Research article

Drug Testing and Analysis

Received: 13 October 2010 Revised: 20 November 2010 Accepted: 23 November 2010 Published online in Wiley Online Library: 21 February 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.246

Stability-indicating methods for the determination of mosapride citrate in the presence of its degradation products according to ICH guidelines

Maha A. Hegazy,* Ali M. Yehia and Azza A. Mostafa

In the present work, different spectrophotometric methods and one spectrofluorimetric method have been developed and validated for the determination of mosapride citrate in the presence of its acid-induced degradation products. The drug was subjected to stress stability study including acid, alkali, oxidative, photolytic, and thermal stress degradation. The developed spectrophotometric methods included the use of first order derivative (^{1}D), derivative of ratio spectra (^{1}DD), mean centring of ratio spectra (MC) and H-point standard additions (HPSAM) spectrophotometric methods. For ^{1}D method, the peaks amplitudes at 282.8 and 319.6 nm were measured, while for ^{1}DD method those at 308 nm and 323 nm were measured. Mean centring of ratio spectra method used the values at 317 nm for calibration, while for HPSAM the absorbance at 273 and 288.6 nm were used. These methods were successfully applied for determination of mosapride in the concentration range of 5–70 μg.ml^{-1}. The spectrofluorimetric method was based on measuring the native fluorescence of mosapride in 0.1 M NaOH using $\lambda_{excitation}$ 276 nm and $\lambda_{emission}$ 344 nm and 684 nm with linearity ranges of 50–3000 ng.ml^{-1} and 50–9000 ng.ml^{-1}, respectively. All the developed methods were validated according to the International Conference on Harmonization (ICH) guidelines and were applied for bulk powder and dosage form. The results obtained were statistically compared to each other using one-way ANOVA testing.

Keywords: mosapride; mosapride degradation products; spectrophotometry; spectrofluorimetry

Introduction

Mosapride citrate is chemically designed as 4-amino-5-chloro-2-ethoxy-N-{[4-(4-fluorobenzyl)morpholin-2-yl]methyl} benzamide.[1] It is a potent gastroprokinetic agent with selectivity for 5-HT4 receptor, used in the treatment of gastrointestinal motility dysfunction associated with non-ulcer dyspepsia,[2,3] it is not like other gastroprokinetic agents, especially metoclopramide, which block dopamine D2 receptors causing several adverse effects, such as central nervous system depression and extra pyramidal syndrome in man.[4–6] In addition, the pharmacological profile of mosapride is different from that of cisapride and metoclopramide. Unlike cisapride it does not stimulate motor activity.[7] Reviewing the literature in hand, there are no reported stability studies on mosapride citrate. Only the intact drug was determined in its bulk powder and in pharmaceutical formulations by HPLC,[8,9] spectrophotometry,[10] spectrofluorimetry,[11] electrochemical methods,[12] purity studies,[13,14] enantiomeric separation,[15–17] determination in biological tissues,[18–20] and separation of mosapride and rabeprazole in their binary mixture by HPLC and TLC.[21]

The aim of this work was to develop new, accurate, simple, sensitive, and rapid stability-indicating spectrophotometric and spectrofluorimetric methods for the determination of mosapride citrate in the presence of its acid degradation products with satisfactory statistical validation measures.

Experimental

Instrumentation

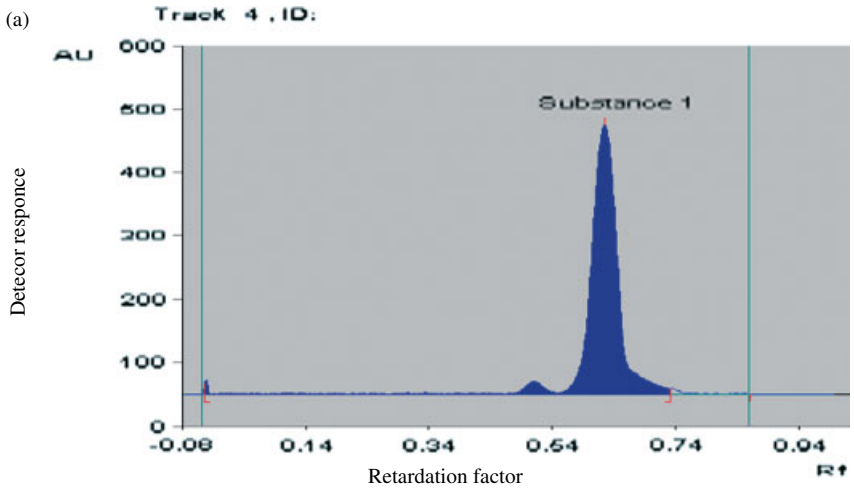
A dual beam Shimadzu (Kyoto, Japan) UV-Vis. spectrophotometer, model UV-1601 PC connected to IBM compatible with an Hp 600 inkjet printer. The bundle software, UV PC personal spectroscopy software version 3.7 (Shimadzu, Kyoto, Japan) was used to process absorption and derivative spectra, the spectral band width was 2 nm and scanning speed was 2800 nm.min^{-1}. MATLAB® 6.5[22] was used for subsequent data manipulation.

TLC system consists of: Camag Linomat autosampler (Sonnen matt strasse 11 CH-4132 Muttenzl Switzerland), Camag micro syringe (100 μl) and Camag TLC scanner 35/N/30319 with winCATS software. UV lamp-short wavelength at 254 nm (Desaga, Wiesloch, Germany). Thin-layer chromatographic plates precoated with silica gel G.F_{254} 20 × 20 cm, 0.25 mm thickness (E.Merck, Darmstadt, Germany).

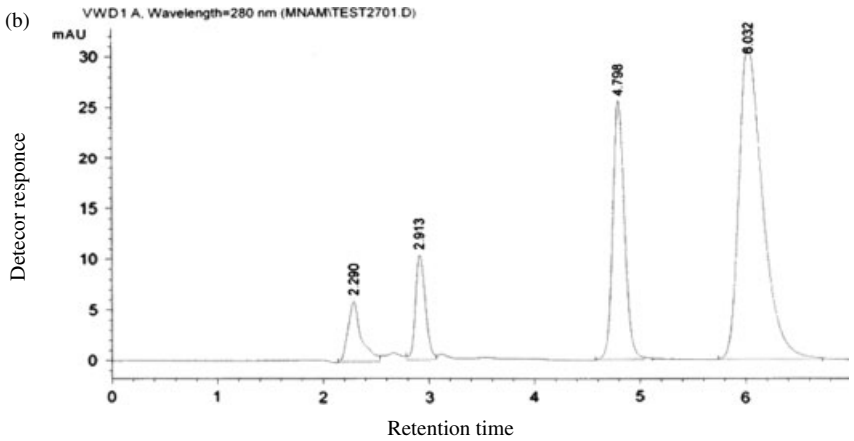
HPLC system consists of: Agilent pump with different flow rates (model 1100 series, Agilent, Germany), equipped with a variable

* *Correspondence to: Maha A. Hegazy, Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, El-Kasr El-Aini St. 11562, Cairo, Egypt. E-mail: mahahgazy@yahoo.com*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, El-Kasr El-Aini St. 11562, Cairo, Egypt



TLC chromatogram of Mosapride (R_f=0.63) and acid degradation products (R_f=0.0 and 0.50) using mobile phase of chloroform-methanol-toluene (5:5:2 v/v/v) and detection at 308 nm.



