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Development and validation of new spectrophotometric ratio H-point standard addition method and application to gastrointestinal acting drugs mixtures

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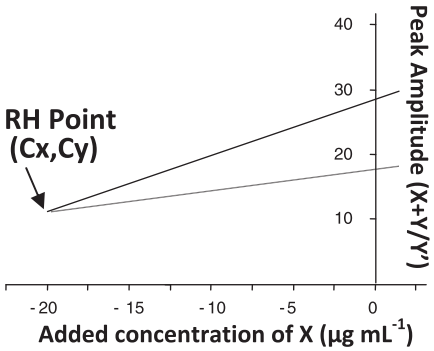
HIGHLIGHTS

- Spectral resolution and simultaneous determination of binary mixtures.
- New developed and validated Ratio H-point standard addition method, and applied on two different binary mixtures.
- The method combines ratio spectrophotometry and H-point standard addition method.

GRAPHICAL ABSTRACT

Ratio H-point standard addition method is a new dual wavelength spectrophotometric method which utilizes the basic principles in ratio spectrophotometry and H-point standard addition method. It was successfully used for simultaneous determination of gastrointestinal drugs mixtures with several advantages over the previously mentioned two conventional spectrophotometric methods.



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ABSTRACT

New, simple, specific, accurate and precise spectrophotometric technique utilizing ratio spectra is developed for simultaneous determination of two different binary mixtures. The developed ratio H-point standard addition method (RHPSAM) was managed successfully to resolve the spectral overlap in itopride hydrochloride (ITO) and pantoprazole sodium (PAN) binary mixture, as well as, mosapride citrate (MOS) and PAN binary mixture. The theoretical background and advantages of the newly proposed method are presented. The calibration curves are linear over the concentration range of 5–60 µg/mL, 5–40 µg/mL and 4–24 µg/mL for ITO, MOS and PAN, respectively. Specificity of the method was investigated and relative standard deviations were less than 1.5. The accuracy, precision and repeatability were also investigated for the proposed method according to ICH guidelines.

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Introduction

Management of gastroesophogeal reflux disease (GERD) can be divided into five stages; the agents used in stage III treatment of GERD include scheduled H2-receptor blocker, prokinetic agents and proton pump inhibitors (PPIs) [1].

Itopride hydrochloride (ITO) is chemically, N-[[4-(2-dimethylaminoethoxy) phenyl]methyl]-3,4-dimethoxybenzamide hydrochloride [2]. Itopride is one of the prokinetic agents; it has anticholinestrase activity as well as dopamine D_2 receptor antagonist activity and it is being used for the symptomatic treatment of various gastrointestinal motility disorders [3,4]. Mosapride Citrate

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(MOS) is chemically, 4-amino-5-chloro-2-ethoxy-N-[[4-(4-fluorobenzyl)morpholin-2-yl]methyl]benzamide; 2-hydroxypropane-1,2, 3-tricarboxylic acid [2]. It is another potent gastroprokinetic agent with selectivity for 5-HT4 receptors and it is used in the treatment of gastrointestinal motility dysfunction associated with nonulcer dyspepsia [5]. Pantoprazole sodium (PAN) is chemically 6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-l*H*-benzimidazole [2]. It is an irreversible PPI. The inhibition of the gastric proton pump or H^+/K^+ ATPase, suppresses gastric acid secretions and hence hyperacidity can be controlled by pantoprazole [6]. The chemical structures of the studied drugs (ITO, MOS and PAN) were presented in Fig. 1.

Combination of antisecretory agent and prokinetic agent may be appropriate in a patient with delayed gastric empting, such as diabetic patients with gastroparesis [1]. In some patients with GERD, PPI monotherapy is insufficient and combination therapy with prokinetics and PPIs is required to achieve symptoms relief [7]. Thus, the complementary pharmacological actions of PAN with ITO or MOS in a combined dosage form is useful for treating various gastrointestinal disorders, in particular for hyperacidity frequently associated with gastrointestinal dysmotility.

The literature comprises few spectroscopic methods for the determination of ITO [8], MOS [9] and PAN [10], alone and other methods for the simultaneous determination of PAN combinations with ITO [11] and with MOS [12,13] in their combined pharmaceutical formulations, while chromatographic methods have been cited in the literature for the separation of these combinations in absence [13] and in presence of their degradation products [14,15].

The aim of this work is to develop a new dual wavelength spectrophotometric method which utilizes the basic principles of two well established spectrophotometric techniques, the ratio spectrophotometry and H-point standard addition method (HPSAM). The proposed ratio H-point standard addition method is validated for the determination of binary mixtures, simultaneously, from *X* and *Y* coordinates of RH-point. Interestingly, the two calibration graphs could be performed at any pair of analytical wavelengths without any restrictions concerning wavelength selection. Moreover, errors produced by the sample matrix are eliminated by applying standard addition technique.

