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Spectrophotometric and LC determination of two binary mixtures containing pyridoxine hydrochloride

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Abstract

Several methods are developed for the determination of two binary mixtures containing pyridoxine HCl together with either metoclopramide HCl (mixture 1) or meclozine HCl (mixture 2). The resolution of binary mixture of pyridoxine HCl and metoclopramide HCl has been accomplished by using partial least squares (PLS-1) and principal component regression (PCR) applied to zero and first order UV spectra of the mixture, respectively. In addition HPLC method depending on using RP18 column with a mobile phase consisting of acetonitrile–water (30:70, v/v, pH 3.2) with UV detection at 305 nm was developed. In mixture 2, the simultaneous determination of pyridoxine HCl and meclozine HCl was carried out by using graphical (second derivative of the ratio spectra) and numerical spectrophotometric methods (principal component regression and partial least squares, PLS-1 and PLS-2, applied to the zero order UV spectra of the mixture). The proposed methods were successfully applied for the determination of the two binary combinations in synthetic mixtures and in commercial tablets and syrup containing several light absorbing excipients.

Keywords: Meclozine; Metoclopramide; Pyridoxine; Chemometrics; Second derivative of the ratio spectra; Reversed phase HPLC

1. Introduction

Two binary mixtures are used to treat nausea and vomiting, namely, pyridoxine HCl–metoclopramide HCl (mixture 1) and pyridoxine HCl–meclozine HCl (mixture 2). No analytical method has been reported for the simultaneous determination of pyridoxine HCl (PR) and metoclopramide HCl (MT) in binary mixture. While various methods have been reported for simultaneous determination of pyridoxine HCl (PR) and meclozine HCl (MC) using HPLC [1,2], orthogonal function [3], first derivative spectra [4] and two-component first derivative equation [5]. Spectrophotometric determination of (MC) in tablets containing (PR) and (MC) combination was reported using ion-pair formation with methyl orange [6].

This work presents PLS and PCR methods for determination of either (MT) or (MC) as minor weakly absorbing components in combinations containing (PR) as major strongly absorbing component. Therefore the strongly absorbing major component (PR) interferes seriously in the

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absorption spectra of the other two minor compounds (MT, MC). In addition, HPLC and second derivative of the ratio spectrum (^{2}DD) methods have been developed for the assay of the components of mixtures 1 and 2, respectively. The proposed methods are simple, sensitive, reduce the duration of the analysis and suitable for routine determination of the components in the studied mixtures.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV–Visible spectrophotometer, model UV-1601 PC equipped with 1 cm quartz cells and connected to an IBM compatible computer. HP 600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). PLS and PCR analysis were carried out by using PLS-Toolbox software version 2.1 PC [7] for use with MATLAB5.

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 μl loop and a SPD-10AVP UV–VIS detector, separation and quantitation were made on a 250 × 4.6 mm (i.d.). Waters XTerra™ RP_{18} column (5 μm particle size). The detector was set at λ 305 nm. Data acquisition was performed on class-VP software.

2.2. Materials and reagents

Pharmaceutical grade of (PR), (MT) and (MC) were used and certified to contain 99.8, 99.9 and 99.7%, respectively. Acetonitrile used was HPLC grade (BDH, Poole, UK). Hydrochloric and phosphoric acids used were analytical grade.

Anausan syrup (RAMEDA, 6th of October city, Egypt) contains 25 mg of (PR) and 5 mg of (MT) per 5 ml, Ezadoxine tablets (Kahira pharmaceutical Co., Egypt) and Vomidoxine tablets (Pharaonia pharmaceuticals, Alexandria, Egypt) contain 50 mg of (PR) and 25 mg of (MC) per tablet were used.

2.3. HPLC conditions

The mobile phase for mixture 1 was prepared by mixing acetonitrile and water in a ratio of 30:70 v/v and the apparent pH was adjusted to 3.2 using phosphoric acid. The flow rate was 0.5 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Standard solutions and calibration

2.4.1. For mixture 1

Standard solutions of each (PR) and (MT) were prepared in 0.1 M hydrochloric acid (for spectrophotometric methods) or mobile phase (for HPLC method) within concentration rang of 5–40 and 3–8 μg ml^{-1} for (PR) and (MT), respectively.

