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Bivariate versus multivariate smart spectrophotometric calibration methods for the simultaneous determination of a quaternary mixture of mosapride, pantoprazole and their degradation products

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The ability of bivariate and multivariate spectrophotometric methods was demonstrated in the resolution of a quaternary mixture of mosapride, pantoprazole and their degradation products. The bivariate calibrations include bivariate spectrophotometric method (BSM) and H-point standard addition method (HPSAM), which were able to determine the two drugs, simultaneously, but not in the presence of their degradation products, the results showed that simultaneous determinations could be performed in the concentration ranges of 5.0–50.0 μg/ml for mosapride and 10.0–40.0 μg/ml for pantoprazole by bivariate spectrophotometric method and in the concentration ranges of 5.0–45.0 μg/ml for both drugs by H-point standard addition method. Moreover, the applied multivariate calibration methods were able for the determination of mosapride, pantoprazole and their degradation products using concentration residuals augmented classical least squares (CRACLS) and partial least squares (PLS). The proposed multivariate methods were applied to 17 synthetic samples in the concentration ranges of 3.0–12.0 μg/ml mosapride, 8.0–32.0 μg/ml pantoprazole, 1.5–6.0 μg/ml mosapride degradation products and 2.0–8.0 μg/ml pantoprazole degradation products. The proposed bivariate and multivariate calibration methods were successfully applied to the determination of mosapride and pantoprazole in their pharmaceutical preparations.

1. Introduction

Mosapride, 4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)morpholin-2-yl]methyl}benzamide (The Merck Index 2006) (MW 425.9), is a potent gastroprokinetic agent with selectivity for 5-HT4 receptor and is used in the treatment of gastrointestinal motility dysfunction associated with non-ulcer dyspepsia (Kato et al. 1991). Pantoprazole, 5-[difluoromethoxy]-2-{[(3,4-dimethoxy-2-pyridinyl)methyl] sulfinyl}-l*H*-benzimidazole (The Merck Index 2006) (MW 383.4), is an irreversible proton pump inhibitor. The inhibition of the gastric proton pump or H^+/K^+ ATPase, suppresses gastric acid secretions and hence hyperacidity can be controlled by pantoprazole (Fitton and Wiseman 1995). Thus, the pharmacological action of mosapride and pantoprazole corroborates their use in combination to treat various gastrointestinal disorders in particular for hyperacidity associated with gastrointestinal motility dysfunction.

Several chromatographic methods have been reported for the estimation of mosapride (Krishnaiah et al. 2002) and pantoprazole (Mansour and Sorour 2001). A spectroscopic method has been published for the simultaneous determination of mosapride and pantoprazole in their combined dosage form (Bhatt et al. 2009). Another stability indicating chromatographic method has been reported for simultaneous determination of both drugs in their combined dosage form and spiked human plasma (Hegazy et al. 2011a). However, there is no stability indicating spectrophtometric method for the determination of mosapride and pantoprazole in their binary mixture.

So the aim of this work was to develop bivariate and multivariate calibration methods and compare their abilities in the resolution of a quaternary mixture of mosapride, pantoprazole along with their degradation products. Four spectrophotometric techniques are proposed to resolve the spectral overlap. Two different techniques that utilize two variables as analytical wavelengths (bivariate calibrations) which are bivariate spectrophotometric method (BSM) and H-point standard addition method (HPSAM) for the resolution and determination of the spectral overlap between mosapride and pantoprazole binary mixture. The resolution of component mixtures has been achieved by bivariate method (López-de-Alba et al. 1997) and HPSAM (Sabry and Khamis 2000; Afkhami and Sarlak 2005). Moreover, more advanced statistical methods are needed to handle multivariate data (multivariate calibrations) where computer software will be an essential tool (Beebe 1998; Kramer 1998; Brereton 2003). Therefore, two multivariate calibrations, in which the whole spectral data were used, stability indicating multivariate methods used for the resolution and determination of mosapride, pantoprazole and their degradation products in their quaternary mixtures. Concentration residuals augmented classical least square (CRACLS) and partial least squares (PLS) were proposed as stability indicating multivariate methods.