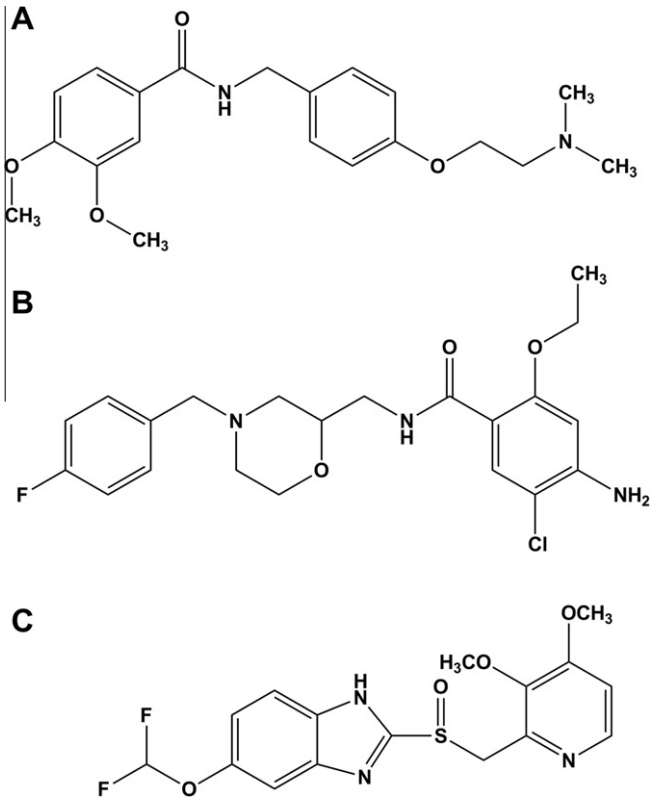

Fig. 1. Chemical structures of (A) itopride, (B) mosapride and (C) pantoprazole.

Experimental

Apparatus 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.

Materials and reagents

Pure standards

ITO, MOS and PAN were kindly supplied by National Organization for Drug Control and Research (NODCAR), Giza, Egypt, their purity were found to be 99.51 ± 1.091%, 99.31 ± 0.931% and 100.11 ± 1.059% according to the reported spectrophotometric methods [8–10], respectively.

Pharmaceutical dosage forms

Pantocid IT® hard gelatin capsules, manufactured by Sun Pharma Sikkim, Mumbai, India, Batch No. BSL0053 and labeled to contain 150 mg itopride hydrochloride (as sustained release) and 40 mg pantoprazole (as enteric coated) per Capsule.

Moza plus® hard gelatin Capsules, manufactured by Intas Pharmaceutical LTD, Mumbai, India, batch No. L090027 and labeled to contain 15 mg mosapride citrate (as sustained release) and 40 mg pantoprazole (delayed release) per tablet.

Solvents

All solvents used throughout the work were of analytical grade; methanol, acetonitrile, 0.1 N aqueous hydrochloric acid solution (Merck, Darmstadt, Germany) and distilled water.

Preparation of solutions

Stock standard solutions

Stock standard solutions of ITO, MOS and PAN (1.0 mg/mL) in methanol.

Working standard solutions

Working standard solutions of ITO, MOS and PAN (100.0 μg/mL) in methanol, prepared by suitable dilution from their respective stock standard solutions.

Synthetic mixture solutions

ITO–PAN binary mixtures. ITO and PAN binary mixtures were prepared by transferring aliquots from their respective stock solutions (1.0 mg/mL) equivalent to 1.50, 2.25 and 3.00 mg of ITO stock solution and 0.4, 0.8 and 1.2 mg from PAN stock solution into nine separate 10-mL volumetric flasks. The prepared nine mixtures contain three different concentration levels of the two components, for ITO (150, 225 and 300 μg/mL) and for PAN (40, 80 and 120 μg/mL).

MOS–PAN binary mixtures. MOS and PAN binary mixtures were prepared by transferring aliquots from their respective stock solutions (1.0 mg/mL) equivalent to 0.50, 0.75 and 1.00 mg of MOS stock solution and 1.0, 1.5 and 2.0 mg from PAN stock solution into nine

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separate 10-mL volumetric flasks. The prepared nine mixtures contain three different concentration levels of two components, for MOS (50, 75 and 100 μg/mL) and for PAN (100, 150 and 200 μg/mL).

Pharmaceutical dosage form solutions

Ten capsules of pantocid IT® or of Moza plus® were evacuated, weighted and finely powdered. An amount of the powdered capsules equivalent to 300 mg of ITO and 80 mg of PAN, in case of Pantocid IT® capsules, or equivalent to 75 mg of MOS and 200 mg of PAN, in case of Moza plus® capsules, was accurately weighted and transferred into 100-mL beaker, sonicated in 30 mL methanol for 10 min and filtered into 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.

Procedures

Spectral characteristics of ITO, MOS and PAN

The zero order absorption spectra (^{0}D) of ITO (30.0 μg/mL), MOS (7.5 μg/mL) and PAN (10.0 μg/mL) were recorded against methanol as a blank over the range 200–350 nm.

Construction of calibration curves

Aliquots equivalent to 50–600 μg of ITO, 50–400 μg MOS and 40–240 μg PAN were accurately transferred from their corresponding working standard solutions (100.0 μg/mL), into three separate series of 10-mL volumetric flasks and the volumes were completed with methanol. The zero order absorption spectra (^{0}D) of the three drugs were recorded against methanol as a blank, and then divided by the normalized PAN spectrum. The peak amplitudes at 258 nm and 290 nm were measured for ITO and at 274 nm and 290 nm were measured for MOS, while at 258 nm, 274 nm and 290 nm were measured for PAN. The peak amplitudes were plotted against the corresponding concentrations of each drug and the regression parameters were computed.