HPLC chromatogram of Mosapride (t_R=6.03) and acid degradation products (t_R=2.91 and 4.80) using zorbax ODS (5µm, 250mm x 4.6mm i.d.) column, mobile phase of acetonitrile - methanol - phosphate buffer pH 6 (5:2:3 v/v/v), flow rate 1 ml/min and detection at 280 nm.

Figure 1. TLC and HPLC chromatograms of mosapride and its degradation products.

wavelength detector and a 20 µl injection loop. Zorbax ODS (5 µm, 250 mm × 4.6 mm i.d.) column was used as stationary phase. The samples were injected with 50 µl Hamilton analytical syringe.

WPA pH combined electrode model C D 740 was used for pH measurements.

Materials

Pure standard

Mosapride citrate was kindly supplied by Wester Pharmaceutical Industries, Cairo, Egypt. Its purity was found to be 100.06 ± 1.459% according to a reported HPLC method (8).

Pharmaceutical dosage form

Mosapride® 2.5 mg (Wester Pharmaceutical Industries, Cairo, Egypt) Batch No. 08122, labelled to contain 2.5 mg mosapride citrate per tablet.

Mosapride® 5.0 mg (Wester Pharmaceutical Industries, Cairo, Egypt) Batch No. 08061, labelled to contain 5 mg mosapride citrate per tablet.

Degradation products sample

50 mg of mosapride citrate bulk powder was accurately weighed, dissolved in 50 ml of 2 M aqueous hydrochloric acid, then 10-ml portions of the solution were transferred into 10-ml ampoules sealed and heated for 4 h at 120 °C.

Chemicals and reagents

All chemicals used throughout the work were of analytical grade and solvents were of spectroscopic and HPLC grade.

1. Methanol Adwic-ElNasr, 1st Industrial zone (Merck, Germany)
2. Acetonitrile (Merck, Germany)
3. Hydrochloric acid (E. Merck, Darmstadt, Germany); 2M aqueous solution
4. Sodium hydroxide (BDH); 0.1M and 0.2M aqueous solutions
5. Toluene (R.P. NORMARAPUR AR Adwic, Egypt)
6. Monobasic potassium phosphate: Riedel-deHaen (Sigma-Aldrich Germany)
7. Phosphate buffer solution pH 6.0[23]
   28.5 ml of 0.2 M NaOH was added to 250 ml of 0.2 M KH_2PO_4 then diluted to 1000 ml with double distilled water.

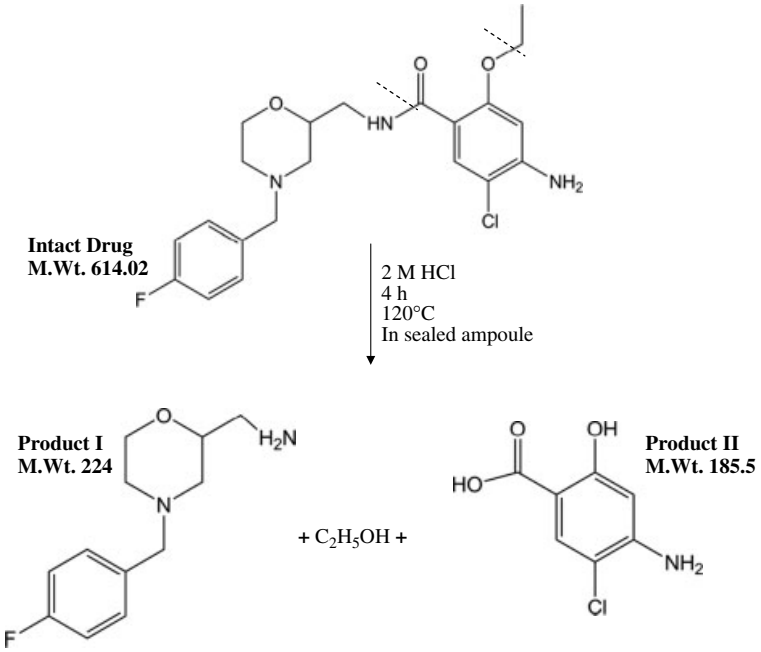


Figure 2. Suggested scheme for the acid hydrolysis of mosapride to its main degradation products.

8. Chloroform (Egypt-Adwic-ElNasr pharmaceutical chemical company- Obour City-1st Industrial zone)
9. Double distilled deionized water

Solutions

Stock solutions

- Mosapride citrate stock standard solution (1.0 mg.ml^{-1}) was prepared by accurately weighing 50 mg of mosapride bulk powder into 50-ml volumetric flask; 25 ml methanol was added, shaken for a few min. and diluted to the volume with methanol.
- Mosapride degradation products stock solution (equivalent to 1.0 mg.ml^{-1} mosapride) was prepared as mentioned above.

Working solutions for spectrofluorimetric method

Mosapride and its degradation products working solutions (10 μg.ml^{-1}) were prepared by transferring 1 ml aliquot from their stock solutions (1.0 mg.ml^{-1}) into two separate 100-ml measuring flasks then the volumes were completed to the mark with 0.1M NaOH.

Laboratory-prepared mixtures

Solutions containing different ratios of mosapride and its degradation products were prepared by transferring aliquots from their stock standard solutions (1.0 mg.ml^{-1}) or from their working solutions (10 μg.ml^{-1}) into a series of 10-ml volumetric flasks and the volume of each was completed to the mark with either methanol or 0.1 M NaOH for spectrophotometry and spectrofluorimetry, respectively.

Procedures

Construction of the calibration curves

For ^1D spectrophotometric method. Aliquots equivalent to 50–700 μg of mosapride were accurately measured and transferred from its standard stock solution (1.0 mg.ml^{-1}) into a set of 10-ml volumetric flasks and the volumes were completed to the mark with methanol. The zero order (^0D) absorption spectrum of each solution was recorded against methanol as a blank, then the first derivative (^1D) spectra were computed using scaling factor = 10 and $\Delta\lambda$ = 4 nm. The peaks amplitudes at 282.8 and 319.6 nm were recorded, plotted each against its corresponding concentration, and the regression parameters were computed.

For ^1DD spectrophotometric method. The scanned zero order (^0D) absorption spectra of mosapride (5–70 μg.ml^{-1}) were divided by a standard spectrum of its degradation products (50 μg.ml^{-1}). The first derivative of the obtained ratio spectra (^1DD) were computed using scaling factor=1 and $\Delta\lambda$ = 4 nm. Two calibration curves were constructed relating the peak amplitudes of the ^1DD spectra at 308 and 323 nm to the corresponding drug concentrations and the regression parameters were computed.

For mean centring of ratio spectra (MC) spectrophotometric method. The scanned zero order (^0D) absorption spectra mosapride (5–70 μg.ml^{-1}) in the range 200–350 nm and a standard spectrum of the degradation products (50 μg.ml^{-1}) were transferred to MATLAB® for subsequent calculation. Each spectrum of the drug was divided by the standard spectrum of the degradation products, the obtained ratio spectra were mean centred with respect to wavelength. The new mean centred ratio spectra having a maximum value at 317 nm, these values were recorded, plotted each against its corresponding concentration, and the regression parameters were computed.