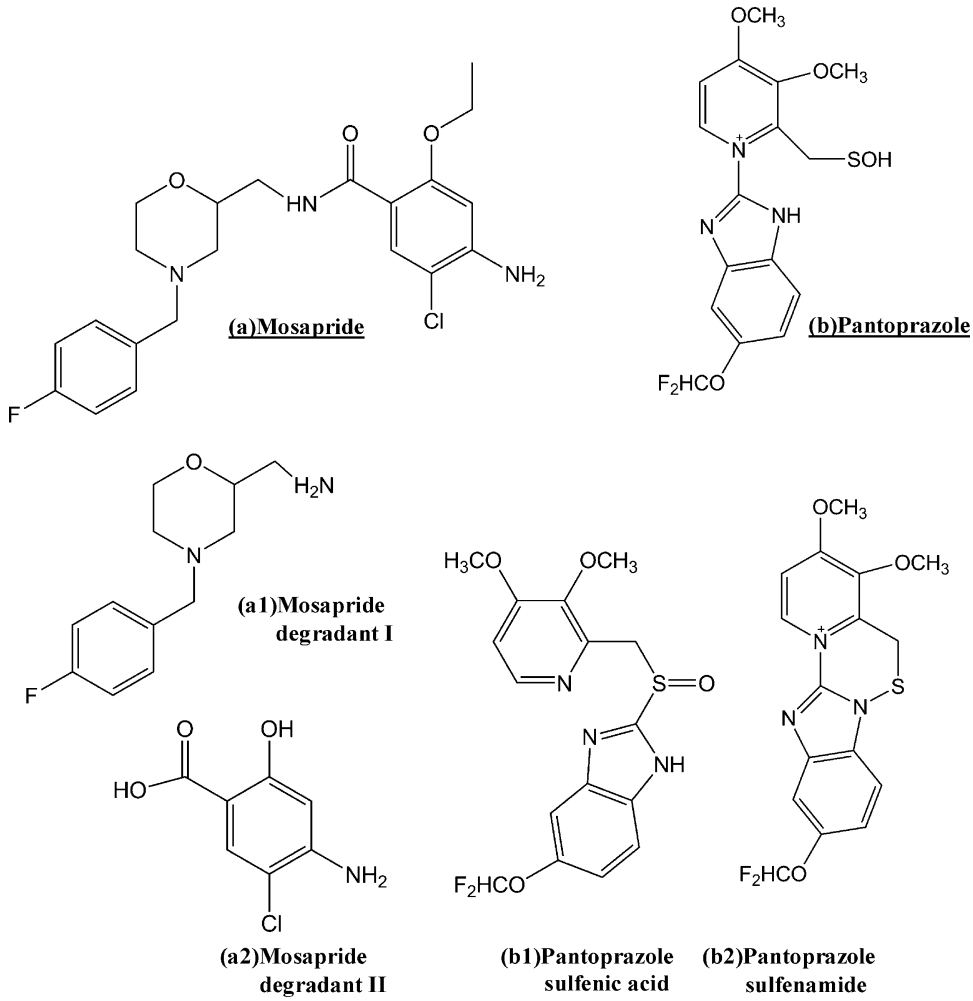

Fig. 1: Chemical structures of (a) mosarpide (b) pantoprazole and possible degradation products of mosapride (a1&a2) and pantoprazole (b1&b2)

CRACLS is a recently developed method for the resolution of complex mixtures, while PLS is a conventional chemometric method which is used for the purpose of comparison with the proposed CRACLS.

2. Investigations, results and discussion

According to previous studies, mosapride and pantoprazole are unstable in acidic media (Hegazy et al. 2011a), as mosapride contains amide and ether linkages susceptible to acid hydrolysis (Hegazy et al. 2011b). Likewise, pantoprazole and some other proton pump inhibitors (PPI) undergo decomposition under acidic stress conditions (Qaisi et al. 2006; Tutunji et al. 2006). Chemical structures of intact molecules and acid induced degradation products of mosapride and panroprazole are shown in Fig. 1.

Resolving complex spectral overlap of binary mixtures with no prior separation is difficult, and bivariate statistics were used as a satisfactory tool for the resolution of mosapride and pantoprazole overlapped spectra (Fig. 2(a)). The first bivariate method is BSM, which is a simple mathematical algorithm that uses the data derived from four linear regression equations, two calibrations for each of mosapride and pantoprazole at a pair of carefully selected wavelengths using the method of Kaiser (Massart et al. 1998). Moreover, the second bivariate method is HPSAM which permits both proportional and constant errors produced by the matrix of the sample mixture to be corrected directly. This method combines the principle of dual wavelength

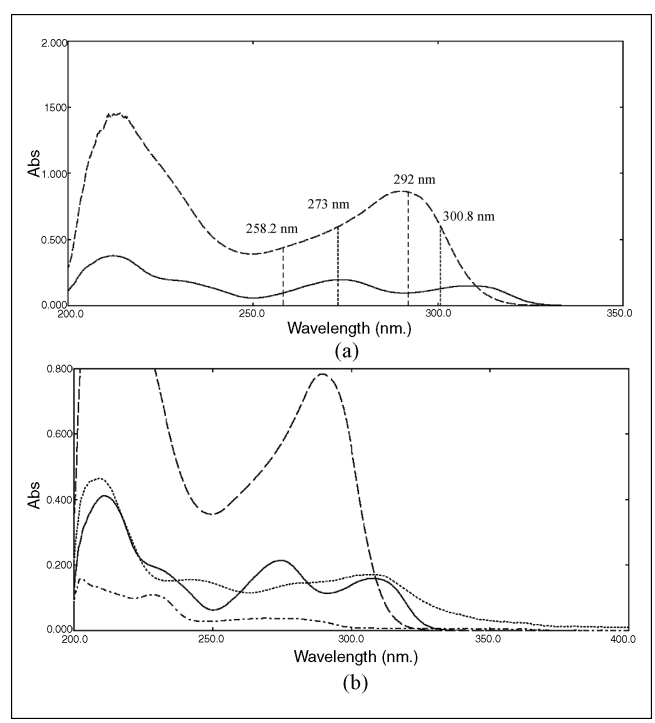

Fig. 2: Zero order absorption spectra of 7.50 μg/ml of mosapride (------), 20.00 μg/ml of pantoprazole (-----), 3.75 μg/ml of mosapride degradation products (.....)and 5.00 μg/ml of pantoprazole degradation products (. . . . .) using methanol as a blank

spectrophotometry along with the standard addition technique, where the analytical signal due to one of the two drugs is constant at the selected pair of wavelength.

Multivariate calibration spectophotometric methods for the determination of mosapride and pantoprazole along with their degradation products without previous chemical separation are developed as the previously applied bivariate calibrations failed to resolve the sever overlap of mosapride, pantoprazole and their degradation products in a complex mixture (Fig. 2(b)). Multivariate methods (CRACLS and PLS), in which the whole data obtained from UV spectra are used for determination of the drugs and their degradation products, succeeded in the resolution and determination of each component in this multicomponent mixture and can be used as stability indicating method.

2.1. Bivariate calibrations

2.1.1. BSM

This method is based on a simple mathematical algorithm that allows the resolution of the mosapride and pantoprazole binary mixture by measuring the absorbance of the mixtures at the two wavelengths and using the parameters of the linear regression functions evaluated individually for each component at two wavelengths selected using the Kaiser method (Massart et al. 1998). For mosapride (X) and pantoprazole (Y) mixture determination, six different wavelengths (260.0, 270.0, 280.0, 290.0, 300.0, 310.0 nm) were taken. To select the optimum pair of wavelengths, the slope values of the linear regression were estimated for the respective components at the selected six wavelengths and then the obtained regression parameters were used to calculate the sensitivity matrices using the following determinants

$$K = \begin{vmatrix} m_{X1} & m_{Y1} \\ m_{X2} & m_{Y2} \end{vmatrix}$$

Where, $m_{X1,2}$, $m_{Y1,2}$ are the sensitivity parameters of the components X, Y at the two selected wavelengths (1, 2). It was decided to use the value of the linear regression calibration slopes as the sensitivity factor. The determinants of these matrices were calculated, where 270 and 290 nm were the selected pair of wavelengths as it gave the highest matrix determinant value (Table 1).

