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ORIGINAL PAPER

Liquid Chromatography Coupled with Fluorimetric Detection and Third Derivative Synchronous Fluorescence Spectroscopy as two Analytical Methods for the Simultaneous Determination of Rabeprazole Sodium and Domperidone After Derivatization with 4-Chloro-7-Nitrobenzofurazan

F. Ibrahim · M. E. K. Wahba

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Abstract Two simple, sensitive, rapid, economic and validated methods, namely reversed phase liquid chromatography (method I) and third derivative synchronous fluorescence spectroscopy (method II) have been developed for the simultaneous determination of rabeprazole sodium and domperidone in their laboratory prepared mixture after derivatization with 4-Chloro-7-nitrobenzofurazan. Reversed phase chromatography was conducted using a Zorbax® SB-Phenyl column (250.0 mm × 4.6 mm id) combined with a guard column at ambient temperature with fluorimetric detection at 540 nm after excitation at 483 nm. A mobile phase composed of a mixture of distilled water with methanol and acetonitrile in a ratio of 50:20:30 adjusted pH to 4 has been used at a flow rate of 1 mL/min. Sharp well resolved peaks were obtained for domperidone and rabeprazole sodium with retention times of 5.5 and 6.4 min respectively. While in method II, the third-derivative spectra were estimated at 507 and 436 nm for rabeprazole sodium and domperidone respectively. Linearity ranges for rabeprazole sodium and domperidone respectively in both methods were found to be 0.15–2.0 and 0.1–1.5 μg/mL. The proposed methods were successfully applied for the analysis of the two compounds in their binary mixtures, and laboratory prepared tablets. The obtained results were favorably compared with those obtained by the comparison method. Furthermore, detailed validation procedure was also conducted.

F. Ibrahim · M. E. K. Wahba (✉)
Department of Analytical Chemistry Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
e-mail: marywahba@ymail.com

Keywords Liquid chromatography · Derivative synchronous fluorescence spectroscopy · Rabeprazole · Domperidone

Introduction

Rabeprazole sodium (RPZ), chemically 2-[4-(−3methoxypropoxy)-3-methyl-2-pyridinyl] methyl] sulfinyl]-1H-benzimidazole sodium salt [1] (Fig. 1), a pro drug metabolized by P450 or CYP450, acts as a selective and irreversible proton-pump inhibitor which suppresses gastric acid secretion by specific inhibition of the gastric hydrogen–potassium adenosine triphosphatase H^+, K^+ ATPase enzyme system at the secretory surface of gastric parietal cells. It inhibits the final transport of hydrogen ions (via exchange with potassium ions) into the gastric lumen [2].

The literature reveals that several chromatographic methods have been reported for the determination of RPZ in pharmaceutical dosage forms (as single component) by HPLC [3–5], stability- indicating HPLC in the presence of its degradation products [6, 7] and TLC densitometric determinations [4, 8]. Several analytical methods shave been described for the simultaneous determination of RPZ with many drugs of pharmaceutical interest using HPLC, HPTLC [9–11], and spectrophotometric methods [12, 13].

Domperidone (DOM), 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl) propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazole2-one [14] (Fig. 2) is a dopamine antagonist used as an antiemetic for the short-term treatment of nausea and vomiting of various etiologies. Domperidone, is indicated for treating symptoms associated with upper gastrointestinal motility disorders caused by chronic and sub-acute gastritis. It is a gastrointestinal emptying adjunct, a peristaltic