Assay of blind pure samples

Standard addition of different aliquots of ITO working standard solutions (100.0 μg/mL) was applied, separately, to six different blind concentrations of ITO and PAN, and the volumes were completed to the mark with methanol. The zero order absorption spectra (^{0}D) of each standard addition series were recorded against methanol as a blank, and then divided by the normalized PAN spectrum. The peak amplitudes at 258 nm and 290 nm were measured then plotted against the corresponding added ITO concentrations and the regression parameters at the two selected wavelengths were computed.

Likewise, six different blind concentrations of MOS and PAN were analyzed by the proposed method after the application of standard addition of different aliquots of MOS working standard solutions (100.0 μg/mL) on each concentration, separately, and the volumes were completed to the mark with methanol. The same procedure was followed and then peak amplitudes at 274 nm and 290 nm were measured then plotted against the corresponding added MOS concentrations and the regression parameters at the two selected wavelengths were computed.

Assay of synthetic mixtures

One mL aliquots from each previously prepared synthetic mixture were transferred into separate sets of 10-mL volumetric flasks and then standard addition of different aliquots of ITO and MOS working standard solution was applied to ITO–PAN and MOS–PAN binary mixtures, respectively. The volumes were completed to the mark with methanol. The procedure under calibration was followed for each mixture's standard addition series.

Assay of pharmaceutical formulation

Ten fold dilutions were applied to the prepared pharmaceutical dosage form, then 1.0 mL aliquots were transferred from these diluted pharmaceutical dosage form solutions into separate series of 10-mL volumetric flasks. Standard addition were applied to the transferred aliquots, such that, standard addition of ITO and MOS working standard solutions were applied to pantocid IT® and Moza plus® transferred aliquots, respectively. The volumes were completed to the mark with methanol. The general procedures previously described under calibration were followed to determine the concentration of the drugs in the prepared dosage form solutions.

Result and discussion

The scanning profile of the three drugs showed severe interference between each of ITO and MOS spectra with PAN spectrum, which prevents the direct determination of each component, Fig. 2. The maximum wavelengths (λ_{max}) of the three drugs, which were 258 nm for ITO, 274 nm for MOS and 290 nm for PAN were also presented in the same figure.

This work presents a newly developed "ratio H-point standard addition method" which is a type of dual wavelength spectrophotometry combining the principles of ratio spectrophotometry and HPSAM. The constants generated in the ratio spectra [16] can be revealed and determined using the fact that H-point standard addition method could determine the proportionality constants present in mixtures [17,18]. The new Ratio H-point standard addition method (RHPSAM) has the power of simultaneous determination of both components in the mixture using the peak amplitudes of the ratio spectra at any two wavelengths along the ratio spectra to be plotted versus the added concentrations of one component.

Theoretical background of ratio H-point standard addition method

For two components X and Y, first, the spectrum of the mixture ($X + Y$) is divided by a standard normalized Y spectrum (Y'), the peak amplitudes ($X + Y/Y'$) at two selected wavelengths where then plotted versus the added component (X) concentration, two straight lines are obtained that have a common point with coordinates ($-C_x$, C_y), where C_x and C_y are the unknown concentrations of X and Y components, respectively.

Consider an unknown sample containing mixture of the two components, X and Y. The simultaneous determination of both by the proposed RHPSAM under these conditions requires successive addition of known amounts of component (X) to the mixture, then

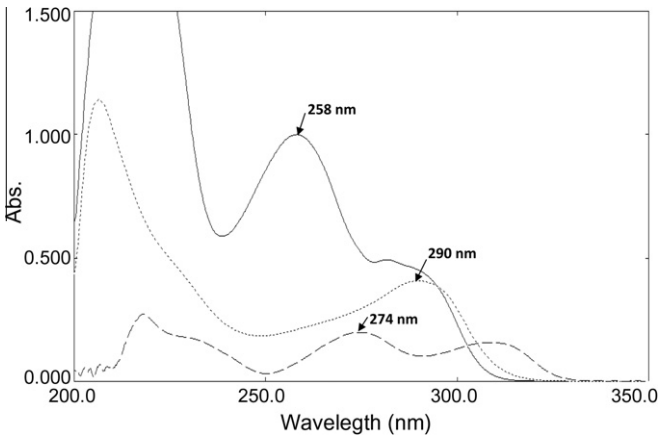

Fig. 2. Zero order absorption spectra of ITO, MOS and PAN, using methanol as a blank.

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the selection of suitable divisor of normalized spectrum of (Y) component, obtained by dividing certain spectrum of Y component by its concentration to obtain the normalized spectrum (Y'). As the generated constant (Y/Y') is extended all over the ratio spectrum, so any two wavelengths could be used to perform the calibrations. Plot the resulting peak amplitude at the two selected wavelengths (λ_1 and λ_2) to the corresponding added concentrations of (X) component. Eqs. (1) and (2) represent this relation

$$P_1 = (X/Y')_1 + (Y/Y') + M_1C_x \quad (1)$$

$$P_2 = (X/Y')_2 + (Y/Y') + M_2C_x \quad (2)$$

where, P_1 and P_2 are the peak amplitudes of the ratio spectra recorded at the two wavelengths λ_1 and λ_2, $(X/Y')_1$ and $(X/Y')_2$ are the amplitude of X/Y' ratio at λ_1 and λ_2, respectively; (Y/Y') is the amplitude of Y/Y' ratio which is a constant value at the two wavelengths; M_1 and M_2 are the slopes of the standard addition calibration lines obtained on applying the RHPSAM at λ_1 and λ_2, respectively; C_x is the added analyte (X) concentration.