The regression equations at the two selected wavelengths λ_1 (270.0 nm) and λ_2 (290.0 nm) for a binary mixture of X and Y are represented by:

$$A_{XY1} = m_{X1}C_X + m_{Y1}C_Y + e_{XY1}$$
$$A_{XY2} = m_{X2}C_X + m_{Y2}C_Y + e_{XY2}$$

Table 1: Application of the Kaiser method* for the selection of wavelength set for the mixture of mosapride and pantoprazole

λ_2 / λ_1	260	270	280	290	300	310
260	0	787	620	3326	2344	1285
270		0	2223	6301	4857	1080
280			0	4521	2865	2791
290				0	1756	5715
300					0	4706
310						0

*Massart et al. (1998).
The absolute values of determinations of sensitivity matrices ($K \times 10^{-7}$)

where, e_{XY1}, e_{XY2} are the sum of the intercepts of the linear calibration at two wavelengths ($e_{XY1} = e_{X1} + e_{Y1}$), m_X, m_Y are the slopes of the linear regression. C_X and C_Y are the concentrations of the mosapride and pantoprazole, respectively.

At these two selected wavelengths, the one-component calibration curves were obtained in the range of 5.0–50.0 μg/ml for mosapride, and 10.0–40.0 μg/ml for pantoprazole**.** The linear regression equations parameters were used in the following equations for calculating the concentration of mosapride C_X and pantoprazole C_Y

$$C_X = \frac{A_{XY1} - e_{XY1} - m_{Y1}C_Y}{m_{X1}}$$
$$C_Y = \frac{m_{X2}(A_{XY1} - e_{XY1}) + m_{X1}(e_{XY2} - A_{XY2})}{m_{X2}m_{Y1} - m_{X1}m_{Y2}}$$

where A_{XY1} and A_{XY2} are the measured absorbance at 270.0 and 290.0 nm, respectively, $e_{XY1} = 0.541$, $e_{XY2} = 0.0327$, $m_{X1} = 0.0246$, $m_{X1} = 0.0129$, $m_{Y1} = 0.0219$ and $m_{Y2} = 0.0371$. Those values were calculated using the regression equation parameters (slopes and intercepts) of the four constructed calibration curves at 270.0 and 290.0 nm for both drugs.

2.1.2. HPSAM

Consider an unknown sample mixture containing analyte (X) and interferent (Y). The determination of concentration of (X) by HPSAM under these conditions requires the selection of two wavelengths; λ_1 and λ_2 at which the interferent (Y) has the same absorbance values. Then known amounts of (X) are successively added to the mixture and the resulting absorbances are measured at the two selected wavelengths and expressed by the following equations

$$A_{\lambda 1} = A_{1X} + A_{1Y} + M_{\lambda 1}C_i$$
$$A_{\lambda 2} = A_{2X} + A_{2Y} + M_{\lambda 2}C_i$$

Where, A_{1X} and A_{2X} are the absorbance of X at λ_1 and λ_2, respectively, A_{1Y} and A_{2Y} are the absorbances of Y at the same wavelengths, $M_{\lambda 1}$ and $M_{\lambda 2}$ are the slopes of the standard addition calibration lines obtained on applying the HPSAM to λ_1 and λ_2, respectively, C_i is the added analyte (X) concentration and $A_{\lambda 1}$ and $A_{\lambda 2}$ are the absorbance measured at the two wavelengths for difference points of the HPSAM.

By plotting the analytical signal versus added analyte concentration, two straight lines are obtained that intercept at the so-called H point. At the H-point, since $A_{\lambda 1} = A_{\lambda 2}$, $C_i = -C_H$, so equations for calculating C_i can be obtained

$$A_{1X} + A_{1Y} + M_{\lambda 1}(-C_H) = A_{2X} + A_{2Y} + M_{\lambda 2}(-C_H)$$
$$-C_H = [(A_{2X} - A_{1X}) + (A_{2Y} - A_{1Y})]/(M_{\lambda 1} - M_{\lambda 2})$$
$$C_i = -C_H = -C_x = (A_{2X} - A_{1X})/(M_{\lambda 1} - M_{\lambda 2})$$

The term $M_{\lambda 1} - M_{\lambda 2}$ determines the sensitivity of the method. A great difference between the two slopes can be achieved by choosing two wavelengths sufficiently distant from the maximum of Y.

For the determination of mosapride and pantoprazole by the proposed HPSAM, two pairs of wavelengths were selected; one pair (273.0 and 300.8 nm) used for mosapride determination where pantoprazole gives constant absorbance and the other pair (258.2 and 292.0 nm) used for pantoprazole determination as mosapride gives constant absorbance as shown in Fig. 2(a). The plotted calibration graphs for each drug at its corresponding two

wavelengths are given by the following regression equations

$$A_{\lambda 1} = M_{\lambda 1}C_1 + Y_1 \quad (\text{at}\,\lambda 1)$$

$$A_{\lambda 2} = M_{\lambda 2}C_2 + Y_2 \quad (\text{at}\,\lambda 2)$$

Where, A is the absorbance, M is the slope, C is the concentration and Y is the intercept.

The point at which each pair of calibrations intersect is the H point with a coordinate ($-C_H$, A_H), where C_H is the unknown analyte concentration (mosapride or pantoprazole) and A_H is the analytical signal due to interferent (pantoprazole or mosapride).where C_H and A_H could be calculated by the following equations

$$\mathbf{C_H} = Y_2 - Y_1/(M_{\lambda 1} - M_{\lambda 2})$$

$$\mathbf{A_H} = (M_{\lambda 1}\,Y_2 - M_{\lambda 2}Y_1)/(M_{\lambda 1} - M_{\lambda 2})$$

So, ($\mathbf{C_H}$) the constant amount of analyte in those mixtures can be directly calculated by substituting the measured absorbances at the two selected wavelengths directly in the previously mentioned equation, while the calculated ($\mathbf{A_H}$) can be used for further determination of the ineterferent drug concentration by substitution in an individual regression equation of the intereferent drug at any of the two selected wavelengths.