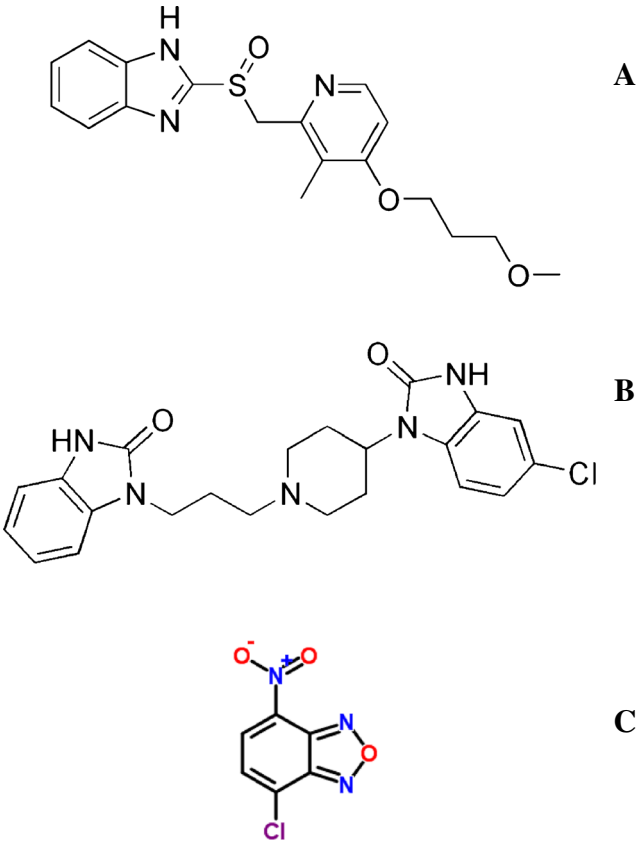

Fig. 1 Structural formulae of: **A**) RPZ, **B**) DOM, **C**) NBD-Cl

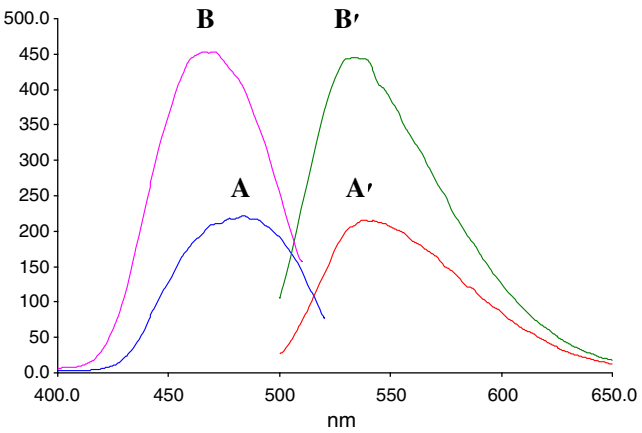

Fig. 2 Fluorescence spectra of: **A** and **A'** are excitation and emission spectra of the reaction product of RPZ (0.4 μg/mL) with NBD-Cl, **B** and **B'** are excitation and emission spectra of the reaction product of DOM (0.6 μg/mL) with NBD-Cl

stimulant, and also exhibits antiemetic properties. Domperidone is also used to prevent stomach problems associated with the use of certain medications used to treat Parkinson's disease. Several analytical methods were reported for quantitative determination of DOM including spectrophotometry [15], high performance liquid chromatography [16–18] and anodic differential pulse voltammetry. [19].

However, most of the methods reported for the assay of the studied drugs require expensive and sophisticated instruments and are time consuming. Hence it is worthwhile to develop simple and sensitive methods for their analysis.

It was reported that RPZ and DOM were previously determined applying HPLC coupled with UV detection [20] and derivative spectrophotometry [21]. The reported HPLC method [20] utilized 0.01 M ammonium acetate buffer: methanol: acetonitrile (40:30:30 *v/v*, pH 7.44) as the mobile phase at a flow rate of 1 mL/min. at ambient temperature and UV detection at 287 nm. The retention time of RPZ and DOM were found to be 6.13 and 8.38 min., respectively. The linearity ranges of the two drugs were in the range of 0.2–2.0 μg/mL and 0.3–3.0 μg/mL, respectively. However, the proposed HPLC method carries the advantages of being more rapid (retention times of RPZ and DOM are 5.51, 6.44 respectively), more sensitive, with wider linearity ranges; more than ten times fold (0.15–2.0 μg/mL, 0.1–1.5 μg/mL for RPZ and DOM respectively), using a simply prepared mobile phase.

4-Chloro-7-nitrobenzofurazan (NBD-Cl; Fig. 3) is a suitable labeling agent which reacts with both primary and secondary amines producing colored products which exhibit strong fluorescence. Stable adducts with low background noise and higher signal/noise ratio are provided by this agent [22]. To our knowledge there was not any report about its application in derivatization of the drugs under investigation.

Literature survey revealed neither liquid chromatography with fluorimetric detection nor third derivative synchronous fluorescence spectroscopy (TDSFS) after using NBD-Cl as derivatizing agent was applied for simultaneous determination of the titled drugs in binary mixture; which encouraged us to perform this study.