By plotting the peak amplitudes of the ratio spectra versus the added (X) concentration, two straight lines are obtained that intercept at the so-called RH point having ($-C_{RH}$; P_{RH}) coordinate Fig. 3.

At this point, since $P_1 = P_2 = P_{RH}$, so Eqs. (1) and (2) are equal, and Eqs. (3) and (4) can be obtained

$$(X/Y')_1 + (Y/Y') + M_1(C_x) = (X/Y')_2 + (Y/Y') + M_2(C_x) \quad (3)$$

$$M_1(C_x) - M_2(C_x) = (X/Y')_2 + (Y/Y') - (X/Y')_1 - (Y/Y')$$

$$C_x(M_1 - M_2) = (X/Y')_2 - (X/Y')_1$$

$$C_x = -C_{RH} = (X/Y')_2 - (X/Y')_1/(M_1 - M_2) \quad (4)$$

At the same RH-point, the y-coordinate (P_{RH}), can be calculated as follow using Eqs. (1) and (2), (5) and (6) can be obtained

$$C_x = [P_1 - (X/Y')_1 - (Y/Y')]/M_1 \quad (5)$$

$$C_x = [P_2 - (X/Y')_2 - (Y/Y')]/M_2 \quad (6)$$

$$[P_1 - (X/Y')_1 - (Y/Y')]/M_1 = [P_2 - (X/Y')_2 - (Y/Y')]/M_2$$

$$M_1[P_2 - (X/Y')_2 - (Y/Y')] = M_2[P_1 - (X/Y')_1 - (Y/Y')]$$

$$M_1P_2 - M_1(X/Y')_2 - M_1(Y/Y') = M_2P_1 - M_2(X/Y')_1 - M_2(Y/Y')$$

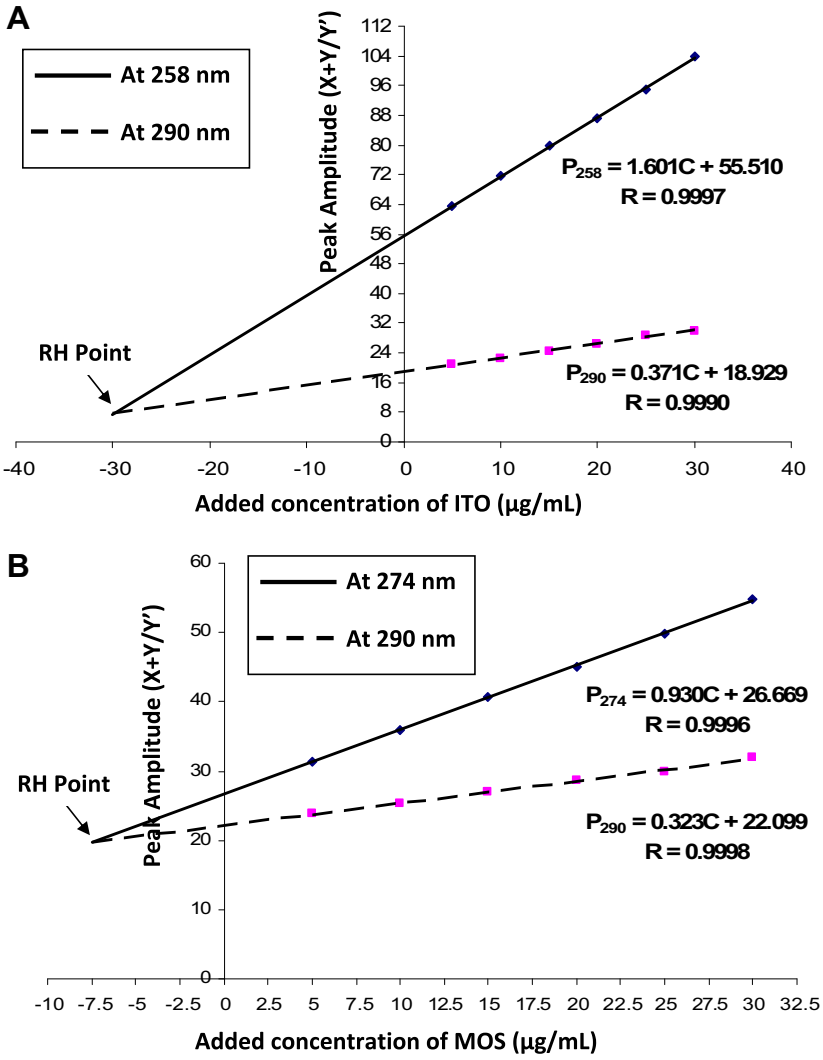

Fig. 3. Plots of ratio H-point standard addition method between the peak amplitudes ($X = Y/Y'$) and the added (X) concentration: (A) added concentration of ITO to mixture of 30.0 µg/mL ITO and 8.0 µg/mL PAN; (B) added concentration of MOS to mixture of 7.5 µg/mL MOS and 20.0 µg/mL PAN.