For HPSAM spectrophotometric method. Synthetic samples were prepared into a set of 10-ml volumetric flasks, in which each sample is a mixture of mosapride and its degradation products in the concentration of 100 μg.ml^{-1} and 200 μg.ml^{-1}, respectively. Aliquots from mosapride equivalent to 100–600 μg.ml^{-1} were accurately transferred and added to the prepared synthetic samples

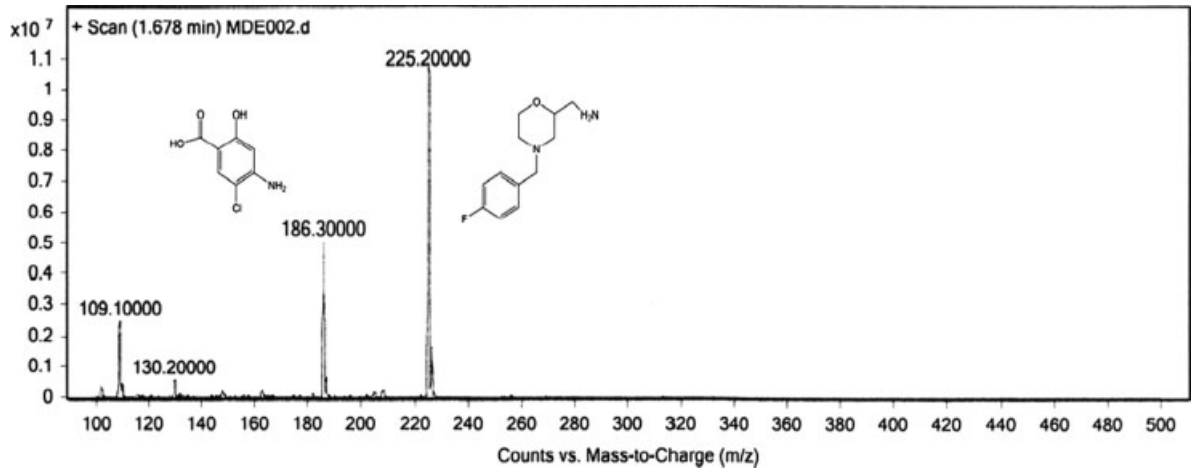


Figure 3. LC-MS of mosapride degradation products.

and the volumes were completed to the mark with methanol. The zero order (^{0}D) absorption spectra of the synthetic mixtures were recorded against methanol as a blank. The absorbance at the selected pair of wavelengths (273and 288.6 nm) was measured, plotted against the corresponding concentrations of the added mosapride, and the regression parameters were computed.

For spectrofluorimetry method. Aliquots from mosapride working solution (10 μg.ml^{-1}) equivalent to 0.5–90 μg were accurately measured and transferred into a set of 10-ml volumetric flasks and the volumes were completed with 0.1 M NaOH. The emission spectra of these solutions were scanned upon excitation at 276 nm ($\lambda_{excitation}$) and the fluorescence intensity (I_f) at 344 nm and 684 nm were recorded ($\lambda_{emission}$), plotted against the corresponding concentration and the regression parameters were calculated.

Assay of laboratory-prepared mixtures

The absorption and emission spectra of the laboratory-prepared mixtures were scanned, processed as under calibration for each of the proposed methods and the concentration of mosapride in each mixture was calculated using the specified regression equation.

Application to pharmaceutical formulations

Ten tablets of Mosapride® 2.5 mg were accurately weighed, decoated by washing with water, dried using filter paper, and finely powdered. Another ten tablets of Mosapride® 5 mg were accurately weighed and finely powdered as well.

Accurately weighed portions of the powdered tablets equivalent to 10 mg of mosapride citarate were separately weighed, transferred into two 100-ml beakers, sonicated in 30 ml methanol for 10 min, and filtered into two 100-ml volumetric flasks. The residues were washed three times each with 10 ml methanol and the solution was completed to the mark with the same solvent. Aliquots of 1.0 ml or 0.1 ml were transferred from the prepared solutions to 10-ml volumetric flasks and diluted with either methanol or 0.1N NaOH for the spectrophotometric or spectrofluorimetric determination of mosapride. The general procedure previously described under each method was followed to determine the concentration of mosapride in the prepared dosage form solutions.

Results and discussion

Analytical methods for the determination of a compound in presence of its degradation products without previous chemical separation are always of interest. However, by reviewing the literature concerned with the stability of mosapride citrate, it was found that no stability-indicating assay was described.

In this study, mosapride citrate was subjected to different stress conditions, namely acid, alkaline, oxidative, thermal, and photo-degradation as stated by the International Conference on Harmonization (ICH) guidelines. To study the effect of acids and alkali, mosapride was heated with different concentrations of hydrochloric acid and sodium hydroxide. The drug was insoluble in aqueous concentrated solution of NaOH and was found to be stable upon refluxing with 1M methanolic NaOH solution up to 8 h. Also, it showed stability in 30% H_2O_2 after 10 h reflux, in a dry oven at 120 °C for 10 h and on exposure to UV light for 10 h.

The acid degradation process was carried out by dissolving the drug in 1, 2, 4 M HCl and heated in sealed ampoules in a dry oven of temperature 120 °C for 4 h. Complete degradation was obtained by using 2 MHCl after 4 h. Heating at 120 °C oven and was confirmed by the disappearance of the intact molecule's spot or peak. While the degradation products remain stable even after 4 h heating in 4 M HCl, complete degradation was confirmed by TLC and HPLC (through the disappearance of the intact molecule's spot or peak, respectively) (Figures 1A and 1B).

Mosapride structure shows an amide and an ether group; many amide-containing drugs undergo degradation through acidic or alkaline hydrolysis with cleavage of the amide linkage and cleavage of ether linkage as well which is usually unstable in acidic medium.[24] The suggested scheme of acid degradation is shown in Figure 2. For confirmation of the suggested mechanism, the products of acidic hydrolysis were subjected to liquid chromatography-mass spectrometry (LC-MS) analysis. The LC-MS chromatogram showed two main peaks at *m/z* 225.2 and 186.3 corresponding to the protonated molecular ions of the suggested two degradation products (Figure 3).

Mosapride and its acid degradation products spectra are severely overlapped as shown in Figure 4; direct determination of mosapride is impossible, while upon the application of ^{1}D, ^{1}DD, MC, or HPSAM methods, the spectra can be resolved and determination of mosapride in presence of its degradation products could be achieved.