2.4.1.1. For PLS and PCR methods. A training set of 30 synthetic mixtures with different concentrations of (PR) and (MT) in range 5–40 μg ml^{-1} for (PR) and 3–8 μg ml^{-1} for (MT) within concentration ratio ranged from 1:1 to 1:8 for (MT):(PR) were prepared in 0.1 M hydrochloric acid.

The UV absorption and first derivative spectra using $\Delta\lambda = 11$ nm were recorded over the range 300–320 nm. The data points of the spectra were collected at every 0.2 nm. The computations were made in PLS-Toolbox software version 2.1.

PLS-1 model was applied to the UV absorption spectra of these mixtures using two latent variables for determination of (PR) and three latent variables for determination of (MT).

PCR model was applied to the first derivative UV spectra of these mixtures using two principal component for determination of (PR) and (MT).

2.4.1.2. For HPLC method. Triplicate 20 μl injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations. Linear relationship was obtained.

2.4.2. For mixture 2

Standard solutions of each of (PR) and (MC) were prepared in 0.1 M hydrochloric acid within concentration range of 5–40 and 6–20 μg ml^{-1} for (PR) and (MC), respectively.

2.4.2.1. For PLS and PCR methods. A training set of 30 synthetic mixtures with different concentrations of (PR) and (MC) in range 5–40 μg ml^{-1} for (PR) and 6–20 μg ml^{-1} for (MC) within concentration ratio ranged from 1:0.5 to 1:4 for (MC): (PR) were prepared in 0.1 M hydrochloric acid.

The UV absorption was recorded over the range 220–300 nm. The data points of the spectra were collected at every 1 nm. The computations were made in PLS-Toolbox software version 2.1.

PLS-1, PLS-2 and PCR models were applied to the UV absorption spectra of these mixtures using two latent variables (or principal component) for determination of (RP) and (MC).

2.4.2.2. For ^{2}DD method.

2.4.2.2.1. For determination of (PR):. The UV absorption spectra of standard solutions of (PR) were divided by a normalized spectrum of (MC) [a spectrum of unit concentration]. The second derivative was calculated for the obtained ratio spectra with $\Delta\lambda = 4$ nm. The second derivative of the ratio spectra obtained was smoothed with eight experimental points. The amplitudes at 309 nm were measured and found to be proportional to the concentration of (PR).

2.4.2.2.2. For determination of (MC). The UV absorption spectra of standard solutions of (MC) were divided by a normalized spectrum of (PR).The second derivative was calculated for the obtained ratio spectra with $\Delta\lambda = 4$ nm. The second derivative of the ratio spectra obtained was smoothed with eight experimental points and scaling factor of 10. The amplitudes at 245.8 nm were measured and found to be proportional to the concentration of (MC).

2.5. Sample preparation

2.5.1. For mixture 1 syrup

A volume of the syrup equivalent to 25 mg of (PR) and 5 mg of (MT) was diluted to 50 ml with distilled water. Further dilution was carried out with 0.1 M hydrochloric acid (for spectrophotometric method) or mobile phase (for HPLC method) to provide a solution of 25 μg ml^{-1} of (PR) and 5 μg ml^{-1} of (MT). The general procedures for PLS-1, PCR and HPLC methods described under calibration were followed and the concentrations of (PR) and (MT) were calculated.

2.5.2. For mixture 2 tablets

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to about 40 mg of (PR) and 20 mg of (MC) was weighed accurately, dissolved and diluted to 100 ml with 0.1 M hydrochloric acid. The sample solution was filtered. Further dilution of the filterate was carried out with the same solvent to provide a solution of 20 μg ml^{-1} of (PR) and 10 μg ml^{-1} of (MC). The general procedures for PLS-1, PLS-2, PCR and ^{2}DD methods described under calibration were followed and the concentrations of (PR) and (MC) were calculated.

