.**

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Conflict of interests: The authors declare that they have no any conflict of interests.

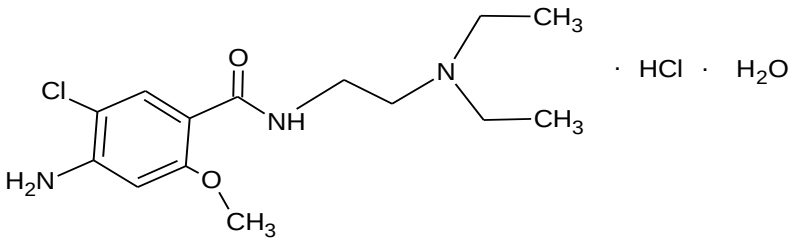

Fig. 1: Structure of MCP-HCl

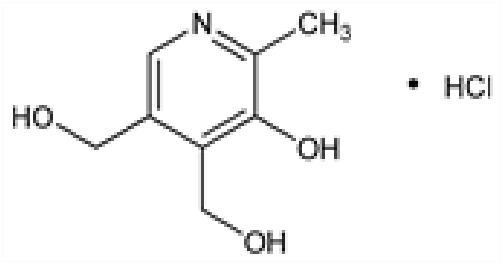

Fig. 2: Structure of VB6

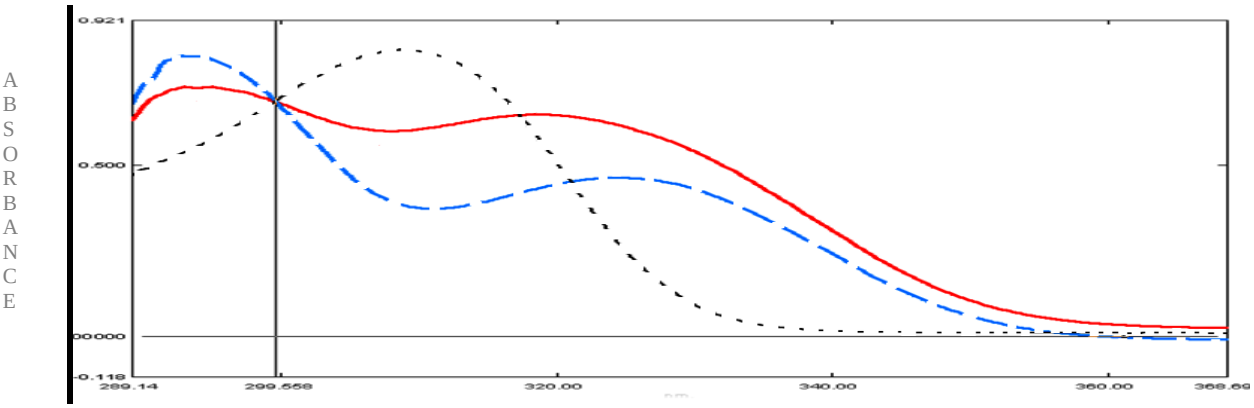

Fig. (3): Zero order spectra of MCP-HCl (60 µg/mL) (…..), VB6 (40 µg/mL) (----) and MCP-HCl / VB6 mixture (50: 50) (⁻), using water as a blank.

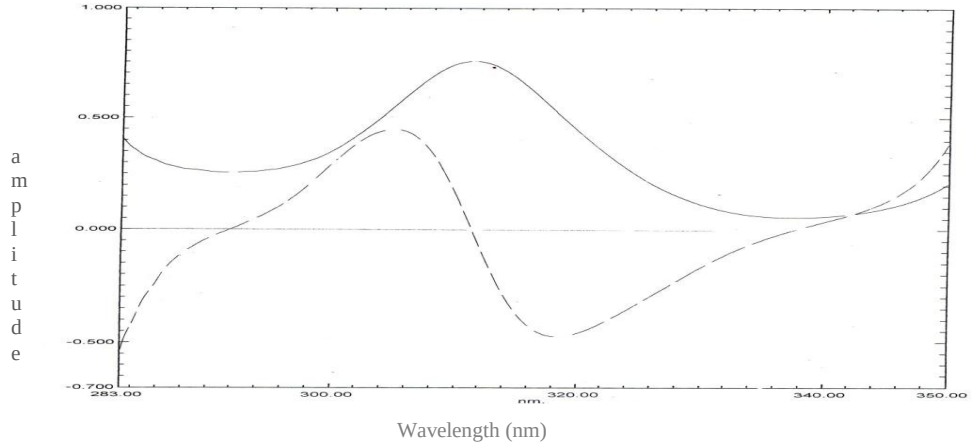

Fig. (4): Ratio spectrum of MCP-HCl (20µg/mL) using VB6 (50 µg/mL) spectrum as a divisor (-) and its first derivative spectrum (----), showing maximum responses at 305 & 318.5 nm.