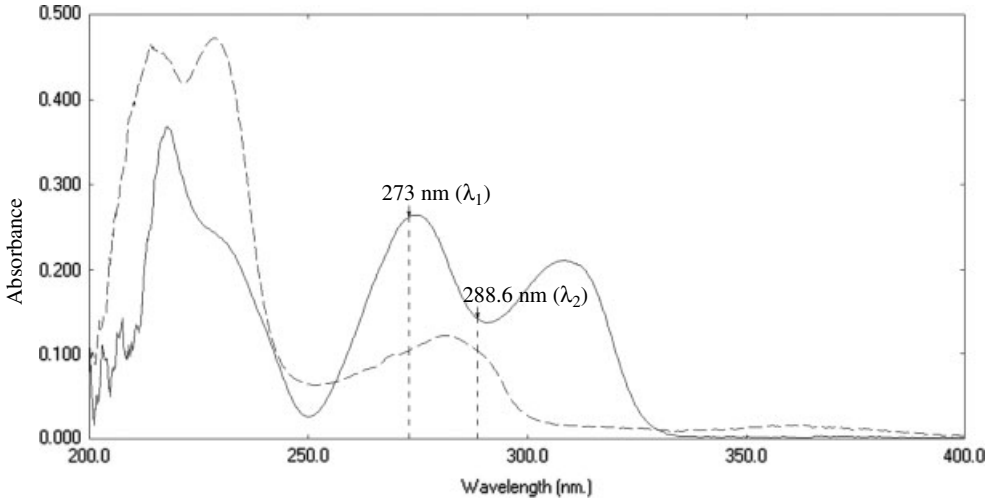


Figure 4. Zero order absorption (^{0}D) spectra of 10 μg.ml^{-1} mosapride (———) and its degradation products 20 μg.ml^{-1} (- - - -) in methanol.

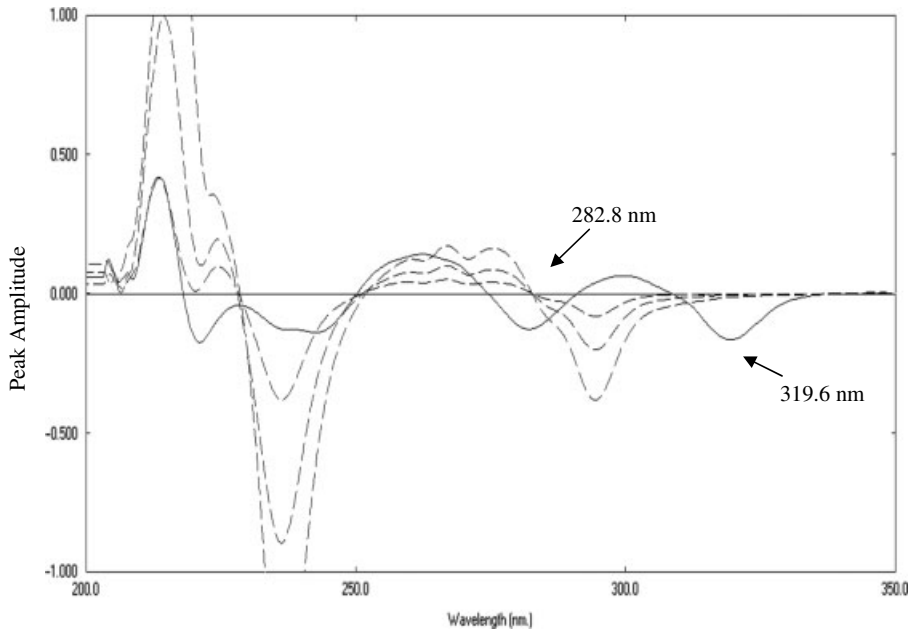


Figure 5. First derivative (^{1}D) spectra of 10 μg.ml^{-1} mosapride (———) and its degradation products (- - - -) 20, 50 and 100 μg.ml^{-1} in methanol.

Taking the advantage of the native fluorescence of the intact molecule, a highly sensitive spectrofluorimetric method was developed as a stability-indicating method for mosapride determination.

^{1}D method

Derivative spectrophotometry was first suggested during the last decade and soon became a well established technique for the assay of drugs in mixtures and pharmaceutical dosage forms.[25] It is considered a good technique that is capable of enhancing the resolution of overlapped spectral bands.[26] Figure 5 shows that ^{1}D could be applied for the determination of mosapride in the presence of its degradation products by measuring the peak amplitude at 282.8 nm (zero crossing point of mosapride degradation products) and 319.6 (nearly no contribution of mosapride degradation products).

A linear correlation was obtained between the values of peak amplitude at 282.2 and 319.6 nm against the corresponding concentration of mosapride.

^{1}DD method

Another method for resolving binary mixtures without previous separation is the derivative ratio spectrophotometric method (^{1}DD), which was developed by Salinas *et al*.[27] In this method, the absorption spectrum of the mixture is obtained and divided by the absorption spectrum of a standard solution of the degradation product and then the first derivative of the ratio spectrum is obtained. This method permits the determination of mosapride in its mixtures at the wavelengths corresponding to a maximum or minimum.

In order to optimize the developed ^{1}DD method, the influence of different variables was studied; these variables include divisor

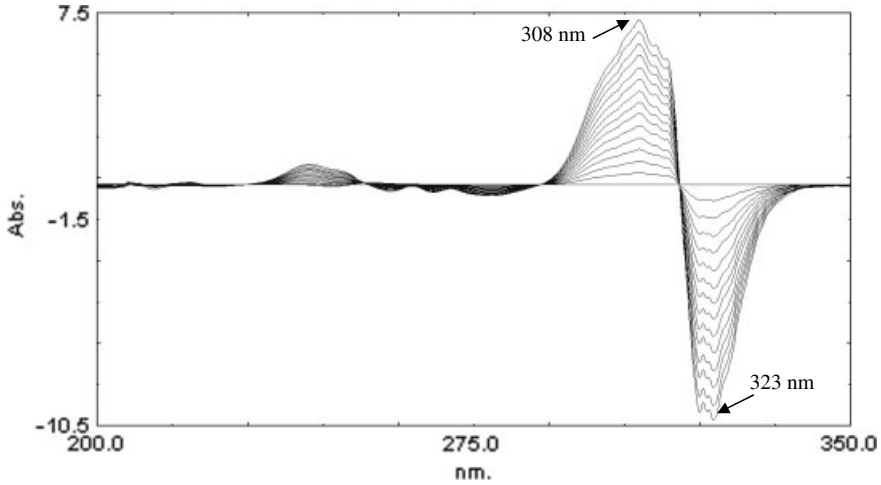


Figure 6. First derivative of ratio spectra (^{1}DD) of mosapride in the range of 5–70 μg.ml^{-1} in methanol using the spectrum of 50 μg.ml^{-1} of degradation products as a divisor.

concentration, $\Delta\lambda$ and smoothing factor. The careful choice of the divisor and the working wavelengths were of great importance; three different concentrations of the degradation products solutions (20, 50 and 70 μg.ml^{-1}) were tried as divisors. It was found that minimum noise and better selectivity were obtained when using 50 μg.ml^{-1} of the degradation products spectrum. Two calibration curves were constructed representing the relationship between the peak amplitudes of ^{1}DD at 308 and 323 nm and the corresponding concentrations (Figure 6).

MC method

It is a new spectrophotometric method that was developed for the simultaneous determination of binary and ternary mixtures without preliminary separation. This method is based on the mean centring of ratio spectra instead of calculating the derivative and therefore signal-to-noise ratio is enhanced.[28] It was used in many applications as kinetic analysis of binary mixtures[29] and determination of reactions rate constants.[30]

To explain the mean centring expression, let us consider a three-dimensional vector, *y*

$$y = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix}$$

We mean centre (MC) this vector by subtracting the mean of three numbers calling *y*

$$y = \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix}$$

$$\mathrm{MC(Y)} = y - y = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix} - \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix} = \begin{bmatrix} +2 \\ -2 \\ 0 \end{bmatrix}$$

It could be proved that if the vector *y* is multiplied by *n* (a constant number), the mean centred vector is also multiplied by *n* and also if a constant number is added to the vector *y*, the mean centre of this vector is not changed.