3. Results and discussion

3.1. For mixture 1

3.1.1. PLS and PCR methods

Fig. 1 shows the UV absorption spectra of (PR), (MT) and strongly absorbing excipients of syrup at their nominal concentrations. The excipients include methylparaben, propylparaben, ethylenediamine tetra acetate disodium, quinoline yellow, sorbitol, propylene glycol, citric acid and sodium citrate. As can be seen, (MT) contributes very little to overall absorption of the sample; also, the absorption bands of (PR) and the excipients are extensively overlapped with (MT) spectrum. The simultaneous determination of (PR) and (MT) in syrup by conventional, derivative and derivative ratio spectrophotometric methods is hindered by strong spectral overlap throughout the wavelength range. If the concentration of one or more absorbing component (excipient) is omitted, the predicted absorbance will be incorrect, for these reasons the classical least squares is not recommended in this case. Therefore, PLS or PCR

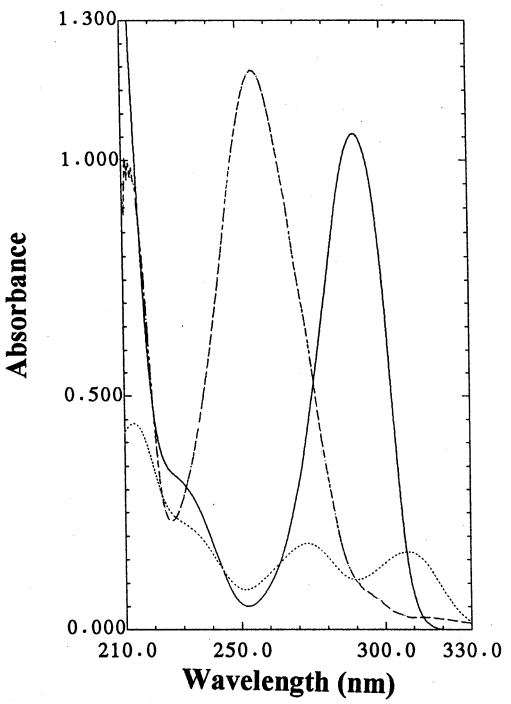

Fig. 1. UV absorption spectra of 25 μg ml^{-1} of pyridoxine HCl (—), 5 μg ml^{-1} of metoclopramide HCl (-----) and strongly absorbing excipients of syrup (— - —) in 0.1 M hydrochloric acid.

calibration methods were necessary for such determination due to the presence of interferences or matrix effect.

The quality of multicomponent analysis results is dependent on the wavelength range and spectral mode used [8,9]. Spectral resolution was assayed with absorbance and first derivative spectra for PLS-1 and PCR methods, respectively, measured at 0.2 nm intervals over the range 300–320 nm. Wavelengths less than 300 nm were rejected due to the differences between the synthetic mixture spectra and pharmaceutical syrup spectra. These differences due to the strongly absorbing excipients of syrup. Wavelengths more than 320 nm were not used because (PR) does not absorb in this region, so any absorbance values obtained at these wavelengths would have introduced a significant amount of noise in the calibration matrix, thereby decreasing the precision. $\Delta\lambda = 11$ nm was selected for best resolution of spectra in first derivative.

To select the number of factors in the PLS-1 and PCR algorithms, a cross-validation method leaving out one sample at a time [10] was employed using a training (calibration) set of 30 calibration spectra. The predicted concentrations of the components in each sample were compared with the actual concentrations in this training sample and the root mean square error of prediction (RMSEP) was calculated for each method. The RMSEP was used as a diagnostic test for examining the errors in the predicted concentrations.

Number of factors of 2 and 3 were obtained as optimum for the (PR) and (MT), respectively by the PLS-1 method. A number of 2 factors was found to be optimum for both components by the PCR method. The RMSEP obtained by optimizing the calibration matrix of the absorption and first derivative spectra for the PLS-1 and PCR methods, respectively, are shown in Table 1 indicating good accuracy and precision. Satisfactory correlation coefficient (r) values between actual and predicted concentrations are obtained for both components in the training set by PLS-1 and PCR optimized models (Table 1).