Experimental

Instrumentation

- The fluorescence spectra and measurements were recorded using a Perkin Elmer LS 45 Luminescence Spectrometer equipped with a 150 W Xenon arc lamp. A 1 cm quartz cell was used. Derivative spectra were evaluated using Fluorescence Data Manager (FLDM) software, Perkin Elmer Buck i.e. FL WINLAB, version 400.02.
- Separations were performed using a Merck Hitachi L-7100 chromatograph equipped with a Rheodyne injector valve with a 20 μL loop and a L-7400 UV detector (Darmstadt, Germany). Chromatograms were recorded on a Merck Hitachi D-7500 integrator. Mobile phases were degassed using Merck L-7612 solvent degasser.
- Hanna pH-Meter (Bucharest, Romania) was used for pH adjustments.

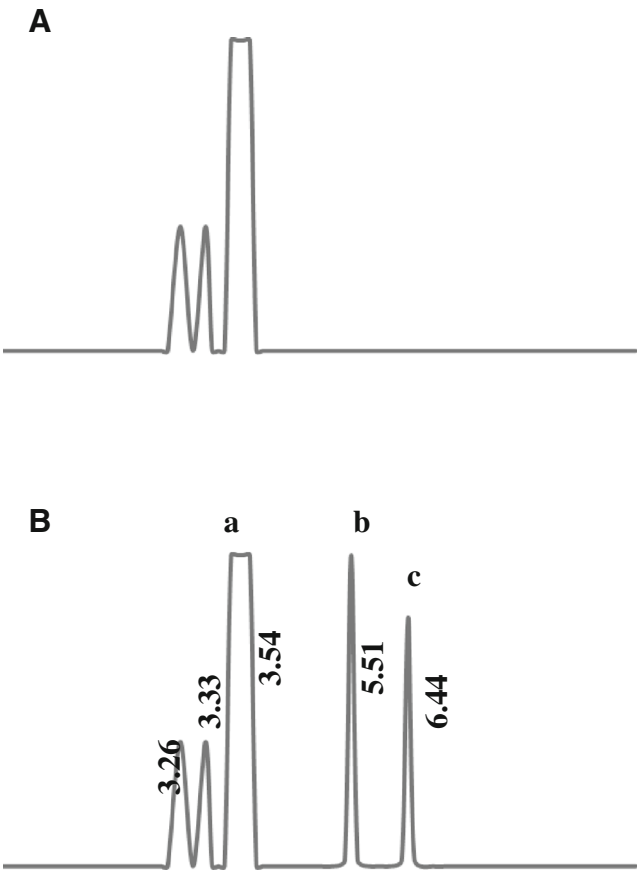

Fig. 3 **A**) Blank chromatogram **B)** typical chromatogram of laboratory prepared mixture of the studied drugs where: **a**) Solvent front **b**) DOM (0.6 μg/mL), **c**) RPZ (0.4 μg/mL)

Materials and reagents

- Rabeprazole sodium (RPZ); Domperidone (DOM) of purities 98.74 % and 99.44 % respectively were kindly provided by Sigma and Epico Pharmaceutical Companies, Cairo, Egypt.
- Methanol, acetonitrile (Sigma-Aldrich), HPLC grade.
- Ortho phosphoric acid (0.2 M); (Riedel de Häen, Seelze, Germany).

Development of HPLC method

Separation was achieved on a Zorbax® SB-Phenyl column (250.0 mm × 4.6 mm id) combined with a guard column (Merck, Darmstadt, Germany). The column was operated at ambient temperature. The analytical system was washed daily with 60 mL of 1:1 mixture of water and methanol to eliminate the mobile phase; this did not cause any change in the column performance. The mobile phase was prepared by mixing distilled water with methanol and acetonitrile in a ratio of 50:20:30 and adjusting pH to 4 using 0.2 M ortho phosphoric acid. The mixture was then sonicated for 30 min. The resulting mobile phase was filtered through a 0.45 μm membrane filter (Millipore, Ireland).

Standard solutions and calibration

Stock solutions containing 1.0 mg/mL of RPZ and DOM were prepared by dissolving in 10 mL methanol and further diluting with the mobile phase (method I), or with same solvent (method II) to obtain a final working concentration of 10.0 μg/mL. The solutions were found to be stable for at least 2 weeks when kept in the refrigerator.