Consider a mixture of two components; *X(analyte)* and *Y(interferent)*. If there is no interaction among the compounds and Beer's Law is obeyed for each, it can be written:

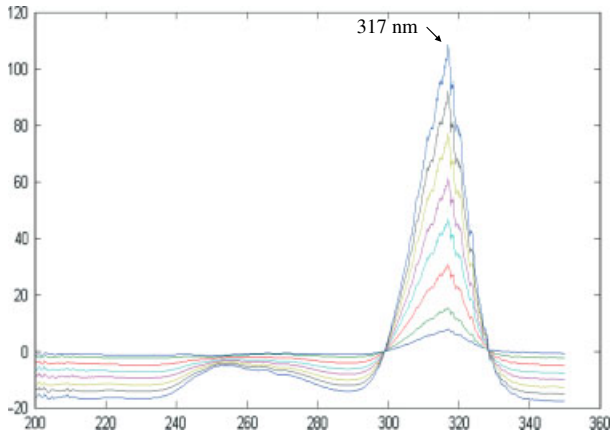


Figure 7. Mean centering of ratio (MC) spectra of mosapride, 5 – 70 μg.ml^{-1} in methanol using the spectrum of 50 μg.ml^{-1} of degradation products as a divisor.

$$Am = \alpha_X C_X + \alpha_Y C_Y \quad (1)$$

where, *Am* is the vector of the absorbance of the mixture, α_X and α_Y are the molar absorptivity vectors of *X* and *Y* and C_X and C_Y are the concentrations of *X* and *Y*, respectively.

If Eqn (1) is divided by α_Y corresponding to the spectrum of a standard solution of the interferent *Y*, the first ratio spectrum is obtained in the form of Eqn (2) (for possibility of dividing operation, the zero values of α_Y should not be used in the divisor):

$$B = Am/\alpha_Y = \alpha_X C_X/\alpha_Y + C_Y \quad (2)$$

If Eqn (2) is mean centred (MC), since the mean centring of a constant (C_Y) is zero, Eqn (3) would be obtained:

$$\mathrm{MC}(B) = \mathrm{MC}(\alpha_X C_X/\alpha_Y) \quad (3)$$

Eqn (3) is the mathematical foundation of multicomponent analysis that permits the determination of concentration of the active compounds in the solution (*X* in this equation) without

interfering from the degradation compounds of the binary system (*Y* in this equation). As Eqn (3), shows there is a linear relation between the amount of MC(*B*) and the concentration of *X* in the solution.

A calibration curve could be constructed by plotting MC(*B*) against concentration of *X* in the standard solutions of *X*. To increase the sensitivity of the method, the amount of MC(*B*) corresponding to maximum or minimum wavelength should be selected; the maxima at 234 nm were plotted against mosapride concentration (Figure 7).

A linear correlation was obtained between the amplitude values at 317 nm against the corresponding concentrations of the drugs.

H-point standard additions method(HPSAM)

HPSAM[31] permits both proportional and constant errors produced by the matrix of the sample to be corrected directly. It is based on the principle of dual wavelength spectrophotometry and the standard addition method. The great advantage of HPSAM is that it can remove the errors resulting from the presence of an interfering substance and blank reagent. It uses the analytical signal data at the two accurately selected wavelengths corresponding to constant absorbance of the interfering substance to be plotted versus the added analyte concentration. The following principles have to be followed for selection of appropriate wavelengths for applying HPSAM:

(1) At the two selected wavelengths, the signal of interfering substance must remain the same, even if the analyte is changed.
(2) The analytical signals of the mixture composed from analyte and the interfering substance should be equal to the sum of the individual signals of two species.
(3) The slope difference of the two straight lines obtained at two selected wavelengths must be as large as possible in order to get good accuracy.

By plotting the absorbance at the two selected wavelengths versus the added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, A_H), where C_H is the unknown analyte concentration and A_H is the analytical signal due to the interferent. The method has been applied to binary mixtures of drugs with overlapped absorption spectra.[32,33]

Consider an unknown sample containing mosapride (analyte X) and degradation products (interferent Y). The determination of concentration of X by HPSAM under these conditions requires the selection of two wavelengths λ_1 (273 nm) and λ_2 (288.6 nm) at which the degradation products (Y) have the same absorbance values (Figure 4). Then, a known amount of mosapride (X) is successively added to the mixture and the resulting absorbance is measured at the two selected wavelengths and expressed by Eqns (4) and (5)

$$A_{\lambda 1} = b_0 + b + M_{\lambda 1} C_i \quad (4)$$

$$A_{\lambda 2} = A_0 + A + M_{\lambda 2} C_i \quad (5)$$

where, b_0 and A_0 are the absorbance of X at λ_1 and λ_2, respectively; b and A are the absorbance of Y at the same wavelengths; M_1 and M_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 mosapride (X) concentration; and A_1 and A_2 are

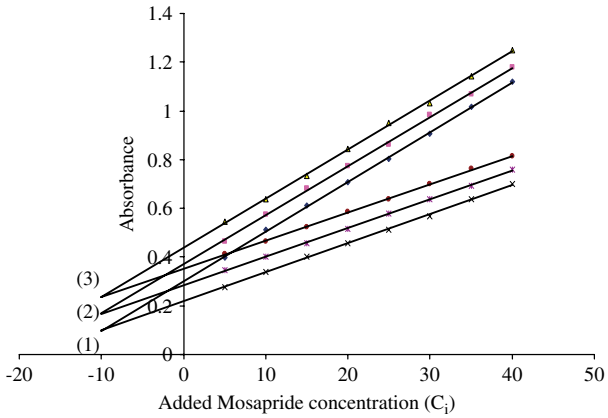


Figure 8. Plots of H-point standard addition method between the absorbance at 273 nm and 288.6 nm and the added concentration of mosapride to mixture of fixed mosapride (10 μg.ml^{-1}) and degradation product (1) 20 μg.ml^{-1} (2) 40 μg.ml^{-1} and (3) 60 μg.ml^{-1}.

the absorbance measured at the two wavelengths for difference points of HPSAM.

By plotting the analytical signal versus added mosapride (analyte) concentration, two straight lines are obtained that intercept at the so-called H point (Figure 8).

At the H-point, since $A_{\lambda 1} = A_{\lambda 2}$, $C_i = -C_H$, so Eqns (6) and (7) can be obtained

$$b_0 + b + M_{\lambda 1}(-C_H) = A_0 + A + M_{\lambda 2}(-C_H) \quad (6)$$

$$-C_H = [(A_0 - b_0) + (A - b)]/(M_{\lambda 1} - M_{\lambda 2})$$

$$C_i = -C_H = -C_x = (A_0 - b_0)/(M_{\lambda 1} - M_{\lambda 2}) \quad (7)$$

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.