3.1.2. HPLC method

The developed HPLC method has been applied for the simultaneous determination of (PR) and (MT). To optimize the HPLC assay parameters, the mobile phase composition and pH were studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile–water (30:70, v/v). Increasing acetonitrile concentration to more than 50% led to inadequate separation of the two drugs. At lower acetonitrile concentration ($< 15\%$) separation occurred but with excessive tailing for (MT) peak. Variation of apparent pH of the mobile phase resulted in maximum capacity factor (K') value at apparent pH 5.5, with loss of peak symmetry for (PR). At apparent pH 2.9–3.5 improved resolution for the two drugs was observed. However at apparent pH 3.2 optimum resolution with reasonable retention time was observed. Quantitation was achieved with UV detection at 305 nm based on peak area. The specificity of the HPLC method is illustrated in Fig. 2 where complete separation of the two drugs was noticed. The average retention

Table 1
Cross validation results for simultaneous determination of pyridoxine HCl (PR) and metoclopramide HCl (MT) by PLS-1 and PCR; and (PR) and meclozine HCl (MC) by PLS-1, PLS-2 and PCR methods

	(RMSEP)			(*r*)		
	PLS-1	PLS-2	PCR	PLS-1	PLS-2	PCR
Mixture 1						
(PR)	0.0161		0.0168	0.9999		0.9999
(MT)	0.0121		0.0126	0.9998		0.9998
Mixture 2						
(PR)	0.0156	0.0157	0.0152	0.9999	0.9999	0.9999
(MC)	0.0125	0.0127	0.0124	0.9998	0.9998	0.9998

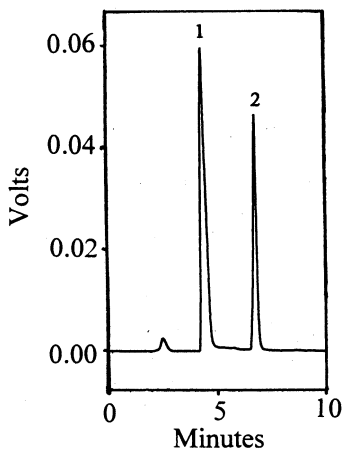

Fig. 2. Typical HPLC chromatogram of 20 μl injection of syrup sample containing 25 μg ml^{-1} of pyridoxine HCl (1) and 5 μg ml^{-1} of metoclopramide HCl (2).

time ± standard deviation for (RP) and (MT) were found to be 4.4 ± 0.025 and 6.8 ± 0.030 min, respectively, for ten replicates. Characteristic parameters for regression equations of the HPLC method and correlation coefficient obtained by least squares treatment of the results were given in Table 2.

3.2. For mixture 2

3.2.1. PLS and PCR methods

(MC) possesses a low absorption in the UV region while (PR) exhibits a large absorption in the same region (Fig. 3). The conventional UV method for the assay of (MC) is susceptible to interference from (PR). PLS or PCR calibration methods can be used to overcome this problem. To select the number of factors in the PLS-1, PLS-2 and PCR algorithms, a cross-validation method, leaving out one sample at a time [10] was employed using a training set of 30 calibration spectra. A number factor of 2 was found to be optimum for both components by the PLS-1, PLS-2 and PCR methods. The RMSEP obtained by optimizing the calibration matrix for each method is shown in Table 1 indicating good accuracy and precision. Satisfactory correlation coefficient (*r*) values between actual and predicted concentrations are obtained for both components in the training set using PLS-1, PLS-2 and PCR models (Table 1).

3.2.2. For ^{2}DD method

To optimize the simultaneous determination of (PR) and (MC) by using ^{2}DD method, it is necessary to test the influence of the divisor standard concentration, the $\Delta\lambda$ and smoothing function. $\Delta\lambda = 4$ nm was selected as the optimum value. From several tests for correct choice of the divisor standard concentration, the best results in terms of signal to noise ratio, sensitivity and repeatability followed using normalized spectra as divisor. Due to the extent of the noise levels on the ratio spectra, a smoothing function was used and eight experimental points were considered as suitable.