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$$M_1P_2 - M_2P_1 = M_1(X/Y')_2 + M_1(Y/Y') - M_2(X/Y')_1 - M_2(Y/Y')$$

$$P_{RH} = P_1 = P_2$$

So

$$P_{RH}(M_1 - M_2) = (Y/Y')(M_1 - M_2) + M_1(X/Y')_2 - M_2(X/Y')_1$$

$$P_{RH} = (Y/Y') + [M_1(X/Y')_2 - M_2(X/Y')_1]/(M_1 - M_2) \quad (7)$$

As $(X/Y')_1/M_1 = (X/Y')_2/M_2$, therefore, $M_1(X/Y')_2 = M_2(X/Y')_1$, so the numerator $(M_1(X/Y')_2 - M_2(X/Y')_1)$ in Eq. (7) equals to zero, and so Eq. (7) can be written as

$$P_{RH} = (Y/Y') = C_Y \quad (8)$$

As explained before in Eq. (4) and (7), C_x and C_y can be calculation from the two calibrations as follow:

$$C_X = B - A/(M_1 - M_2)$$

$$C_Y = (M_1B - M_2A)/(M_1 - M_2)$$

where A and B are the intercepts of the two regressions, such that $A = (X/Y')_1 + (Y/Y')$ and $B = (X/Y')_2 + (Y/Y')$.

Application of RHPSAM to the determination of the studied binary mixtures

In order to apply RHPSAM for the determination of ITO–PAN and MOS–PAN binary mixtures, the selection of the divisor is a critical step. Several normalized PAN concentrations (5, 10, 15 μg/mL) were chosen as a divisor where the normalized 10 μg/mL PAN spectrum was the most suitable divisor as it gives the least noise, and so it was used as a divisor for the analysis of the binary mixtures by the proposed method.

Another factor to be studied was the estimation of the analytical wavelength pair. In order to choose the optimum two wavelengths to perform the calibrations, certain mixture was selected to be analyzed at three different pairs of wavelengths. The λ_{max} (258 nm or 274 nm) of the added component was compared to three other wavelengths (280, 290, and 300 nm). Tables 1 and 2 showed the obtained results from the calculated calibration at each wavelength (slope and intercept) and the found concentrations of each component, as well. The recovery percentages showed no significant difference in the accuracy of drugs determinations using any of the three pairs, which justifies the applicability of the proposed RHPSAM using wide range of wavelengths.

The determination of ITO, MOS and PAN samples by the proposed method was done by plotting ratio H-point standard addition calibrations. Fig. 4 showed the plotting of calibrations, at 258 nm and 290 nm, between the peak amplitudes and added ITO concentrations to three pure samples of ITO (15, 22.5 and 30 μg/mL) and PAN (4, 8, and 12 μg/mL), likewise, Fig. 5 illustrated the plotting of calibrations, at 274 nm and 290 nm, between the peak amplitudes and added MOS concentrations to three pure samples of MOS (5, 7.5 and 10 μg/mL) and PAN (10, 15, and 20 μg/mL).

Fig. 6 presented the resulting RH-points obtained after the analysis of ITO–PAN and MOS–PAN mixtures, the obtained calibrations for these mixtures by applying the proposed method were conducted in Tables 3 and 4, where each drug's concentration could be calculated using the regression parameters of each pair of equations, the same table also showed the found concentrations in each mixture.

Table 1
Results of several experiments using different wavelength pairs for the analysis a synthetic mixture of ITO (15 μg/mL) and PAN (4 μg/mL).

Wavelength (nm)	Regression equation[a]	258 nm (ITO–PAN)			
		Found (μg/mL)		Recovery %	
		ITO	PAN	ITO	PAN
280	$P = 0.486C + 11.461$	15.17	4.09	101.13	102.25
290	$P = 0.369C + 9.628$	15.22	4.01	101.47	100.25
300	$P = 0.261C + 7.912$	15.28	3.92	101.87	98.00

[a] The regression equation at 258 nm is $P = 1.582C + 28.091$.

Table 2
Results of several experiments using different wavelength pairs for the analysis a synthetic mixture of MOS (5 μg/mL) and PAN (10 μg/mL).

Wavelength (nm)	Regression equation[a]	274 nm (MOS–PAN)			
		Found (μg/mL)		Recovery %	
		MOS	PAN	MOS	PAN
280	$P = 0.677C + 13.381$	4.90	10.06	98.00	100.60
290	$P = 0.322C + 11.527$	5.08	9.89	101.6	98.90
300	$P = 0.564C + 12.754$	5.09	9.88	101.8	98.80

[a] The regression equation at 274 nm is $P = 0.934\ C + 14.638$.