In this special system, analyte is mosapride and its degradation products are as interfering substance. Several wavelength pairs were examined and the wavelength pair of 273 and 288.6 nm was selected. Under optimum conditions, determination of mosapride and its degradation products was carried out using HPSAM. The concentration of the interfering substance was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H- point (AH). Several synthetic mixtures with different concentration ratios of mosapride and its degradation products were analyzed by the proposed method. The results are given in Table 1.

A linear correlation was obtained between the absorbance against the added mosapride concentration at the two selected wavelengths 273 nm and 288.6 nm and the correlation is represented by two regression equations at the two selected wavelengths as follows:

$$A_1 = M_{\lambda 1} C_1 + Y_1 \quad \text{(regression equation at } \lambda 1)$$

$$A_2 = M_{\lambda 2} C_2 + Y_2 \quad \text{(regression equation at } \lambda 2)$$

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

For the application of the method to the analysis of mosapride in laboratory-prepared mixtures and in pharmaceutical dosage form, the absorbance of the prepared solutions was recorded

Table 1. Results of several experiments for the analysis of mosapride degradation products mixtures at different concentration ratios by HPSAM

A-C Equation	R	Present (μg.ml^{-1})		Found (μg.ml^{-1})	
		Mosapride	Degradation products	Mosapride	Degradation* products
$A_{273} = 0.0203\ C_i + 0.3009$	0.9998	10	20	10.06	19.76
$A_{288.6} = 0.0120\ C_i + 0.2174$	0.9997				
$A_{273} = 0.0202\ C_i + 0.3697$	0.9995	10	40	10.17	39.47
$A_{288.6} = 0.0118\ C_i + 0.2843$	0.9997				
$A_{273} = 0.0201\ C_i + 0.4385$	0.9996	10	60	10.11	60.35
$A_{288.6} = 0.0116\ C_i + 0.3526$	0.9998				
$A_{273} = 0.0202\ C_i + 0.5091$	0.9999	20	20	20.33	20.35
$A_{288.6} = 0.0119\ C_i + 0.3404$	0.9991				
$A_{273} = 0.0203\ C_i + 0.7070$	0.9997	30	20	30.02	20.35
$A_{288.6} = 0.0119\ C_i + 0.4555$	0.9995				

* Calculated from individual calibration curve of the degradation products at 273 nm, where its regression is A = 0.0034C + 0.0298.

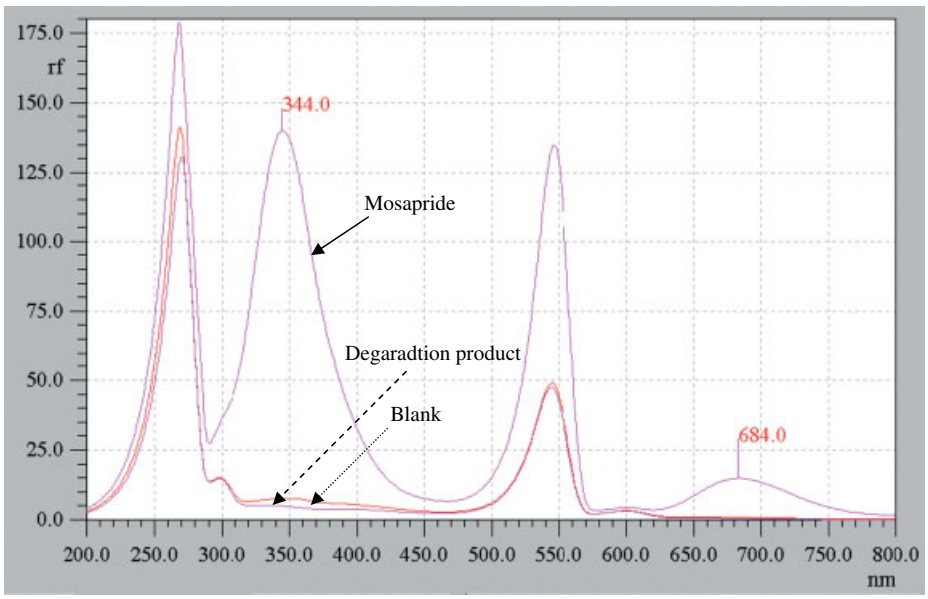


Figure 9. The emission spectra of mosapride 300 ng.ml^{-1}, its degradation products 300 ng.ml^{-1} and blank of 0.1 M NaOH using $\lambda_{excitation}$ 276 nm.

at the two selected wavelengths. The concentration of both the analyte (mosapride) and the interfering substance (degradation products) could be obtained.

For C_H calculation

At H point $A_1 = A_2$

$M_{\lambda 1}C_1 + Y_1 = M_{\lambda 2}C_2 + Y_2$

$M_{\lambda 1}C_1 - M_{\lambda 2}C_2 = Y_2 - Y_1$

$C_1 = C_2 So$

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

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

Then, by substituting directly in the previous equation, the concentration of mosapride in either laboratory-prepared mixtures and in pharmaceutical formulation could be obtained.

For A_H calculation

$C_1 = (A_1 - Y_1)/M_{\lambda 1}$

$C_2 = (A_2 - Y_2)/M_{\lambda 2}$

At H point $C_1 = C_2$

$(A_1 - Y_1)/M_{\lambda 1} = C_2 = (A_2 - Y_2)/M_{\lambda 2}$

$M_{\lambda 1}\ (A_2 - Y_2) = M_{\lambda 2}(A_1 - Y_1)$

$M_{\lambda 1}\ A_2 - M_{\lambda 1}\ Y_2 = M_{\lambda 2}\ A_1 - M_{\lambda 2}\ Y_1$

$M_{\lambda 1}\ A_2 - M_{\lambda 2}\ A_1 = M_{\lambda 1}\ Y_2 - M_{\lambda 2}\ Y_1$

$A_H = A_1 = A_2\ So$

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

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

Then AH was used for the determination of the concentration of interfering substance by substituting in its regression equation of its pure form.

Table 2. Regression and validation parameters of the proposed spectrophotometric and spectrofluorimetric methods for the determination of mosapride citrate bulk powder

	Spectrophotometry							Spectrofluorimetry	
	^{1}D		^{1}DD		MC	HPSAM			
Parameter	282.8 nm	319.6 nm	308 nm	323 nm	317 nm	273 nm	288.6 nm	344 nm	684 nm
Linearity									
Range*	5–65	5–70	5–70	5–70	5–70	5–65		50–3000	50–9000
Intercept	0.0085	−0.0002	0.0126	0.0035	−0.03203	0.3009	0.2174	64.58	5.7318
SE of intercept	0.00516	0.00327	0.01451	0.03095	0.29503	0.00477	0.00339	3.50267	0.55023
Slope	0.0123	0.0163	0.1023	0.1458	1.5111	0.0203	0.012	0.2957	0.0231
SE of slope	0.00013	0.00008	0.00035	0.00074	0.00705	0.00019	0.00013	0.00207	0.00011
Correlation coefficient(r)	0.9997	0.9999	0.9999	0.9999	0.9999	0.9998	0.9997	0.9999	0.9999
Accuracy	100.08 ± 0.993	100.56 ± 0.386	100.27 ± 0.472	100.84 ± 0.688	100.46 ± 0.469	100.32 ± 0.952		99.72 ± 0.772	99.73 ± 0.990
Precision**									
Repeatability	±0.203	±0.127	±0.203	±1.160	±0.650	±0.706		±0.461	±0.496
Intermediate precision	±0.379	±0.211	±0.379	±0.379	±1.359	±1.314		±0.800	±0.703
LOD	1.87	0.83	0.64	0.96	0.89	0.84	1.01	47.75	140.89
LOQ	5.67	2.76	1.95	2.92	2.69	2.79	3.36	144.71	426.94

* The range is in µg.ml^{-1} in spectrophotometric methods and ng.ml^{-1} in spectrofluorimetric method.
** The precision (n=3), average of three concentrations (10, 20, 30µg.ml^{-1}) and (500, 1000, 2000 ng.ml^{-1}) in spectrophotometric and spectrofluorimetry methods, respectively.