The second derivative of the ratio spectra was preferred than the first derivative for a better

Table 2
Characteristic parameters of the calibration equations for the proposed HPLC method for simultaneous determination of pyridoxine HCl (PR) and metoclopramide HCl (MT); and the proposed second derivative of ratio spectra (^{2}DD) method for simultaneous determination of (PR) and meclozine HCl (MC)

Parameters	HPLC		^{2}DD	
	(PR)	(MT)	(PR)	(MC)
Calibration range (μg ml^{-1})	5–40	3–8	5–40	6–20
Detection limit (μg ml^{-1})	8.92×10^{-3}	7.07×10^{-3}	6.33×10^{-3}	8.05×10^{-3}
Quantitation limit (μg ml^{-1})	29.70×10^{-3}	23.56×10^{-3}	20.88×10^{-3}	26.81×10^{-3}
Regression equation (Y)[a]: Slope (b)	40.99×10^{3}	12.50×10^{4}	57.46×10^{-3}	47.55×10^{-3}
Standard deviation of the slope (S_b)	1.71×10^{2}	4.13×10^{2}	1.70×10^{-4}	1.79×10^{-4}
Relative standard deviation of the slope (%)	0.42	0.33	0.30	0.38
Confidence limit of the slope[b]	40.57×10^{3}–41.41×10^{3}	12.40×10^{4}–12.60×10^{4}	57.04×10^{-3}–57.88×10^{-3}	47.11×10^{-3}–47.99×10^{-3}
Intercept (a)	-6.05×10^{3}	-2.30×10^{3}	-1.32×10^{-3}	1.21×10^{-3}
Standard deviation of the intercept (S_a)	4.32×10^{3}	2.31×10^{3}	4.29×10^{-3}	2.5×10^{-3}
Confidence limit of the intercept[b]	(-16.63×10^{3})–4.51×10^{3}	(-6.53×10^{3})–4.76×10^{3}	(-11.83×10^{-3})–9.19×10^{-3}	(-4.81×10^{-3})–7.25×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	5.55×10^{3}	1.77×10^{3}	5.51×10^{-3}	2.31×10^{-3}

[a] $Y = a + bC$, where C is the concentration of drug in μg ml^{-1} and Y is the peak area or ^{2}DD amplitude for HPLC and ^{2}DD methods, respectively.
[b] 95% confidence limit.

resolution of the spectra and more accurate and precise results. The second derivative of the ratio peak amplitudes at 309 and 245.8 nm were measured and found to be proportional to the concentration of (PR) and (MC), respectively (Figs. 4 and 5). The characteristic parameters for

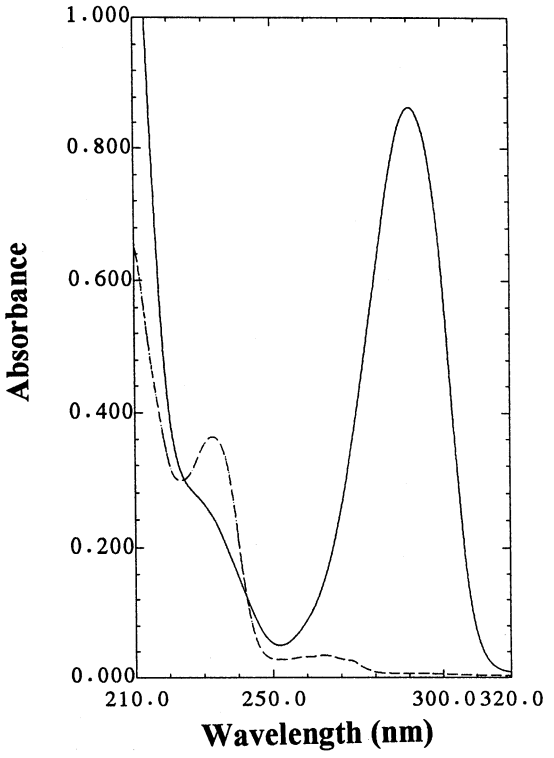

Fig. 3. UV absorption spectra of 20 μg ml^{-1} of pyridoxine HCl (—) and 10 μg ml^{-1} of meclozine HCl (-----) in 0.1 M hydrochloric acid.