Procedure for individual fluorescent determination of RPZ and DOM after derivatization with NBD-Cl

Suitable aliquot volumes of the final working solutions were transferred into 10 mL volumetric flasks so that the final concentrations will be within the linearity ranges of 0.15–2.0 μg/mL for RPZ or 0.1–1.5 μg/mL for DOM. 3 mL of 0.2 M borate buffer of pH 9 followed by 2 mL of 0.1 % methanolic solution of NBD-Cl were added. The solutions were heated at 70 °C for 10 min, and cooled in ice-water, after which 0.2 mL of HCl 12 M were added, and dilution to the mark with methanol was performed. The relative fluorescence intensities were measured at $\lambda_{em/ex}$ of 540/483 nm, 535/467 nm for RPZ and DOM respectively. A blank experiment was performed simultaneously.

Construction of calibration graphs of HPLC method.

Suitable aliquot volumes of the final working solutions were transferred into 10 mL volumetric flasks so that the final concentrations will be within the linearity ranges of 0.15–2.0 μg/mL for RPZ or 0.1–1.5 μg/mL for DOM. Derivatization was performed as mentioned before, after which; dilution to the mark with the mobile phase was carried out. Twenty μL aliquots were injected (in triplicates) and eluted with the mobile phase under the described chromatographic conditions. The calibration curves were constructed by plotting the peak areas against the final concentrations of the drugs (μg/mL). Alternatively, the corresponding regression equations were derived.

Construction of calibration graphs of TDSFS method

The synchronous fluorescence spectra of the derivatized solutions were recorded by scanning both monochromators at ($\Delta\lambda$) =40 nm and a scan rate of 600 nm/min using 10 nm excitation and emission windows. The third-derivative fluorescence spectra of RPZ and DOM were derived from the normal synchronous spectra using FLDM software. For best resolution and smoothing 99 points were used for deriving the third-derivative spectra. The fluorescence intensities of the third-derivative spectra were estimated at 507 and 436 nm for RPZ and DOM respectively. A blank experiment was

performed simultaneously. The corrected peak amplitude of the third derivative technique was plotted versus final concentration of the drug (μg/mL) to obtain the calibration graphs. Alternatively, the corresponding regression equations were derived.

Analysis of laboratory prepared mixtures

Aliquots of RPZ and DOM standard solutions in the ratio of 2:3 were transferred into a series of 10 mL volumetric flasks, derivatized as previously mentioned, and diluted either with the mobile phase (method I) or methanol (method II) to the mark. Then, the steps described under "Construction of calibration graphs of HPLC method or TDSFS method" were preceded. The percentage recoveries were calculated by referring to the calibration graphs previously prepared or by applying the regression equations.

Analysis of laboratory prepared co formulated tablets

Laboratory-prepared tablets containing 20.0 mg RPZ and 30.0 mg DOM were prepared by mixing the drugs with the tablet excipients: lactose (15 mg), starch (15 mg), talc (20 mg), and magnesium stearate (10 mg) per each tablet. An accurately weighed quantity of the mixed contents of 10 prepared tablets equivalent to 2.0 mg RPZ and 3.0 mg DOM (according to their pharmaceutical ratio) was transferred into a small conical flask and extracted with 3×30 mL of methanol. The extract was filtered into 100 mL volumetric flask. The conical flask was washed with few milliliters of methanol; the washings were added to the filterate and completed to the mark with the same solvent. The procedure was followed as described under "Construction of calibration graphs of HPLC method or TDSFS method". The nominal contents of the laboratory prepared tablets were calculated using either the calibration graphs or the corresponding regression equations.

Results and discussion

Since RPZ is nonfluorescent [4], derivatization with NBD-Cl was carried out yielding a highly fluorescent derivative measured at λ_{em} of 540 nm after excitation at λ_{ex} of 483 nm . On the contrary, DOM is characterized by its highly native fluorescence [23]. However, throughout the experimental trials it was found that upon addition of NBD-Cl, DOM undergoes a complete reaction under the same experimental conditions used for RPZ giving rise to a highly fluorescent derivative measured at λ_{em} of 535 nm after excitation at λ_{ex} of 467 nm (Fig. 2). This reaction was further used to develop two sensitive, rapid, and economic methods for the simultaneous determination of the studied drugs; namely HPLC coupled with fluorimetric detection and TDSFS.

HPLC method

Under the described chromatographic conditions clear base line separation with satisfactory resolution between the produced chromatographic peaks was achieved in a short chromatographic run; less than 6.5 min (Fig. 3) permitting quantification of the studied drugs The proposed method was assessed for selectivity, linearity, precision, accuracy, stability, and recovery.