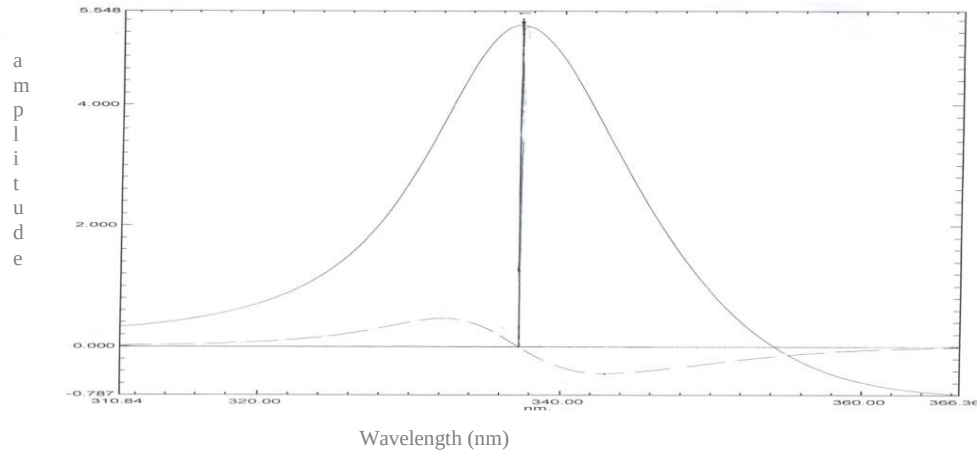

Fig. (5): The ratio spectrum of VB6 (34 µg/mL) using MCP-HCl (20 g/mL) spectrum as a divisor (⁻) an its first derivative spectrum (---).

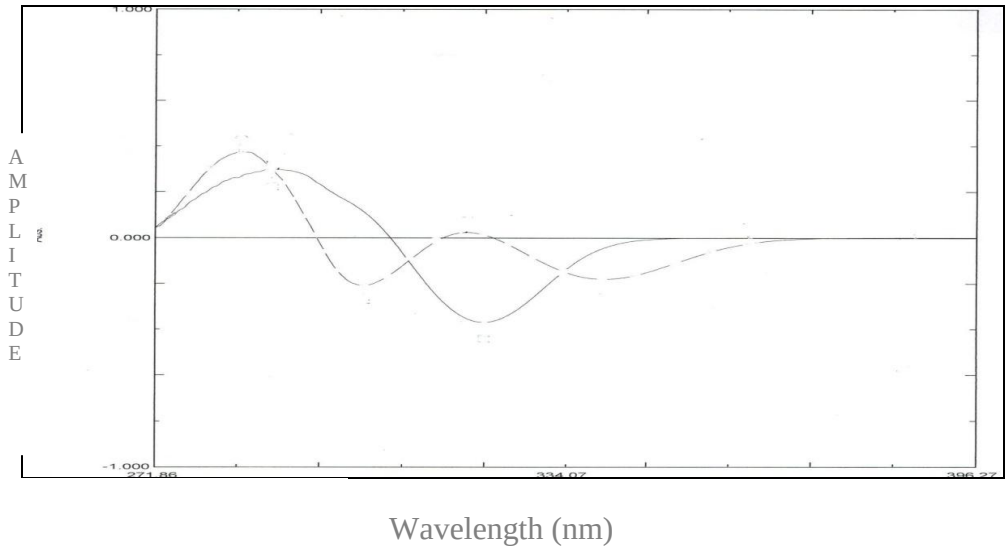

Fig 6: The first order spectra of MCP-HCl (60 µg/mL) (—) and VB6 (40 µg/mL) (---) aqueous solutions.