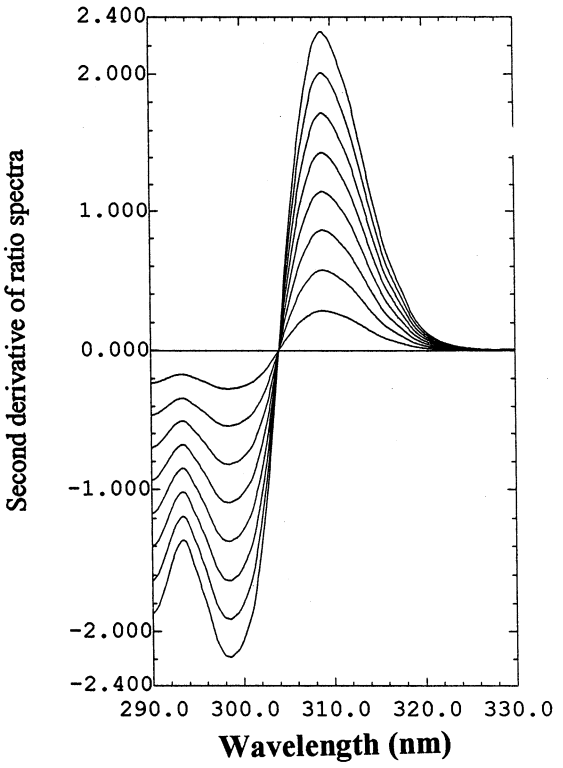

Fig. 4. Second derivative of ratio spectra for different concentrations (5, 10, 15, 20, 25, 30, 35, 40 μg ml^{-1}) of pyridoxine HCl, using normalized spectrum of meclozine HCl as divisor.

regression equations for the ^{2}DD method and correlation coefficient were given in Table 2.

3.3. Analysis of pharmaceutical products

The proposed PLS-1, PCR and HPLC methods were applied to the simultaneous determination of (PR) and (MT) in commercial syrup. Seven replicates determinations were made. Satisfactory results were obtained for both drugs in good agreement with the label claims (Table 3). No published method has been reported for simultaneous determination of (PR) and (MT). So that the results of the proposed PLS-1 and PCR methods were compared with those of the proposed HPLC method.

The proposed PLS-1, PLS-2, PCR and ^{2}DD methods were applied to the simultaneous determination of (PR) and (MC) in commercial tablets. Seven replicates determinations were made. Satisfactory results were obtained (Table 3). These results were compared with those of the published HPLC method [1].

Statistical comparison between the results was performed with regards to accuracy and precision using Student's *t*-test and the *F*-ratio at 95% confidence level (Table 3). There is no significant difference between the results.

3.4. Validation of the methods

3.4.1. Linearity

The linearity of the HPLC method for determination of (PR) and (MT); and ^{2}DD method for determination of (PR) and (MC) was evaluated by analysing a series of different concentrations of each drug. According to the International Conference on Harmonization [11], at least five concentrations must be used. In this study eight

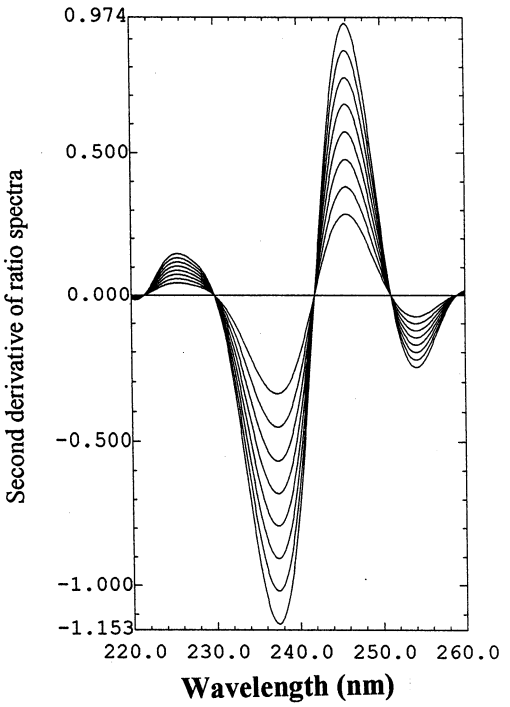

Fig. 5. Second derivative of ratio spectra for different concentrations (6, 8, 10, 12, 14, 16, 18, 20 μg ml^{-1}) of meclozine HCl, using normalized spectrum of pyridoxine HCl as divisor.