Different experimental parameters affecting the separation selectivity of the liquid chromatographic system have been investigated and optimized. Hence, the method was applied to the determination of the studied drugs in their laboratory prepared co formulated tablets.

Selection and optimization of the chromatographic conditions

Well-defined symmetrical peaks were obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials.

Three columns were used for performance investigations, including: EC nucleosil C18-SN: 4115568 column, Hibar prepacked column RT-250–4-L-100-RP8, and Zorbax® SB-Phenyl column (250 mm × 4.6 mm id). Experimental studies revealed that, the third column was appropriate, giving symmetrical, well defined peaks and allowing good separation of peaks of the studied drugs.

Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of type and ratio of the organic modifier, the pH and the flow rate. The results achieved are summarized in Table 1. Methanol and acetonitrile were investigated for selection of the proper organic modifier for the assay, when either of them was used alone; inadequate separation of RPZ and DOM was attained. On the other hand; a mixture of acetonitrile: methanol (30:20, v/v) was chosen, since it yields symmetrical, narrow and well-resolved peaks within reasonable retention times.

The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing organic modifier; (acetonitrile: methanol 30:20, v/v): water in a ratio of (30:70–70:30, v/v). Ratios lower than 45 % of organic modifier resulted in long un acceptable retention times of both drugs. Meanwhile, mobile phases containing ratios higher than 55 % of organic phase caused elution of DOM with solvent front. Finally, mobile phase consisting of acetonitrile: methanol: water mixture in the ratio of 30:20:50, v/v/v (of final pH 4), was considered the optimal one as it gave a good compromise between retention times, resolution, number of theoretical plates, and peak shape.

To investigate the effect of the final pH of the mobile phase on the selectivity and retention times of the test solutes, mobile

Table 1 Optimization of the chromatographic conditions for separation of RPZ and DOM by proposed HPLC method

Parameter		Number of theoretical plates (N)		Capacity factor K'		Selectivity factor α
		DOM	RPZ	DOM	RPZ	
Ratio of organic modifier: water	70:30	2220	6605	1.14	1.43	1.25
	60:40	2885	8723	0.86	1.07	1.24
	55:45	7461	9756	0.53	0.83	1.57
	50:50	7387	9666	0.52	0.84	1.62
	45:55	7298	9634	0.51	0.84	1.65
	40:60	2116	8774	0.29	0.43	1.48
	30:70	1032	8934	0.09	0.14	1.59
Ratio of acetonitrile: methanol	Methanol alone	5342	7823	0.71	0.77	1.09
	Aetonitrile alone	5634	8567	0.68	0.72	1.06
	10:40	5587	8834	0.48	0.79	1.65
	20:30	6231	9432	0.49	0.81	1.65
	30:20	7387	9666	0.52	0.84	1.62
	40:10	6754	8675	0.51	0.82	1.61
pH	2.5	4213	6234	0.51	0.81	1.59
	3.0	6203	8231	0.52	0.81	1.56
	3.5	7452	9612	0.51	0.84	1.65
	4.0	7387	9666	0.52	0.84	1.62
	4.5	7732	9512	0.53	0.85	1.61
	5.0	6321	7125	0.52	0.84	1.62
	5.5	4115	5539	0.51	0.85	1.67
	6.0	2034	4431	0.55	0.84	1.53

phases of pH values ranging from 2.5 to 6.0 were tested. Phosphate buffer and Britton Robinson Buffer were tried, but over the entire pH range, distorted base line was obtained, hence water was used instead.

RPZ has PKa values of 4.24 and 9.35. Being amphoteric in nature, RPZ remains in ionic form in the entire pH range. At pH lower than 3.5, the carboxylic group of RPZ is protonated and therefore not charged, therefore, RPZ just bears one positive charge and its retention time increases. In the intermediate pH range (pH 3.5-4.5), both, the carboxylic acid group and the amino group, are ionized. Due to the fact that there is now a dual charge on the molecule, the retention time is intermediate. When pH is higher than 4.5, the amino group is deprotonated, the molecule bears only a single negative charge, and thus the retention increases again. On the other hand, PKa of DOM is 7.9 manifesting its alkaline nature due to the presence of tertiary amino groups. Hence, at pH lower than 3.5 the retention time decreases, and DOM is eluted with the solvent front. Meanwhile, at pH higher than 4.5, the retention time increases and it is not well resolved from RPZ.