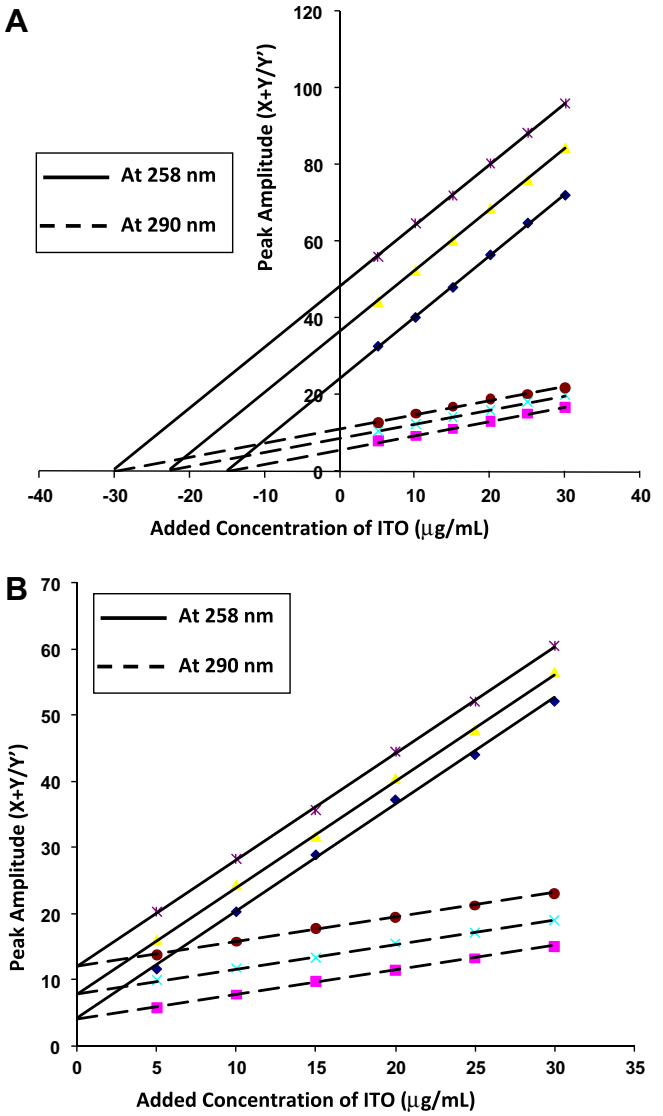

Fig. 4. Plot of ratio H-point standard addition method for blind pure samples of (A) ITO and (B) PAN.

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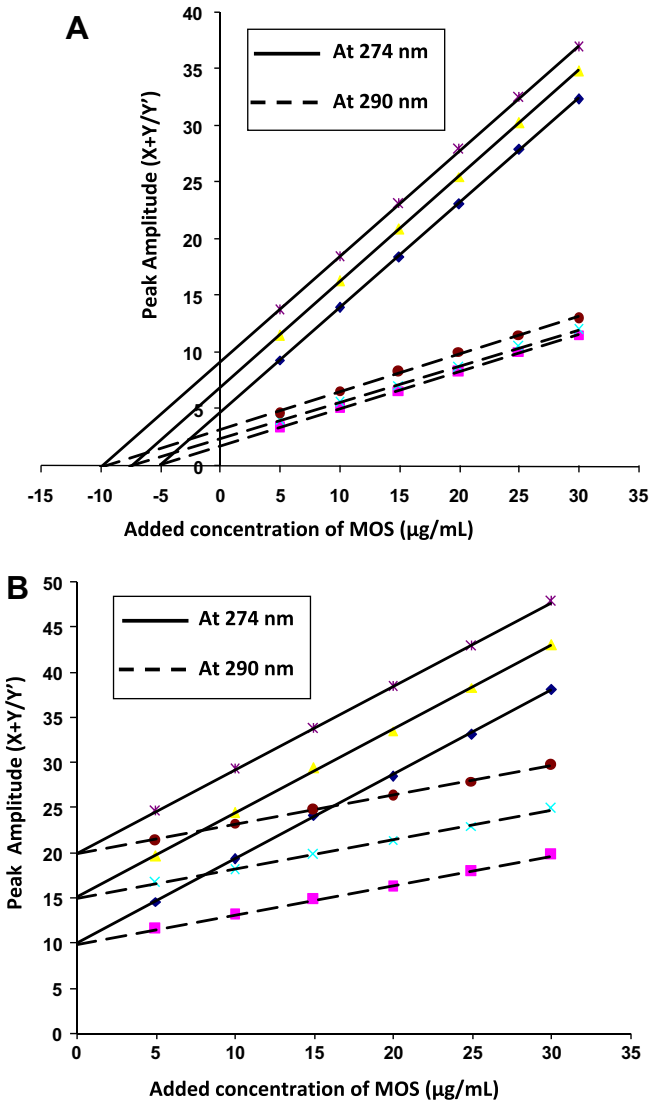

Fig. 5. Plot of ratio H-point standard addition method for blind pure samples of (A) MOS and (B) PAN.

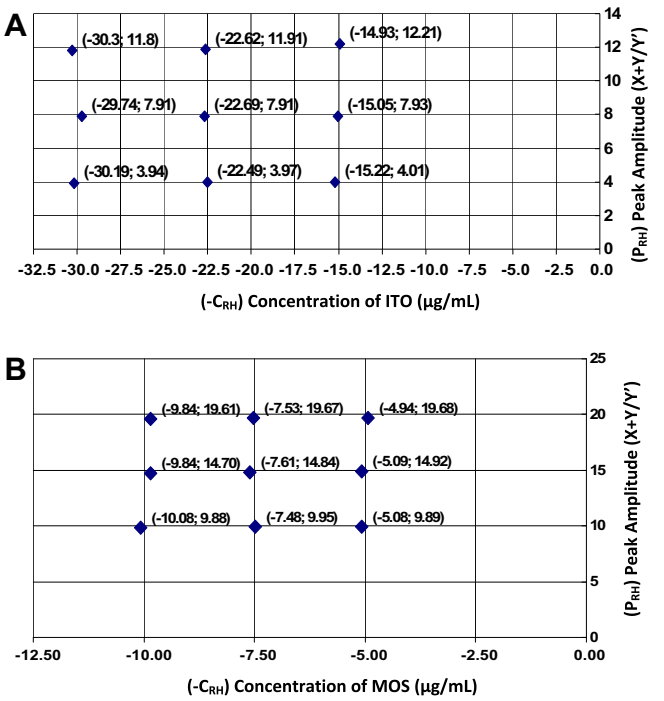

Fig. 6. The resulting RH-points from the analysis of different synthetic mixtures of (A) ITO–PAN and (B) MOS–PAN.