Table 3. Determination of mosapride citrate in the laboratory-prepared mixtures by the proposed spectrophotometric and spectrofluorimetric methods

	Recovery % of mosapride							
	^{1}D		^{1}DD				Spectrofluorimetry	
% of Degradation	282.8 nm	319.6 nm	308 nm	323 nm	MC 317 nm	HPSAM	344 nm	684 nm
10	99.81	99.13	100.21	100.79	100.28	98.83	99.11	98.64
30	98.25	99.26	99.73	100.63	98.87	98.38	99.70	98.94
50	100.96	101.74	98.15	98.61	99.15	100.89	100.69	99.95
70	101.37	101.47	101.27	101.81	99.94	99.39	101.3	100.41
80	101.72	101.90	100.98	101.65	100.86	101.44	103.35*	103.29*
90	102.92*	103.80*	101.78	101.84	101.19	101.54	105.88*	105.79*
Mean ± SD	100.42 ± 1.406	100.70 ± 1.383	100.35 ± 1.306	100.89 ± 1.233	100.05 ± 0.919	100.08 ± 1.383	100.20 ± 0.981	99.49 ± 0.833

* Rejected.

Table 4. Determination of mosapride citrate in its dosage form by the proposed spectrophotometric and spectrofluorimetry methods.

	Recovery* % ± SD of the claimed mean * (mg/tablet)*							
	^{1}D		^{1}D				Spectrofluorimetry	
Pharmaceutical formulation	282.8 nm	319.6 nm	308 nm	323 nm	MC 317 nm	HPSAM	344 nm	684 nm
Mosapride® 2.5 mg Batch No. 08122, labelled to contain 2.5 mg/tablet	99.71 ± 1.611	99.77 ± 1.007	99.49 ± 1.226	99.25 ± 1.522	99.94 ± 1.119	99.69 ± 1.486	99.13 ± 0.926	99.24 ± 1.052
Mosapride® 5.0 mg Batch No. 08061, labelled to contain 5 mg/tablet	99.98 ± 1.360	99.77 ± 0.907	99.92 ± 1.047	99.35 ± 1.272	99.97 ± 1.058	99.52 ± 1.325	99.14 ± 0.955	99.06 ± 1.031

* Average of three determinations.

Spectrofluorimetric method

A spectofluorimetric method (11) was reported for the determination of mosapride citrate in its dosage form. In this method, mosapride in methanol showed emission at 360 nm after excitation at 331 nm.

In our work, the developed spectrofluorimetric method was applied as a stability-indicating method. In order to optimize the method, the effect of different solvents, namely, methanol, water, acetonitrile, 0.1N H_2SO_4 and 0.1N NaOH, was studied. The highest fluorescence intensity (I_f) was obtained when using 0.1N NaOH as a diluting solvent. The excitation was done at $\lambda_{excitation}$ 276 nm and the emission was recorded at $\lambda_{emission}$ were 344 nm and 684 nm (Figure 9).

A linearity correlation was obtained between I_f and the concentration in the range of 50–3000 ng.ml^{-1} and 50–9000 ng.ml^{-1} at the two emission wavelengths 344 nm and 684 nm, respectively.

Validation of the proposed methods was done according to the ICH guidelines.[34] The accuracy and the regression equations parameters obtained from the proposed methods are given in Table 1. Repeatability and intermediate precision were evaluated by assaying freshly prepared solutions in triplicate on the same day and on three successive days, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope using the calibration curve was followed (Table 2). The selectivity of the methods was tested by analyzing different laboratory-prepared mixtures of mosapride citrate and its degradation products (Table 3). Satisfactory results were obtained, indicating the high selectivity of the methods.

The proposed methods were successfully applied to the determination of mosapride citrate in its pharmaceutical formulation as shown in Table 4. The validity of the proposed methods was assessed by applying the standard addition technique. The results obtained were reproducible with low standard deviation as shown in Table 5.

The results obtained by applying the proposed methods for the determination of mosapride citrate in bulk powder were statistically compared with the reported method (8). The t-value and F-value were less than the theoretical ones which indicates that there is no significant difference between the proposed methods and the reported one regarding both accuracy and precision, as shown in Table 6.

The suggested methods for the determination of mosapride in the presence of its degradation products were statistically compared using one-way ANOVA. The data showed that there was no significant difference between them where the calculated F value (0.294) was less than the tabulated one (2.24) (p=0.938)

Table 5. Application of standard addition technique for the determination of mosapride citrate in its dosage form

		Spectrophotometry													Spectrofluorimetry			
		^{1}D 282.8 nm		^{1}D 319.6 nm		^{1}DD 308 nm		^{1}DD 323 nm		MC 317 nm		HPSAM			344 nm		684 nm	
Pharmaceutical formulation	Pure added µg.ml^{-1}	Pure found µg.ml^{-1}	Recovery* %	Pure found µg.ml^{-1}	Recovery* %	Pure found µg.ml^{-1}	Recovery* %	Pure found µg.ml^{-1}	Recovery* %	Pure found µg.ml^{-1}	Recovery* %	Pure found µg.ml^{-1}	Recovery* %	Pure added µg.ml^{-1}	Pure found µg.ml^{-1}	Recovery* %	Pure found µg.ml^{-1}	Recovery* %
Mosapride® 2.5 mg																		
	5	4.93	98.68	4.96	99.20	4.97	99.42	4.96	99.24	4.98	99.64	4.91	98.28	**0**.5	0.502	100.40	0.497	99.40
	10	9.87	98.72	10.08	100.80	9.93	99.26	9.92	99.18	9.97	99.71	9.83	98.33	**1**	0.999	99.90	0.992	99.20
	20	20.26	101.29	19.92	99.60	20.19	100.97	20.24	101.22	20.08	100.38	20.39	101.97	**2**	2.030	101.50	2.016	100.80
	Mean ± SD	99.56 ± 1.495		9.87 ± 0.833		99.88 ± 0.942		99.88 ± 1.161		99.91 ± 0.406		99.53 ± 2.116		**Mean ± SD**	100.60 ± 0.819		99.80 ± 0.872	
Mosapride® 5.0 mg																		
	5	4.95	99.06	4.99	99.80	5.01	100.26	4.99	99.82	5.01	100.22	4.96	99.22	**0**.5	0.505	101.00	0.504	100.80
	10	9.91	99.11	10.11	101.10	9.96	99.61	9.94	99.38	10.04	100.39	9.88	98.84	**1**	1.012	101.20	0.997	99.70
	20	20.22	101.09	20.13	100.65	19.97	99.87	19.93	99.65	20.10	100.49	20.25	101.27	**2**	1.990	99.50	2.028	101.40
	Mean ± SD	99.75 ± 1.158		100.52 ± 0.660		99.91 ± 0.327		99.62 ± 0.222		100.37 ± 0.137		99.78 ± 1.304		**Mean ± SD**	100.57 ± 0.929		100.63 ± 0.862	

* The values of % recovery is an average of three determinations.