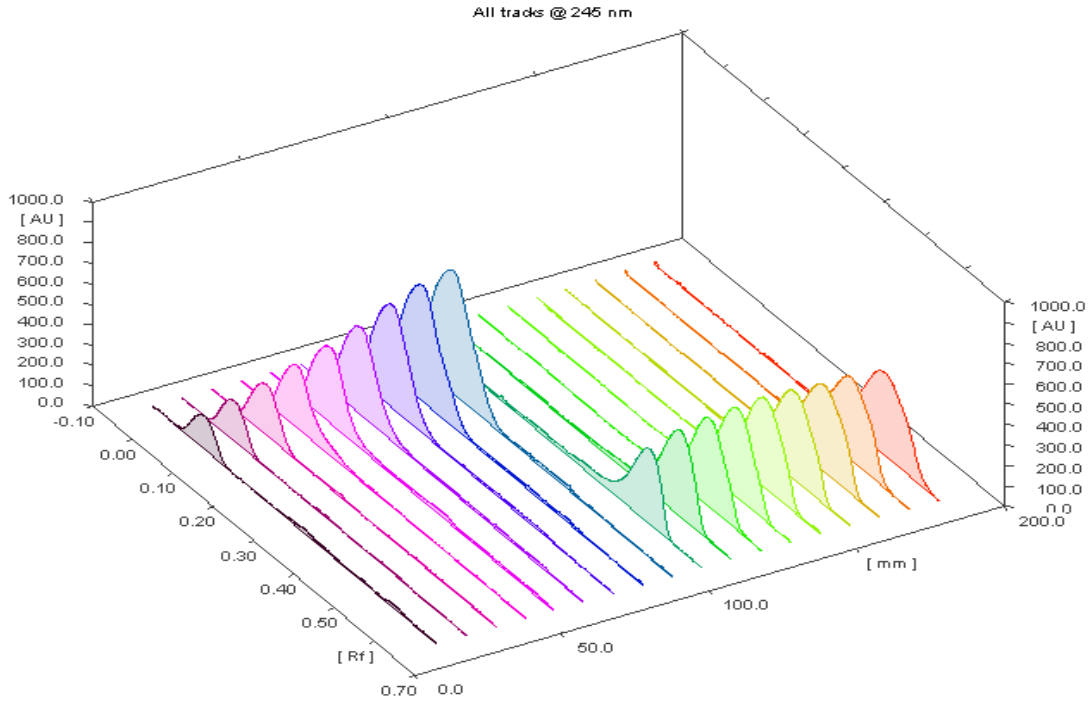

Figure 7: Scanning profile of the TLC chromatogram of MCP-HCl and VB6.

Table (I): Summary of linearity parameters of the proposed methods for determination of MCP-HCl and VB6 in binary mixture.

The Drug	Conc. Range	Calibration Equation	R^2
	Method A		
MCP-HCl (305 nm)	20-60*	y = 2.2 x + 0.2	0.9998
MCP-HCl (318.5 nm)	20-60*	y = 2.3 x + 0.1	1
VB6 (327.5 nm)	14-22*	y = 0.35 x + 0.01	0.9998
VB6 (303 nm)	14-40*	y = 3.6 x + 0.01	0.9999
	Method B		
MCP-HCl (321.5 nm)	10-60*	y = 7.3 x + 0.2	0.9998
VB6 (299.5 nm)	10-40*	y = 0.252 x + 0.2	0.9998
	Method C		
MCP-HCl	15-45**	y = 0.138 x + 0.42	0.9998
VB6	20-90**	y = 3.15 x + 0.01	0.9996

* Concentration range in µg/mL
** Concentration range in µg/Spot

Table (II): Precision of method A for determination of MCP-HCl and VB6 in binary mixture.

Metoclopramide hydrochloride						pyridoxine		
Wavelength 305 nm			Wavelength 318.5 nm			Wavelength 327.5 nm		
Mean R % *	SD (±)	RSD	Mean R % *	SD (±)	RSD	Mean R % *	SD (±)	RSD
Intra-day precision								
100.00	0.00	0.00	100.74	0.834	0.828	100.33	1.166	1.162
100.01	0.708	0.708	100.30	0.170	0.169	100.11	0.020	0.080
100.01	0.122	0.122	99.99	0.378	0.378			
Inter-day precision								
100.76	0.054	0.053	99.99	0.662	0.662	99.67	0.462	0.463
100.00	00.00	00.00	100.00	0.622	0.662	99.91	1.702	1.704
100.00	0.244	0.244	100.00	0.234	0.233			

* Mean of 3 experiments

Table (III): Precisions of method B for determination of MCP-HCl/ VB6 mixture.

Metoclopramide Hydrochloride				Pyridoxine			
Conc. of sample (µg/mL)	Mean R % *	SD (±)	RSD	Conc. of sample (µg/mL)	Mean R % *	SD (±)	RSD
Intra-day precision							
26.50	99.43	0.224	0.226	30.50	101.30	0.112	0.111
36.00	100.57	0.143	0.142	41.50	100.08	0.143	0.143
47.00	99.86	0.310	0.311	52.50	100.24	0.113	0.113
Inter-day precision							
28.00	99.36	00.00	00.00	30	99.67	0.462	0.463
33.50	101.01	0.391	0.387	36.50	101.28	1.726	1.704
48.50	100.16	0.126	0.125				

* Mean of 3 experiments

Table (IV): Precision of method C for determination of MCP-HCl/ VB6 mixture.