concentrations were chosen, ranging between 5 and 40 μg ml^{-1} for (PR) and 3–8 μg ml^{-1} for (MT) in binary mixture 1, and 5–40 μg ml^{-1} for (PR) and 6–20 μg ml^{-1} for (MC) in binary mixture 2. Each concentration was repeated three times, the repeated runs were genuine repeats and not just repetitions at the same reading; this approach will provide information on the variation in peak area and ^{2}DD values between samples of same concentration. The linearity of the calibration graphs and adherence of the system to beer's law were validated by the high value of the correlation coefficient and the intercept value which was not statistically ($P < 0.05$) different from zero (Table 2).

3.4.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for each drug. The data for each concentration level were evaluated by one-way ANOVA. An 8 days × 2 replicates design was performed. Statistical comparison of the results was performed using the P-value of the F-test. Three univariate analyses of variance for each concentration level were made. Since the P-value of the F-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

3.4.3. Range

The calibration range was established through consideration of the practical range necessary, according to each drug concentration present in pharmaceutical product, to give accurate, precise and linear results. The calibration range of the proposed HPLC and ^{2}DD methods are given in Table 2.

3.4.4. Detection and quantitation limits

According to ICH recommendations [11] the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in Table 2.

3.4.5. Selectivity

Methods selectivity was achieved by preparing different mixtures of the studied drugs at various concentrations within the linearity range. The synthetic mixtures were analysed according to the previous procedures described under the proposed methods. Satisfactory results were obtained (Table 3), indicating the high selectivity of the proposed methods for determination of the studied drugs in their mixture.

3.4.6. Accuracy

This study was performed by addition of known amounts of the studied drugs to a known concentration of the commercial pharmaceutical products (standard addition method). The resulting mixtures were assayed and results obtained were compared with the expected results. The excellent recoveries of standard addition method (Table 3) suggest that good accuracy of the proposed methods.

Table 3
Determination of pyridoxine HCl (PR), metoclopramide HCl (MT) and meclozine HCl (MC) in synthetic mixtures and commercial pharmaceutical products using the proposed methods

	Mean found ± S.D.[a]				
	PLS-1	PLS-2	PCR	^{2}DD	HPLC
Binary mixture (1)					
Synthetic mixtures					
For (PR)	100.0 ± 0.66		100.1 ± 0.61		100.0 ± 0.44
For (MT)	100.0 ± 0.88		100.0 ± 0.74		100.0 ± 0.51
Commercial syrup					
For (RP)	100.5 ± 0.59		100.4 ± 0.66		100.3 ± 0.53
t	0.67		0.31		(2.18)[b]
F	1.24		1.55		(4.28)[b]
For (MT)	100.2 ± 0.62		100.1 ± 0.69		100.0 ± 0.50
t	0.66		0.31		(2.18)[b]
F	1.54		1.90		(4.28)[b]
Recovery[c]					
For (PR)	100.0 ± 0.41		100.0 ± 0.51		100.0 ± 0.35
For (MT)	100.1 ± 0.63		100.0 ± 0.65		100.1 ± 0.43
Binary mixture (2)					
Synthetic mixtures					
For (PR)	100.0 ± 0.71	100.0 ± 0.70	100.0 ± 0.87	100.1 ± 0.56	
For (MC)	100.0 ± 0.90	100.0 ± 0.89	100.2 ± 0.88	100.0 ± 0.51	
Commercial tablets					
Ezadoxine tablets					
For (PR)	100.4 ± 0.53	100.3 ± 0.50	100.3 ± 0.61	100.3 ± 0.69	100.2 ± 0.55
t	0.69	0.36	0.32	0.30	(2.18)[b]
F	1.08	1.21	1.23	1.57	(4.28)[b]
For (MC)	100.0 ± 0.57	100.2 ± 0.65	100.2 ± 0.52	100.0 ± 0.66	100.1 ± 0.51
t	0.35	0.32	0.36	0.32	(2.18)[b]
F	1.25	1.62	1.04	1.67	(4.28)[b]
Recovery[c]					
For (PR)	100.1 ± 0.55	100.1 ± 0.65	100.0 ± 0.49	100.1 ± 0.51	
For (MC)	100.0 ± 0.71	100.0 ± 0.63	100.1 ± 0.73	100.1 ± 0.61	
Vomidoxine tablets					
For (PR)	100.3 ± 0.61	100.1 ± 0.52	100.3 ± 0.63	100.3 ± 0.69	100.2 ± 0.53
t	0.33	0.36	0.32	0.30	(2.18)[b]
F	1.32	1.04	1.41	1.69	(4.28)[b]
For (MC)	100.1 ± 0.51	100.2 ± 0.66	100.1 ± 0.62	100.2 ± 0.65	100.0 ± 0.59
t	0.34	0.60	0.31	0.60	(2.18)[b]
F	1.34	1.25	1.10	1.25	(4.28)[b]
Recovery[c]					
For (PR)	100.1 ± 0.51	100.2 ± 0.55	100.1 ± 0.61	100.1 ± 0.49	
For (MC)	100.0 ± 0.72	100.0 ± 0.61	100.0 ± 0.53	100.0 ± 0.51	