Hence, in chromatographic reversed phase separation, both hydrophobic and ionic interactions will take part. This is in compliance with the observation reported that the functional dependence of retention factor on pH of mobile phase differs from the ideal sigmoidal shape of predicted hydrophobic interactions, indicating additional donor–acceptor interactions. These interactions of protonated amino group moiety with residual silanol groups of stationary phase in acidic media often cause problems in chromatographic determination of RPZ and DOM, manifesting mainly with bad peak shape; peak broadening and tailing. Based on these facts, pH 4 was the most appropriate one giving well shaped separated peaks of both drugs.

The effect of flow rate on the separation of peaks of the studied drugs was investigated and a flow rate of 1 mL/min was found to be the optimal one for good separation within a reasonable time. Flow rates greater than 1 mL/min caused broad peaks, while, flow rates lower than 1 mL/min caused long retention times.

Third derivative synchronous fluorescence spectroscopy method

Synchronous Fluorescence spectra of RPZ and DOM

It is necessary to record first the normal synchronous spectra of RPZ and DOM to derive the third-derivative synchronous spectra. Figure 4 shows the synchronous fluorescence spectra of RPZ at 499 nm in presence of DOM, and DOM at 454 nm in presence of RPZ. There is still a great overlap of the

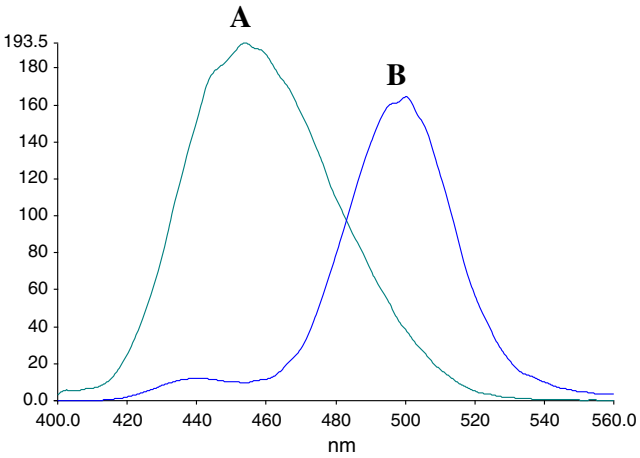

Fig. 4 Synchronous fluorescence spectra at (Δλ =40) of: **A**) DOM (0.6 μg/mL) **B**) RPZ (0.4 μg/mL)

spectra of both drugs in normal synchronous spectroscopy; this encouraged us to perform TDSFS technique for the simultaneous determination of RPZ and DOM in their binary mixture without prior extraction or separation step. It is worth to mention that the authors tried lower derivative techniques (FDSFS, SDSFS) to separate the two drugs, but overlap in their spectra still exist, hence, TDSFS was utilized. The fluorescence spectra of RPZ and DOM were separated entirely using TDSFS with a zero crossing point technique of measurement. Figure 5 shows that RPZ and DOM could be measured at 507 nm and 436 nm respectively. The proposed method was applied to the simultaneous determination of RPZ with DOM in laboratory prepared mixtures containing different concentrations of both drugs in a ratio of 2:3 as present in their co-formulated dosage forms.

Optimization of Experimental Conditions

Different experimental parameters affecting the performance of the proposed method were carefully studied and optimized.

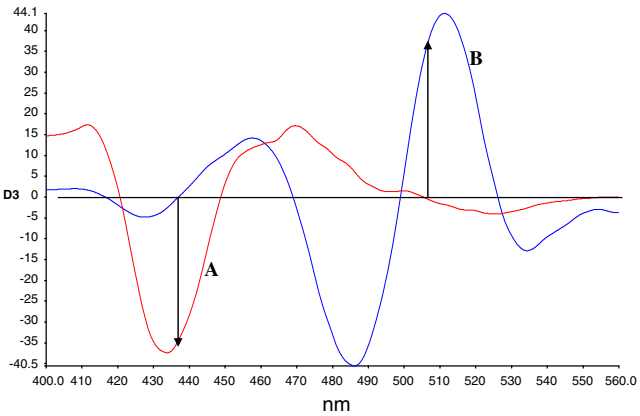

Fig. 5 Third derivative synchronous fluorescence spectra of a mixture of: **A**) DOM (0.6 μg/mL) **B**) RPZ (0.4 μg/mL)

Such factors were changed individually, while others were kept constant.

*Selection of optimum Δ λ.