Metoclopramide Hydrochloride				Pyridoxine			
Conc. of sample (µg/spot)	Mean R % *	SD (±)	RSD%	Conc. of sample (µg/spot)	Mean R % *	SD (±)	RSD%
Intra-day precision							
30	100	0.71	0.71	20	100	0.00	0.00
40	99.79	0.37	0.38	40	99.91	0.12	0.12
45	99.99	0.32	0.32	70	99.96	0.05	0.05
Inter-day precision							
10	102.61	0.36	0.35	20	101.89	1.27	1.25
35	99.99	0.50	0.50	30	101.76	1.60	1.57
40	99.99	0.36	0.34	90	100.02	1.60	1.60

* Mean of 3 experiments

Table (V): Application of standard addition technique on standard solutions of MCP-HCl and VB6 by method A.

Claimed amount taken	Standard added (µg/mL)	R %	Claimed amount taken	Standard added (µg/mL)	R %	Claimed amount taken	Standard added (µg/mL)	R %
MCP-HCl (305 nm)			MCP-HCl (318.5 nm)			VB6 (327.5 nm)		
12.5 (µg/mL)	00.00	100.00	12.5 (µg/mL)	00.00	100.00	12 (µg/mL)	00.00	100.00
	3.50	98.68		3.50	100.50		2.00	98.86
	10.50	99.85		10.50	100.79		5.00	100.09
	18.00	96.51		18.00	98.74		7.00	102.89
Mean		98.76	Mean		100.00	Mean		100.61
S.D (±)		1.395	S.D (±)		0.784	S.D (±)		1.487
RSD		1.413	RSD		0.784	RSD		1.478

Table (VI): Application of standard addition technique on standard solutions of MCP-HCl and VB6 by method B.

Claimed amount taken	Standard added (µg/mL)	R %	Claimed amount taken	Standard added (µg/mL)	R %
Metoclopramide Hydrochloride			Pyridoxine		
10.50 (µg/mL)	00.00	100.27	12.00 (µg/mL)	00.00	100
	33.50	100.01		15.00	100.72
	39.50	99.79		18.00	100.35
	45.00	99.76		21.00	99.62
Mean		100.03	Mean		100.36
S.D (±)		0.239	S.D (±)		0.469
RSD		0.239	RSD		0.467

Table (VII): Application of standard addition technique on standard solutions of MCP-HCl and VB6 by method C.

Claimed amount taken	Standard added (µg/spot)	R %	Claimed mount taken	Standard added (µg/spot)	R %
Metoclopramide Hydrochloride			Pyridoxine		
5 (µg/spot)	12.50	98.85	25 (µg/spot)	40.00	96.90
	15.00	101.16		45.00	100.89
	17.50	103.38		50.00	100.34
Mean		101.13	Mean		99.37
S.D (±)		1.84	S.D (±)		1.76
RSD%		1.82	RSD%		1.77

Table (VIII): Data derived from analysis of MCP-HCl/ VB6 lab. mixtures by the proposed methods to prove selectivity.

Metoclopramide Hydrochloride				Pyridoxine			
Drug % in lab. Mix.	R % Method A	R % Method B	R % Method C	Drug % in lab. Mix.	R % Method A	R % Method B	R % Method C
20			98.83	80			100.46
25			98.48	75			103.09
35			98.48	65			100.32
45	100.00	99.42	100.15	55	99.41	102.18	100.28
50	99.95	99.92	100.43	50	100.06	101.26	103.36
55	100.06	99.36		45	99.98	99.82	
60	99.67	99.75		40	100.10	99.75	
65		99.64		35		99.78	
Mean R%	99.92	99.61	99.27	Mean R%	99.88	100.55	101.50
SD (±)	0.149	0.207	0.844	SD (±)	0.279	0.992	1.410
RSD%	0.149	0.208	0.850	RSD%	0.279	0.987	1.389

Table (IX): Application of the proposed spectrophotometric methods on anausan® syrup.

Anausan® syrup B. NO 10166 (5mg MCP-HCl & 25 mg VB6/ 5 mL)	R%	SD (±)	RSD
Method A			
MCP-HCl	100.99	0.451	0.446
VB6	99.23	0.840	0.846
Method B			
MCP-HCl	98.99	0.850	0.858
VB6	102.03	1.930	1.891
Method C			

Anausan® syrup B. NO 10166 (5mg MCP-HCl & 25 mg VB6/ 5 mL)	R%	SD (±)	RSD
Method A			
MCP-HCl	101.13	1.84	1.82
VB6	99.37	1.76	1.77

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