Table 3
Results of several experiments for the analysis of synthetic mixtures at different concentration ratios of ITO–PAN.

Mix No.	Regression equation[a]	*R*	Taken (μg/mL)		Found (μg/mL)	
			ITO	PAN	ITO	PAN
1	P_{258} = 1.582*C* + 28.091 P_{290} = 0.369*C* + 9.628	0.9995 0.9991	15	4	15.22	4.01
2	P_{258} = 1.599*C* + 32.005 P_{290} = 0.368*C* + 13.475	0.9998 0.9994	15	8	15.05	7.94
3	P_{258} = 1.597*C* + 36.045 P_{290} = 0.371*C* + 17.743	0.9996 0.9990	15	12	14.93	12.20
4	P_{258} = 1.587*C* + 39.678 P_{290} = 0.371*C* + 12.328	0.9999 0.9996	22.5	4	22.49	3.98
5	P_{258} = 1.583*C* + 43.824 P_{290} = 0.366*C* + 16.202	0.9999 0.9998	22.5	8	22.70	7.90
6	P_{258} = 1.605*C* + 48.204 P_{290} = 0.372*C* + 20.312	0.9997 0.9996	22.5	12	22.62	11.90
7	P_{258} = 1.597*C* + 52.175 P_{290} = 0.369*C* + 15.098	0.9998 0.9990	30	4	30.19	3.96
8	P_{258} = 1.601*C* + 55.510 P_{290} = 0.371*C* + 18.929	0.9997 0.9990	30	8	29.74	7.90
9	P_{258} = 1.598*C* + 60.219 P_{290} = 0.368*C* + 22.955	0.9998 0.9990	30	12	30.30	11.81

[a] In regression equations '*C*' is the added concentration of ITO.

Method validation

Validation was done according to ICH guidelines [19].

Linearity

The linearity of the method was evaluated by analyzing different concentrations of ITO (5–60 μg/mL), MOS (5–40 μg/mL) and PAN (4–24 μg/mL). The assay was performed according to the experimental conditions previously described. The linear equations were summarized in Table 5.

Range

The calibration range was established through the consideration of practical range necessary according to adherence to Beer's law and the concentration of ITO, MOS and PAN present in the pharmaceutical combinations to give accurate, precise and linear results, Table 5.

Accuracy

Accuracy was checked by analysis of blind pure samples of ITO, MOS and PAN by the proposed method, were good results were obtained. The mean recoveries and RSD of pure samples analysis are shown in Table 5.

Selectivity

Selectivity of the method was achieved by the analysis of different laboratory prepared mixtures of ITO–PAN and MOS–PAN, with the linearity range and surrounding their pharmaceutical combinations. The recovery percentage and RSD were satisfactory enough to assist the selectivity as shown in Table 5.

Precision

Repeatability. Three different concentrations of ITO (15, 22.5 and 30 μg/mL), MOS (5, 7.5 and 10 μg/mL) and PAN (10, 15, 20 μg/mL)

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Table 4
Results of several experiments for the analysis of synthetic mixtures at different concentration ratios of MOS–PAN.

Mix No.	Regression equation[a]	R	Taken (μg/mL)		Found (μg/mL)	
			MOS	PAN	MOS	PAN
1	$P_{274} = 0.934C + 14.638$ $P_{290} = 0.322C + 11.527$	0.9999 0.9990	5	10	5.08	9.89
2	$P_{274} = 0.933C + 19.663$ $P_{290} = 0.321C + 16.553$	0.9999 0.9990	5	15	5.09	14.92
3	$P_{274} = 0.933C + 24.284$ $P_{290} = 0.333C + 2.963$	0.9998 0.9990	5	20	4.94	19.68
4	$P_{274} = 0.933C + 16.927$ $P_{290} = 0.321C + 12.352$	0.9999 0.9996	7.5	10	7.48	9.95
5	$P_{274} = 0.931C + 21.927$ $P_{290} = 0.320C + 17.278$	0.9999 0.9997	7.5	15	7.61	14.84
6	$P_{274} = 0.930C + 26.669$ $P_{290} = 0.323C + 22.099$	0.9996 0.9998	7.5	20	7.53	19.67
7	$P_{274} = 0.931C + 19.263$ $P_{290} = 0.320C + 13.109$	0.9999 0.9990	10	10	10.08	9.88
8	$P_{274} = 0.932C + 23.876$ $P_{290} = 0.330C + 17.951$	0.9997 0.9990	10	15	9.84	14.70
9	$P_{274} = 0.933C + 28.791$ $P_{290} = 0.321C + 22.773$	0.9996 0.9990	10	20	9.84	19.61

[a] In regression equations 'C' is the added concentration of MOS.

were analyzed three times intra-daily using the proposed method. The relative standard deviations were calculated in Table 5, showing low deviations and high repeatability.