HPSAM was applied to samples containing different concentrations of mosapride (the analyte) and pantoprazole (as interferent) by standard addition of successive mosapride concentrations, and then plotting the obtained absorbances of different solutions at the selected pairs of wavelengths (273.0 and 300.8 nm) to the corresponding added mosapride concentrations. The concentrations of mosapride (C_H) were calculated directly by solving the two-equation system provided by the HPSAM, while the concentration of pantoprazole was calculated by applying Beer's law using the calculated absorbances (A_H) at any of the selected wavelengths (at 273.0 nm) (Table 2). By analogy, other samples containing different concentrations of pantoprazole (the analyte) and mosapride (as interferent) by standard addition of successive pantoprazole concentrations were analysed taking into consideration that the optimal working wavelengths are 258.2 and 292.0 nm. Pantoprazole concentrations were directly calculated using the parameters provided in the two regression equations, while mosapride concentrations were determined by applying the calculated absorbance at this H point in a previously constructed calibration of pure mosapride at 292.0 nm (Table 2).

2.2. Multivariate calibrations

The developed methods were applied for the analysis of mosapride, pantoprazole and their degradation products. Twenty-five mixtures of four factors five levels were prepared using Brereton Design (Brereton 1997), where seventeen quaternary samples were used as a training (calibration) set and the other eight samples were used as an external validation set (Table 3). The training set was designed to give symmetric and orthogonal distribution of the four components in order to allow accurate determination in different concentrations. Using the random subset selection procedure for cross-validation minimized the over-fitting of the model and elevated its prediction ability than when using leave-one-out cross-validation method. Upon optimization of data handling, it was found that the best results were obtained when the spectra were digitized each at 0.2 nm in the range 230.0–358.0 nm, where 641 experimental points were used in the calculation.

The calculation of the absorptivity from absorbance and concentration is given by:

$A = C\hat{S} + E_A$

where, A is the absorbance set, C is the concentration set, $\hat{S}$ is the estimated absorptivity, and E_A is the error of regression.

In classical least squares (CLS), all the components of the measured sample have to be known, a priority which is not always possible (e.g. measuring a degradation sample, and requires that the spectra and the concentration of degradation products to be known in advance), but in CRACLS this condition is not required.

Table 2: Results of several experiments for the analysis of mosapride and pantoprazole in synthetic samples by HPSAM

Analyte	A-C Equation	R	Present (µg/ml)		Found (µg/ml)		Recovery%	
			Analyte	Interferent	Analyte	Interferent*	Analyte	Interferent
Mosapride (273.0 and 300.8 nm)	$A_{273} = 0.0271\,C_i + 0.6740$	0.9999	20	5	20.16	5.05	100.80	101.00
	$A_{300.8} = 0.0169\,C_i + 0.4684$	0.9998						
	$A_{273} = 0.0271\,C_i + 0.5358$	0.9999	15	5	15.20	4.90	101.33	98.00
	$A_{300.8} = 0.0170\,C_i + 0.3823$	0.9999						
	$A_{273} = 0.0271\,C_i + 0.3996$	0.9998	10	5	10.11	4.98	101.10	99.60
	$A_{300.8} = 0.0170\,C_i + 0.2975$	0.9999						
	$A_{273} = 0.0273\,C_i + 0.2605$	0.9999	5	5	4.97	4.94	99.40	98.80
	$A_{300.8} = 0.0171\,C_i + 0.2098$	0.9999						
	$A_{273} = 0.0272\,C_i + 0.4110$	0.9999	5	10	5.06	10.21	101.20	102.10
	$A_{300.8} = 0.0172\,C_i + 0.3604$	0.9998						
	$A_{273} = 0.0270\,C_i + 0.6746$	0.9998	5	20	4.98	19.71	99.60	98.55
Pantoprazole (258.2 and 292.0 nm)	$A_{300.8} = 0.0170\,C_i + 0.6248$	0.9995						
	$A_{258.2} = 0.0367\,C_i + 0.6603$	0.9999	15	5	15.13	4.95	100.87	99.00
	$A_{292} = 0.0175\,C_i + 0.3698$	0.9998						
	$A_{258.2} = 0.0366\,C_i + 0.4761$	0.9998	10	5	10.11	5.02	101.10	100.40
	$A_{292} = 0.0176\,C_i + 0.2840$	0.9998						
	$A_{258.2} = 0.0368\,C_i + 0.2891$	0.9998	5	5	4.95	5.10	99.00	102.00
	$A_{292} = 0.0177\,C_i + 0.1946$	0.9999						
	$A_{258.2} = 0.0367\,C_i + 0.3502$	0.9998	5	10	5.07	9.87	101.40	98.70
	$A_{292} = 0.0180\,C_i + 0.2555$	0.9998						
	$A_{258.2} = 0.0366\,C_i + 0.4908$	0.9998	5	20	5.10	20.02	102.00	100.10
	$A_{292} = 0.0179\,C_i + 0.3955$	0.9998						

*calculated from individual calibration curves of mosapride at 292.0 nm (A = 0.0132C + 0.0397) and pantoprazole at 273.0 nm (A = 0.0281C - 0.0138)

Table 3: Concentration of mosapride, pantoprazole and their degradation products in the training and validation sets for the multivariate calibrations

Mixture number	Concentrations in calibration (training) set (μg/ml)				Concentrations in validation set (μg/ml)			
	Mosapride	Pantoprazole	Mosapride degradation products	Pantoprazole degradation products	Mosapride	Pantoprazole	Mosapride degradation products	Pantoprazole degradation products
1	7.500	20.000	3.750	5.000	7.500	8.000	1.500	8.000
2	3.000	8.000	6.000	3.500	3.000	32.000	2.625	8.000
3	12.000	14.000	6.000	5.000	5.250	32.000	3.750	3.500
4	12.000	20.000	2.625	3.500	5.250	14.000	4.875	8.000
5	7.500	14.000	2.625	6.500	5.250	26.000	6.000	6.500
6	9.750	32.000	4.875	5.000	12.000	8.000	4.875	2.000
7	7.500	32.000	6.000	2.000	7.500	26.000	3.750	8.000
8	12.000	32.000	1.500	6.500	12.000	20.000	6.000	8.000
9	3.000	26.000	1.500	5.000	-------	-------	-------	-------
10	9.750	8.000	3.750	6.500	-------	-------	-------	-------
11	3.000	20.000	4.875	6.500	-------	-------	-------	-------
12	7.500	26.000	4.875	3.500	-------	-------	-------	-------
13	9.750	26.000	2.625	2.000	-------	-------	-------	-------
14	9.750	14.000	1.500	3.500	-------	-------	-------	-------
15	5.250	8.000	2.625	5.000	-------	-------	-------	-------
16	3.000	14.000	3.750	2.000	-------	-------	-------	-------
17	5.250	20.000	1.500	2.000	-------	-------	-------	-------