Table 6. Statistical analysis of the results obtained by applying the proposed spectrophotometric and spectrofluorimetric methods with the reported (8) one for the determination of mosapride citrate

Comparison item	^{1}D		^{1}D				Spectrofluorimetry		Reported* HPLC(8)
	282.8 nm	319.6 nm	308 nm	323 nm	MC 317 nm	HPSAM	344 nm	684 nm	
Mean	100.08	100.56	100.27	100.84	100.46	100.32	99.72	99.73	100.58
SD	0.993	0.621	0.472	0.688	0.469	0.952	0.772	0.990	0.884
RSD	0.992	0.618	0.471	0.682	0.467	0.950	0.774	0.993	0.880
V(Variance)	0.990	0.386	0.223	0.473	0.220	0.906	0.596	0.980	0.782
n	5	6	6	6	6	6	5	7	6
t	0.873	0.045	0.757	0.568	0.294	0.490	1.722	1.635	–
	(2.262)	(2.228)	(2.228)	(2.228)	(2.228)	(2.228)	(2.262)	(2.201)	
F	3.142	1.259	3.507	1.653	3.555	1.159	1.312	1.253	–
	(5.19)	(5.05)	(5.05)	(5.05)	(5.05)	(5.05)	(6.26)	(4.95)	

* HPLC method using C-18 analytical column, acetonitrile-0.02Mphosphate buffer pH4(50 : 50v/v) as a mobile phase pumped at 1 ml/min and detection at 274 nm.
The values in the parenthesis are the corresponding theoretical values of t and F at (P = 0.05).

Conclusion

The proposed methods are simple, sensitive, accurate, precise and could be easily applied in quality control laboratories as stability-indicating methods for the determination of mosapride citrate. The developed spectrophotometric methods almost have the same range and accuracy; mean centring method has the advantage over the other ones which is the enhancement of signal to noise ratio as it eliminates the derivative step. The spectrofluorimetric method, besides being a stability-indicating method, showed higher sensitivity.

The proposed methods could be successfully applied for the routine analysis of the studied drug in pure bulk powder and dosage form in quality control laboratories without any preliminary separation step.

References

[1] *The Merck Index,14 edition*, Merck: Whitehouse Station, NJ, **2006**, pp. 1084.
[2] S. Kato, T. Morie, T. Kon, N. Yoshida, T. Karaswa, J. Matsumoto, *J. Med. Chem.* **1991**, *34*, 616.
[3] N. Yoshida, S. Kato, T. Ito, *Drugs Future* **1993**, *18*, 513.
[4] J. C. Reynold, *Gastroenterol. Clin. N.* **1989**, *18*, 437.
[5] A. D. Craig, D.E. Clarke, *J. Pharmacol. Exp. Ther.* **1990**, *252*, 1378.
[6] A. G. Fernandez, D.J. Roberts, *Drugs Future* **1991**, *16*, 885.
[7] S. Kato, T. Morie, T.K. Hino, S. Naruto, N. Yoshida, T. Karaswa, J. Matsumoto, *J. Med. Chem.* **1990**, *33*, 1406.
[8] Y. S. R. Krishnaiah, T. K. Murthy, D. G. Sankar, V. Satyanarayana, *Pharmazie* **2002**, *57*, 814.
[9] Y. S. R. Krishnaiah, T. K. Murthy, D. G. Sankar, V. Satyanarayana, *Anal. Sci.* **2002**, *18*, 1269.
[10] H. D. Revanasiddappa, M. A. Veena, *Eclet. Quim.* **2007**, *32*, 71.
[11] S. J. Rajput, M. G. Sankalia, F. T. Patel, *Indian J. Pharm. Sci.* **2005**, *67*, 582.
[12] R. Jain, K. Radhapyari, N. Jadon, *J. Electrochem. Soc.* **2008**, *155*, F104.
[13] R. N. Rao, D. Nagaraju, S. N. Alvi, S. B. Bhirud, *J. Pharm. Biomed. Anal.* **2004**, *36*, 759.
[14] Y. R. Kumar, J. Moses-Babu, M. S. P. Sarma, B. Seshidhar, S. S. Reddy, G. S. Reddy, K. Vyas, *J. Pharm. Biomed. Anal.* **2003**, *32*, 361.
[15] R. N. Rao, D. Nagaraju, A. Narasaraju, *J. Pharm. Biomed. Anal.* **2006**, *14*, 338.
[16] A. Karlsson, A. Aspergen, *Chromatographia* **1998**, *47*, 189.
[17] I. Yokoyama, Y. Mizuki, T. Yamaguchi, T. Fujii, *J. Pharm. Biomed. Anal.* **1997**, *15*, 1527.
[18] N. V. S. Ramakrishna, K. N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu, D. P. Varma, *Biomed. Chromatogr.* **2005**, *19*, 539.
[19] H. Itoh, T. Nagano, M. Takeyama, *Biol. Pharm. Bull.* **2001**, *24*, 1072.
[20] Y. Aoki, H. Hakamata, Y. Igarashi, K. Uchida, H. Kobayashi, N. Hirayama, A. Kotani, F. Kusu, *J. Chromatogr. B Anayt. Technol. Biomed. Life Sci.* **2007**, *858*, 135.
[21] B. H. Patel, B. N. Suhagia, M. M. Patel, J. R. Patel, *J. Chromatogr. Sci.* **2008**, *46*, 10.
[22] Matlab 6.5, Mathworks Inc., **1999**.
[23] *The British Pharmacopoeia*, Appendix ID, HMSO: London, **2007**, pp. A140.
[24] S. W. Baertschi, K. M. Alsante, *Pharmaceutical Stress Testing Predicting Drug Degradation*, Taylor and Francis Group: Indianapolis, **2005**, pp. 54–57.
[25] S. Altinös, D. Tekeli, *J. Pharm. Biomed. Anal.* **2001**, *24*, 507.
[26] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, *J. Pharm. Biomed. Anal.* **1997**, *16*, 431.
[27] F. Salinas, J. J. B. Nevado, M. A. Espinosa, *Talanta* **1990**, *37*, 347.
[28] A. Afkhami, M. Bahram, *Talanta* **2005**, *66*, 712.
[29] A. Afkhami, M. Bahram, *Anal. Chim. Acta* **2004**, *526*, 211.
[30] M. Bahram, *Anal. Chim. Acta* **2007**, *603*, 13.
[31] F. Bosch-Reig, P. Compins- Falco, *Analyst* **1988**, *113*, 1011.
[32] S. M. Sabry, E. F. Khamis, *Talanta* **2000**, *51*, 1219.
[33] A. Afkhami, N. Sarlak, *Acta Chim. Slov.* **2005**, *52*, 98.
[34] International Conference on Harmonization, *Validation of Analytical Procedures: Methodology (Q2B)*, **1996**, Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073384.pdf [7 December, 2010].