Intermediate precision. The previous procedures were repeated inter-daily on three different days for the analysis of the three previously chosen concentrations. The relative standard deviations were calculated in Table 5, showing high intermediate precision.

Application of the method in assay of pharmaceutical formulation

The proposed method was successfully applied for the determination of the studied drugs in their pharmaceutical dosage form, the mean recoveries ± RSD in Pantocid IT® capsules were 100.82 ± 0.505 and 100.33 ± 0.801 for ITO and PAN, respectively. While, in Moza plus® capsules were 101.11 ± 0.275 and 98.93 ± 0.568 for MOS and PAN, respectively.

Statistical analysis

The results obtained by the proposed method were statistically compared with those obtained by applying the reported methods for ITO–PAN determination [11] and MOS–PAN determination [12]. The calculated t-value and F-value were less than theoretical ones which indicates that there is no significant difference between the proposed and reported methods regarding both accuracy and precision, as shown in Table 6. However, the RHPSAM is relatively simple in addition to its good reproducibility because the noises present in the ratio spectra are easily eliminated by the use of dual wavelength spectrophotometry along with standard addition technique. Moreover, any shift in the selected wavelength will not affect the results. Contrary from other spectrophotometric methods which require an optimum selection of working wavelengths, and any minor shift in these wavelengths yields unaccepted results.

Table 5
Regression and validation parameters for the determination of ITO, MOS and PAN in bulk powder by the proposed method.

Parameter	ITO		MOS		PAN		
	258 nm	290 nm	274 nm	290 nm	258 nm	290 nm	274 nm
Linearity							
Range (μg/mL)	5–60		5–40			4–24	
Slope	1.600	0.369	0.930	0.330	0.999	1.001	0.999
SE of slope	0.006	0.005	0.005	0.004	0.004	0.009	0.008
Intercept	0.267	0.215	−0.010	−0.015	−0.062	0.091	0.048
SE of intercept	0.210	0.156	0.126	0.089	0.068	0.164	0.132
Correlation coefficient (R)	0.9999	0.9996	0.9999	0.9997	0.9999	0.9998	0.9995
LOD (μg/mL)	0.61	1.12	0.53	1.06	0.18	0.45	0.36
LOQ (μg/mL)	1.86	3.41	1.61	3.20	0.56	1.36	1.09
Accuracy (Mean ± RSD)	101.22 ± 0.635		99.04 ± 0.406		101.06 ± 0.629		98.89 ± 0.369
Specificity (Mean ± RSD)	100.38 ± 0.732		100.14 ± 1.371		99.36 ± 1.058		98.72 ± 0.566
Precision (RSD)							
Repeatability	±0.324		±0.294		±0.492		±0.440
Reproducibility	±0.362		±0.311		±0.521		±0.485

Table 6
Statistical comparison between the proposed and reported methods for the determination of ITO–PAN and MOS–PAN binary mixtures.

Value	ITO–PAN				MOS–PAN			
	Proposed		Reported [11][a]		Proposed		Reported [12][b]	
	ITO	PAN	ITO	PAN	MOS	PAN	MOS	PAN
Mean	100.38	99.36	100.82	99.77	100.14	98.72	99.82	99.28
SD	0.732	1.052	1.082	1.104	1.373	0.559	1.123	0.764
RSD	0.732	1.058	1.073	1.106	1.371	0.566	1.125	0.770
n	9	9	9	9	9	9	9	9
V (variance)	0.540	1.107	1.171	1.219	1.885	0.312	1.261	0.584
Student's t test (2.12)[c]	1.01	0.81	–	–	0.54	1.78	–	–
F (3.44)[d]	2.17	1.10	–	–	1.49	1.87	–	–

[a] Gupta et al. [11]; Simultaneous equation method at 289 nm and 258 nm in distilled water.
[b] Bhatt et al. [12]; First derivative spectrophotometry at 252.1 nm for mosapride and 302.4 nm for pantoprazole in acetonitrile.
[c] The corresponding theoretical values of t at ($P = 0.05$).
[d] The corresponding theoretical values of F at ($P = 0.05$).

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Conclusion

The previous discussion revealed that the proposed ratio H-point standard addition method is simple, accurate and precise for resolving the spectral overlapping and the simultaneous determination of PAN binary mixtures with ITO and MOS. However RHPSAM has several advantages over conventional ratio spectrophotometry and HPSAM that can be summarized as follows:

(1) Direct determination of both components in a mixture, simultaneously, which could not be achieved in any other conventional ratio spectra methods, or even in HPSAM which requires two separate procedures and calibration steps for the determination of each component.
(2) There is no constrains in choosing the two specific analytical wavelengths and any pair of wavelengths could be used, as the constant values generated in the ratio curves are extended along the ratio spectra, contrary from conventional HPSAM in which the choice of the two wavelengths is a critical step and limited to two critical wavelengths where the pure interfering component has constant absorbance value.
(3) Both proportional and constant errors produced by the sample matrix are corrected directly, along with any embedded errors produced from any interfering substance and/or blank reagent.

Moreover the proposed spectrophotometric technique could be successfully applied for the routine analysis of the studied drugs in pure form and in their combined dosage form in quality control laboratories without any preliminary separation steps.

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