2.2.1. CRACLS

Unlike CLS, CRACLS is an alternative new method that estimates absorptivity ($\hat{S}$) by a process of repetitive approximation as shown in the following steps

Step 1: $\hat{S} = (C'C)^{-1}\ C'A$

Step 2: $\hat{S}$ is used to predict C': $C' = A\hat{S}'\ (\hat{S}\hat{S}')^{-1}$

Step 3: Error in C': E = C' – C

Step 4: One vector of E is augmented to the original C (E is considered as a new component).

Step 5: Step (1) is repeated using the augmented C until no further improvement in prediction is achieved.

CRACLS model was built for mosapride, pantoprazole and their degradation products, where the estimated pure components spectra that resulted by including mosapride, pantoprazole and their degradation products concentrations and augmenting 17 times.

CRACLS succeeded in estimating the pure spectral profiles of all the four components, the resemblance was observed with the actually scanned UV-spectra (Fig. 3), for mosapride, pantoprazole and their degradation products, even for mosapride degradation products in the range 230.0–300.0 nm which has no spectral characteristics.

2.2.2. PLS

In order to construct PLS calibration, the correct number of latent variables to be used modeling of the data must be determined; a cross-validation with random subset selection procedures (Haaland and Thomas 1988a) was performed for all the samples in the training set. The seventeen calibration samples were randomly divided into five sets; four sets were used for building the model while the fifth was predicted by the model. This procedure was iterated five times, and the average root mean squares error of cross-validation (RMSECV) was calculated. The RMSECV was calculated in the same manner each time a new latent variable was added to the model. This method involves the comparison between RMSECV of all models with that of the model yielding the minimum RMSECV (V*), and the selection of model with the smallest number of variables, such that the RMSECV for the selected model was not significantly greater than that from the model yielding V* (Haaland and Thomas 1988b; Melgaard et al. 2002). Five latent variables were found optimum for the mean centered data.

To test the predictive ability of the developed CRACLS and PLS models, they were challenged with the spectra of the validation set. The predicted concentrations of each component of the validation set were plotted against the true concentrations and the root mean square error of prediction (RMSEP) was calculated. The RMSEP was used as a diagnostic tool for examining the prediction errors, it indicates both accuracy and precision (World 1995). The RMSEP of mosapride was 0.10877 and 0.22553 for CRACLS and PLS methods, respectively, for pantoprazole 0.19695 and 0.21043. The RMSEP of mosapride degradation products determination was 0.06236 and 0.10107 by CRACLS and PLS, respectively, while for pantoprazole degradation products determination was 0.08734 and 0.16400. From the obtained RMSEP values, CRACLS got the lowest errors in prediction with respect to PLS for the analysis of this ternary mixture.

Linear correlations were obtained for all the proposed methods (Table 4), linearity of the bivariate calibrations were obtained between the absorbance, of the ^0D absorption spectra, and the corresponding concentrations (BSM) or the added concentrations (HPSAM) of the drugs. While in multivariate calibrations, linear correlations were obtained between the predicted and the original concentrations of both drugs and their degradation products. The concentration ranges and regression parameters are shown in Table 4. The same table shows the mean recoveries and RSD values of their laboratory prepared mixtures and dosage form analysis. Moreover, LOD and LOQ were also calculated to assist the validity of the proposed methods according to ICH guidelines (ICH 1996).

The results obtained by applying the proposed bivariate and multivariate calibration for the determination of mosapride and pantoprazole in bulk powder were statistically compared with the reported spectrophotometric method (Bhatt et al. 2009). There is no significant difference between the proposed and reported methods regarding both accuracy and precision (Table 5) as the calculated t-values and F-values were less than the theoretical ones, except for mosapride determination by PLS, where the calculated F-value was higher than the theoretical one,