[a] Mean and S.D., percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F*.
[c] For standard addition of 50% of the nominal content.

3.4.7. Robustness

The robustness of a method is its ability to remain unaffected by small change in parameters. Variation of pH of the mobile phase by ±0.1 and its organic strength by ±2% did not have significant effect on chromatographic resolution in

HPLC method. Variation of strength of hydrochloric acid by ± 0.02 M did not have a significant effect on spectrophotometric methods.

3.4.8. Stability

The studied drug solutions in mobile phase or 0.1 M HCl exhibited no chromatographic or absorbance changes for 24 h when kept at room temperature, and for 8 days when stored refrigerated at 5 °C.

4. Conclusion

The joint use of spectrophotometry and multivariate calibration for the resolution of mixtures of analytes with overlapped spectra is an effective choice for developing new analytical methods as well as for the quality control of pharmaceutical preparations and for the avoidance of some separation and extraction steps of classical determination processes. The PLS and PCR approaches used in this work are simple to perform, with adequate software support, and provide a clear example of the high resolving power of this technique. Control analyses on pharmaceutical preparations containing pyridoxine HCl together with either metoclopramide HCl or meclozine HCl using PLS and PCR methods have been proved to be a valid alternative to HPLC and ^{2}DD methods.

References

[1] T. Al-Jallad, Z. Al-Kurdi, A. Badwan, A.M.Y. Jaber, Pharm. Pharmacol. Commun. 5 (1999) 479–483.
[2] R.G. Ramana, S.S.N. Murty, M.K. Rama, Indian Drugs 19 (1982) 451–455.
[3] S.M. Hassan, Fresenius Z. Anal. Chem. 292 (1978) 400–402.
[4] F. Onur, E. Dinc, Eczacilik Fak. Derg. 7 (1990) 77–90.
[5] M.E. Abdel-Hamid, M.H. Barary, M.A. Korany, E.M. Hassan, Sci. Pharm. 53 (1985) 105–110.
[6] F. Onur, E. Dinc, FABAD Farm. Bilimler Derg. 16 (1991) 61–69.
[7] B.M. Wise, N.B. Gallagher, PLS-Toolbox Version 2.1, Eigenvector Research, Inc., 830 Wapato Lake Road, Manson, WA 98831, 2000.
[8] M. Blanco, J. Coello, F. Gonzalez, H. Iturriaga, S. Maspoch, X. Tomas, J. Pharm. Biomed. Anal. 12 (1994) 509–514.
[9] M. Blanco, J. Coello, F. Gonzalez, H. Iturriaga, S. Maspoch, J. Pharm. Sci. 82 (1993) 834–837.
[10] A. Espinosa-Mansilla, F. Salinas, I. De Orbe Paya, Anal. Chim. Acta 313 (1995) 103–112.
[11] The European Agency for the Evaluation of Medical Products. ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.