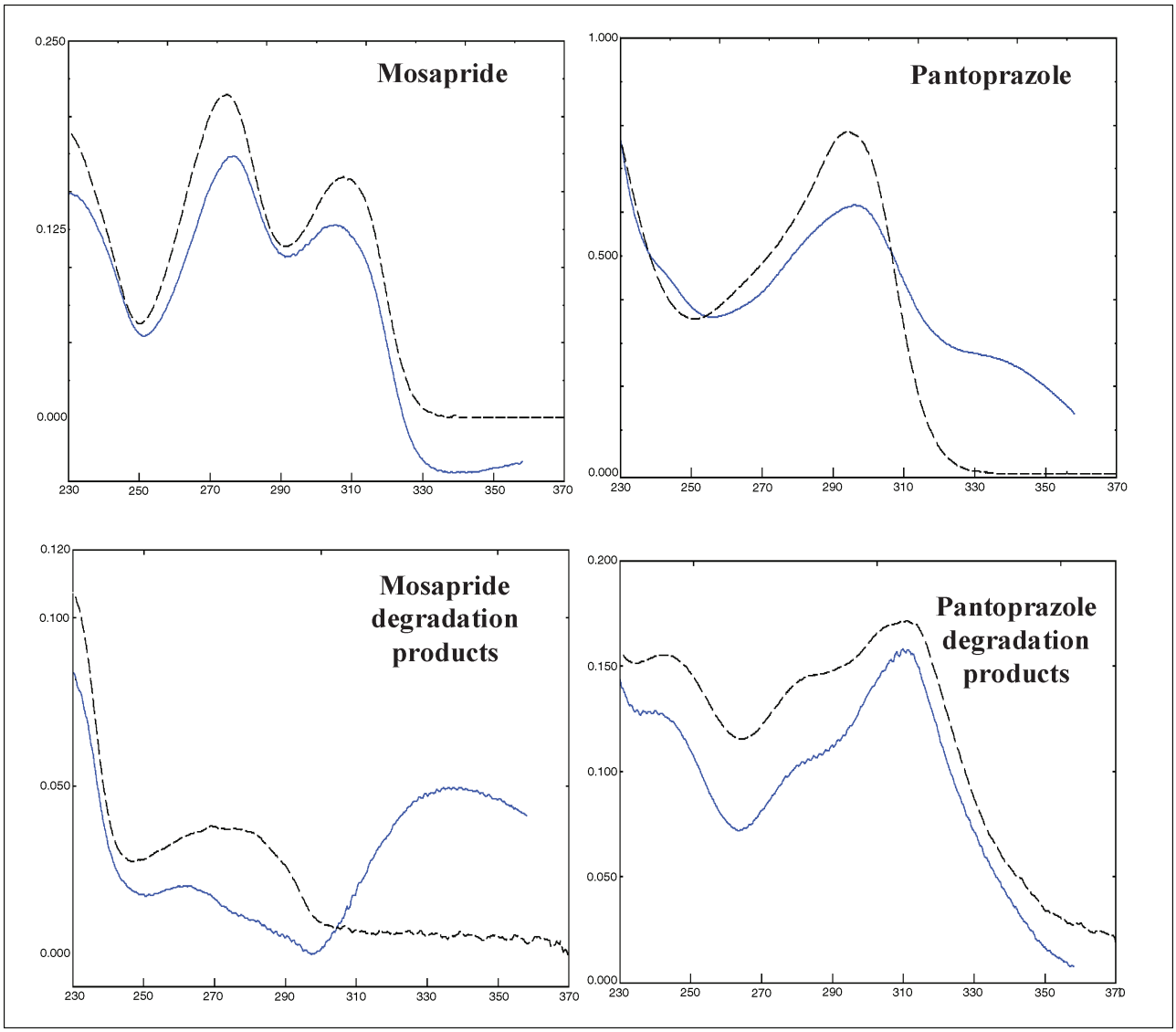

Fig. 3: Zero order absorption spectra of the four components (-----) and their estimated spectra by CRACLS method (------)

so further method of variance comparison which is ANOVA (Analysis of variance) was applied and revealed that there was no significant difference between the two methods' means as the calculated F-value (1.032) was less than the critical one (4.844).

The suggested methods for the determination of mosapride and pantoprazole in laboratory prepared mixtures were compared statistically using one way ANOVA. The calculated F-values for mosapride and pantoprazole determinations were less than the tabulated one (Table 6). So there was no significant difference between the proposed methods for the determination of both drugs.

Finally, the proposed bivariate calibrations methods are simple, accurate and precise for the resolution and determination of mosapride and pantoprazole binary mixtures. On the other hand, the proposed new multivariate calibration (CRACLS) is accurate, precise and highly specific in determination of mosapride, pantoprazole and their degradation products compared to conventional PLS. So, we can conclude that bivariate methods are easily applicable in quality control laboratories for the determination of mosapride and pantoprazole, beside the application of CRACLS multivariate calibration as a stability indicating method for the determination of both drugs in their pure bulk powder, dosage form and in quality control laboratories.

3. Experimental

3.1. *Instruments and software*

A dual beam Shimadzu (Kyoto/Japan) UV-Vis. spectrophotometer, model UV-1601 PC connected to IBM compatible with an Hp 600 inkjet printer. The bundle software, UV PC personal spectroscopy software version 3.7 (SHIMADZU) was used to process absorption and derivative spectra, the spectral band width was 2 nm and scanning speed was 2800 nm/min.

Matlab® (Matlab 2004) for Windows™ version 7.0.1 Mathwork Inc. 2004 was used in calculating multivariate calibrations, for CRACLS, all computation were performed by MATLAB® with previously designed codes (Shehata et al. 2004). The PLS procedure was taken from PLS Toolbox 2.1 Eigenvector Research, Inc.2005 created by B.M. Wise and N.B. Gallagher for use with Matlab®.

3.2. *Materials and reagents*

Pure standards of mosapride citrate and pantoprazole sodium were kindly supplied by Wester Pharmaceutical Industries, Cairo, Egypt, their purity were found to be 99.97 ± 0.625% for mosapride citrate and 99.75 ± 0.974% for pantoprazole sodium, according to a reported spectrophotometric method (Bhatt et al. 2009).

Degradation products samples of mosapride citrate and pantoprazole sodium were prepared according to a reported method (Hegazy et al. 2011a) by weighing and transferring 50.0 mg of each drug bulk powder into two separate 50-ml volumetric flasks, then dissolved in 20 ml of 2.0 M hydrochloric acid and finally the volumes were completed with methanol. Portions of each solution were transferred into 10-ml ampoules sealed and heated in a dry oven for 4 hrs at 120 °C, complete degradation was assessed by disappearance of the intact spot in TLC or its peak in HPLC.

Table 4: Regression of the proposed methods for the determination of mosapride and pantoprazole by bivariate calibrations and the determination of drugs and their degradation products by multivariate calibrations in the validation set

Statistical Parameter	Bivariate calibrations								Mutivariate calibrations							
	BSM				HPSAM				CRACLS				PLS			
	Mosapride		Pantoprazole		Mosapride		Pantoprazole		Mosapride		Pantoprazole		Mosapride		Pantoprazole	
	270.0 nm	290.0 nm	270.0 nm	290.0 nm	273.0 nm	300.8 nm	258.2 nm	292.0 nm	Intact	Degradation products	Intact	Degradation products	Intact	Degradation products	Intact	Degradation products
Linearity Range (μg/ml)	5–50	5–50	10–40	10–40	5–45	5–45	5–45	5–45	3–12	1.5–6	8–32	2–8	3–12	1.5–6	8–32	2–8
Slope	0.0246	0.1419	0.0081	0.018	0.027	0.017	0.0177	0.0368	1.0042	1.0397	0.986	1.0179	0.9713	1.0735	1.0025	0.9379
Intercept	0.0143	−0.0176	0.0293	0.0056	0.6746	0.6248	0.1946	0.2891	0.0163	-0.0768	0.0328	-0.0858	0.3142	-0.2247	-0.0538	0.2618
SE of slope	0.00014	0.00078	0.00006	0.00008	0.00021	0.00022	0.00010	0.00027	0.01472	0.01786	0.00860	0.01613	0.02810	0.02990	0.00933	0.02791
SE of intercept	0.00401	0.03110	0.00177	0.00175	0.00539	0.00560	0.00254	0.00690	0.11994	0.07909	0.19531	0.11095	0.22897	0.13236	0.21177	0.19197
Correlation coefficient(r)	0.9999	0.9999	0.9999	0.9999	0.9998	0.9995	0.9999	0.9998	0.9994	0.9991	0.9998	0.9992	0.9975	0.9977	0.9998	0.9974
Accuracy (Mean ± RSD)	100.33 ± 0.706		100.71 ± 1.093		99.72 ± 0.833		99.45 ± 1.614		100.76 ± 1.707	101.78 ± 2.019	98.84 ± 0.981	100.17 ± 1.938	102.45 ± 5.206	101.09 ± 4.433	99.90 ± 2.027	98.80 ± 3.759
Specificity (Mean ± RSD)	99.35 ± 1.043		100.69 ± 1.010		99.91 ± 1.142		100.91 ± 0.597		-----	-----	-----	-----	-----	-----	-----	-----
Moza plus® capsules (Mean ± RSD)	99.59 ± 0.793		100.79 ± 1.282		98.88 ± 0.429		100.58 ± 1.046		100.11 ± 0.982	-----	100.43 ± 1.514	-----	-----	-----		-----
LOD (μg/ml)	0.69	0.97	0.52	0.36	0.78	1.17	0.56	0.74	0.41	0.22	0.69	0.30	0.79	0.36	0.74	0.58
LOQ (μg/ml)	2.09	2.94	1.59	1.08	2.37	3.91	1.71	2.23	1.23	0.67	2.11	0.93	2.40	1.08	2.24	1.75

Table 5: Statistical analysis of the results obtained by applying the proposed bivariate and mutivariate calibrations and the reported* method for the determination of mosapride and pantoprazole

Value	BSM		Multivariate calibrations						Reported method*	
	BSM		HPSAM		CRACLS		PLS			
	Mosapride	Pantoprazole	Mosapride	Pantoprazole	Mosapride	Pantoprazole	Mosapride	Pantoprazole	Mosapride	Pantoprazole
Mean	100.33	100.71	99.72	99.45	100.65	98.84	102.45	99.90	99.97	99.75
SD	0.708	1.101	0.831	1.605	1.481	0.969	5.334	2.025	0.625	0.972
RSD	0.706	1.093	0.833	1.614	1.471	0.981	5.206	2.027	0.625	0.974
n	6	5	5	5	8	8	8	8	5	5
Variance	0.501	1.212	0.691	2.576	2.193	0.939	28.452	4.101	0.391	0.945
Student's t test	0.895 (2.262)**	1.462 (2.306)**	0.537 (2.306)**	0.357 (2.306)**	1.180 (2.201)**	1.644 (2.201)**	1.301 (2.201)**	1.179 (2.201)**	------	------
F value	1.281 (6.26)**	1.283 (6.39)**	1.767 (6.39)**	2.726 (6.39)**	5.61 (6.09)**	0.99 (6.09)**	72.77*** (6.09)**	4.34 (6.09)**	------	------

* Bhatt et al. 2009; First derivative spectrophotometry at 252.1 nm for mosapride and 302.4 nm for pantoprazole in acetonitrile.
** The values in the parenthesis are the corresponding theoretical values of t and F at (P = 0.05)
*** ANOVA was applied for further means comparison and revealed that there is no significant difference between the methods' mean

Pharmaceutical dosage form, Moza plus® hard gelatin Capsules were manufactured by Intas Pharmaceutical LTD, Mumbai, India, batch No. L090027 and are labeled to contain 15 mg mosapride citrate (as sustained release) and 40 mg Pantoprazole (delayed release) per tablet.
Chemicals and reagents, all chemicals used throughout the work were of analytical grade and solvents were of spectroscopic grade: Methanol, Acetonitrile, Hydrochloric acid; 2.0 M aqueous solution (Merck, Darmstadt, Germany), Ammonia solution 33% (Adwic, Cairo, Egypt).

3.3. Solutions

3.3.1. Stock standard solutions

Stock standard solutions of mosapride and pantoprazole (1.0 mg/ml) were prepared by weighing accurately 50.0 mg of mosapride and pantoprazole powder into two separate 50-ml volumetric flasks; 25.0 ml methanol was added in each, shaken for a few minutes, and diluted to the volume with methanol.

Table 6: One-Way ANOVA of the results obtained from the laboratory prepared mixtures analysis by the bivariate and multivariate calibrations

One-Way ANOVA of mosapride determination
Summary Statistics

Method	N	Mean	SD	SE
BSM	7	99.35429	1.03573	0.39147
HPSAM	7	99.91286	1.14005	0.4309
CRACLS	8	101.7775	2.05491	0.72652
PLS	8	100.96625	4.34834	1.53737

Null Hypothesis: The means of all selected datasets are equal
Alternative Hypothesis: The means of one or more selected datasets are different
ANOVA

Source	Degree of freedom	Sum of squares	Mean square	F value	P value
Model	3	26.2865349	8.76217829	1.29331	0.29767
Error	26	176.149452	6.77497891		

At the 0.05 level,
the population means are not significantly different.

One-Way ANOVA of pantoprazole determination
Summary Statistics

Method	N	Mean	SD	SE
BSM	7	100.68571	1.01785	0.38471
HPSAM	7	100.77143	0.6795	0.25683
CRACLS	8	100.17125	1.9426	0.68681
PLS	8	98.8	3.71315	1.3128

Null Hypothesis: The means of all selected datasets are equal
Alternative Hypothesis: The means of one or more selected datasets are different
ANOVA

Source	Degree of freedom	Sum of squares	Mean square	F value	P value
Model	3	19.0823920	6.36079734	1.25370	0.31073
Error	26	131.914545	5.07363633		

At the 0.05 level,
the population means are not significantly different.

Stock solutions of mosapride and pantoprazole drgradation products (1.0 mg/ml) were prepared by weighing accurately 50.0 mg of mosapride and pantoprazole powder were separately and accurately weighed and dissolved in 50.0 ml 2 M hydrochloric acid, then transferred to ampoules, sealed and placed in an oven at 120 °C for 4 h.

3.3.2. Working solutions

Working solutions for bivariate calibrations methods (100.0 μg/ml) were prepared by transferring 2.5 ml of the drugs stock standard solution (1.0 mg/ml), separately, into two 25-ml measuring flasks and the volume was completed to the mark with methanol.
Mosapride working standard solution (75.0 μg/ml): Prepared by transferring 7.5 ml of the prepared mosapride stock standard solution into 100-ml volumetric flask and the volume was completed with methanol.
Pantoprazole working standard solution (200.0 μg/ml): Prepared by transferring 20.0 ml of the prepared pantoprazole stock standard solution into 100-ml volumetric flask and the volume was completed with methanol.
Working solution of mosapride degradation products (37.5 μg/ml): Prepared by transferring 7.5 ml of the prepared degradated mosapride stock standard solution into 100-ml volumetric flask and the volume was completed with methanol, then further dilution was made by transferring 50.0 ml of the previous solution into 100-ml volumetric flask and the volume was completed with methanol.
Working solution of pantoprazole degradation products (50.0 μg/ml): Prepared by transferring 5.0 ml of the prepared degradated pantoprazole stock standard solution into 100-ml volumetric flask and the volume was completed with methanol.

3.4. Procedures

3.4.1. BSM

Aliquots equivalent to 50.0–500.0 μg of mosapride and 100.0–400.0 μg of pantoprazole were accurately transferred from their corresponding working standard solution (100.0 μg/ml) into two separate sets of 10-ml volumetric flasks and the volumes were completed to the mark with methanol. ^{0}D absorption spectrum of each solution was recorded at the range 200.0–350.0 nm against methanol as a blank. Four calibration curves were constructed relating the absorbance, at 270.0 and 290.0 nm for mosapride and pantoprazole, to the corresponding drug concentrations.
Solutions containing different ratios of mosapride and pantoprazole in methanol were prepared from their respective working solutions and diluted with methanol. The absorption spectra of the laboratory-prepared mixtures were scanned, the absorbance at 270.0 and 290.0 nm were recorded and then the concentrations of mosapride and pantoprazole in each mixture were calculated using the specified parameters of regression equations.

3.4.2. HPSAM

Synthetic samples were prepared by transferring aliquots equivalent to 50.0 μg of mosapride and 50.0 μg of pantoprazole into a set of 10-ml volumetric flasks from their respective working solutions (100 μg/ml). A standard addition of different aliquots of mosapride in the range of 50.0–400.0 μg was added to the previously prepared synthetic samples and the volumes were completed to the mark with methanol. The zero order absorption spectra (^{0}D) of the laboratory prepared mixtures were recorded against methanol as a blank. The absorbance at the selected working pair of wavelength 273.0 and 300.8 nm were measured, then plotted against the corresponding added mosapride concentrations and the regression parameters at the two selected wavelengths were calculated. Similarly, for pantoprazole, while a standard addition of different aliquots of pantoprazole in the range of 50.0–400.0 μg were done to these previously prepared synthetic samples (50.0 μg of mosapride and 50.0 μg of pantoprazole) and the volumes were completed to the mark with methanol. The zero order absorption spectra (^{0}D) of the laboratory prepared mixtures were recorded against methanol as a blank. The absorbance at the other selected working pair of wavelength 258.2 and 292.0 nm, for pantoprazole determination, were measured, then plotted against the corresponding added pantoprazole concentrations and the regression parameters at the two selected wavelengths were calculated. Four calibration curves were constructed relating the absorbance, at 273.0 and 300.8 nm for mosapride and at 2548.2 and 292.0 nm for pantoprazole, to the corresponding added drug concentrations.
Solutions containing different ratios of mosapride and pantoprazole in methanol were prepared from their respective working solutions and diluted with methanol. Standard addition of different aliquots of the drug used as analyte was done in the range of 50.0–400.0 μg to the previously prepared laboratory mixtures and the volumes were completed to the mark with methanol. The absorption spectra of these samples were scanned, processed as under calibration and the concentration of mosapride and pantoprazole were calculated using the specified regression parameters at each pair of corresponding wavelengths.

3.4.3. Multivariate calibration methods (CRACLS& PLS)

The calibration (training) set was designed with 17 synthetic mixtures of different concentration ratios from mosapride, pantoprazole and their degradation products, containing mosapride in the range of 3.0–12.0 μg/ml, pantoprazole in the range of 8.0–32.0 μg/ml, mosapride degradation products in the range of 1.5–6.0 μg/ml and pantoprazole degradation products in the range of 2.0–8.0 μg/ml. The solutions were prepared by mixing different aliquots of drugs and their degradation products working solutions in 10-ml volumetric flasks, and then the volumes were completed with methanol. The UV-spectra of the prepared solutions were recorded over the range 230.0–358.0 nm. The data points of spectra were transferred to Matlab® for subsequent data analysis and multivariate calibration models were constructed. All the spectral data were mean centered before calibration to construct CRACLS and PLS models
The validation set, made up of 8 samples of solution mixtures, was prepared. Aliquots of mosapride, pantoprazole and their degradation products working solutions were mixed in 10-ml volumetric flasks, and then the volumes were completed with methanol. The developed models were then used for determination of each drug along with its degradation products in an external validation set.

3.5. Assay of pharmaceutical formulation

Five capsules of Moza plus® were evacuated and the contents was finely powdered. An amount of the powdered capsules equivalent to 75 mg of mosapride citarate and 200 mg of pantoprazole was accurately weighed and transferred into 100-ml beakers, sonicated in 30 ml methanol for 10 min and filtered into a 100-ml volumetric flask. The residue was washed three times each using 10 ml methanol and the solution was completed to the mark with the same solvent. Aliquots of 0.1 ml were transferred from the prepared solutions to 10-ml volumetric flasks and diluted with methanol. The general procedures previously described under each method were followed to determine the concentration of mosapride and pantoprazole in the prepared dosage form solutions.

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