The synchronous fluorescence spectra of RPZ and DOM were recorded using different Δ λ. The optimum Δ λ value is very important for performing the synchronous fluorescence scanning technique concerning resolution, sensitivity, and features. It can directly influence spectral shape, bandwidth, and signal value. For this reason, a wide range of Δ λ (40, 60, 80, 100, and 120) was examined. When Δ λ was higher than 40 nm, poor separation of the two peaks was obtained in addition to lower fluorescence intensities. Therefore, Δ λ of 40 was chosen as optimal for separation of a mixture of RPZ with DOM, since it resulted in two distinct peaks with good shape, and to minimize the spectral interferences caused by each compound in the mixture.

*Effect of time.

The effect of time on the synchronous fluorescence of the drugs was also studied. It was found that the fluorescence emission developed instantaneously and remained stable for more than 1 hour.

*Effect of diluting solvent.

Dilution with different solvents such as water, methanol, ethanol, acetonitrile, dimethylsulfoxide and dimethylformamide was performed. It was found that the fluorescence intensities of both drugs increased in methanol more than the other solvents; therefore, it was the solvent of choice in this study.

Methods validation

Linearity and range

Under the above described experimental conditions, a linear relationship was established by plotting peak area (method I) or corrected peak amplitude (method II) for the studied drugs against drugs concentrations in μg/mL. The concentration

Table 2 Performance data of the proposed methods

Proposed method	HPLC		TDSFS	
Parameter	RPZ	DOM	RPZ	DOM
Concentration range (μg/mL)	0.15–2.0	0.1–1.5	0.15–2.0	0.1–1.5
LOD (μg/mL)	0.12	0.05	0.15	0.06
LOQ (μg/mL)	0.17	0.08	0.18	0.07
Correlation coefficient (r)	0.9998	0.9996	0.9999	0.9998
Slope	0.78	0.92	0.69	0.58
Intercept	0.058	0.033	−0.065	0.045
$S_{y/x}$, *S.D. of residuals*	0.012	0.064	0.078	0.059
S_a, *S.D. of intercept*	5.3×10^{-3}	6.5×10^{-3}	3.3×10^{-3}	2.5×10^{-4}
S_b, *S.D. of slope*	2.2×10^{-3}	9.9×10^{-4}	6.3×10^{-3}	8.9×10^{-3}

Table 3 Determination of studied drugs in pure form using the proposed methods

Parameter	Taken (μg/mL)	Found (μg/mL)	%Found	Comparison method [21],% Found
HPLC method				
RPZ pure form	0.15	0.149	99.25	100.58
	0.5	0.495	98.94	99.32
	0.8	0.795	99.35	99.18
	1.0	1.003	100.29	
	1.2	1.208	100.64	
	1.5	1.505	100.32	
	2.0	1.996	99.81	
$\bar{X}$ ± SD			99.81±0.59	99.69±0.63
t test			*0.94	
F test			*1.14	
%RSD			0.59	
%Error			0.22	
DOM pure form	0.1	0.099	99.05	99.25
	0.3	0.298	99.46	100.34
	0.5	0.494	98.86	100.75
	0.7	0.705	100.65	
	1.0	1.002	100.18	
	1.2	1.204	100.33	
	1.5	1.506	100.42	
$\bar{X}$ ± SD			99.85±0.66	100.11±0.63
t test			0.51	
F test			1.09	
%RSD			0.51	
%Error			0.19	
TDSFS method				
RPZ pure form	0.15	0.149	99.25	100.58
	0.5	0.496	99.22	99.32
	0.8	0.792	99.06	99.18
	1.0	1.007	100.67	
	1.2	1.203	100.28	
	1.5	1.509	100.66	
	2.0	1.995	99.75	
$\bar{X}$ ± SD			99.84±0.64	99.69±0.63
t test			0.95	
F test			2.27	
%RSD			0.64	
%Error			0.24	
DOM pure form	0.1	0.099	99.36	99.25
	0.3	0.301	100.48	100.34
	0.5	0.503	100.69	100.75
	0.7	0.702	100.25	
	1.0	0.999	99.98	
	1.2	1.19	99.75	
	1.5	1.491	99.36	
$\bar{X}$ ± SD			99.98±0.49	100.11±0.63
t test			0.32	
F test			1.65	
%RSD			0.49	
%Error			0.19	

*1.94, 5.14 are tabulated t and F values at P=0.05 [24]

ranges were found to be 0.15–2.0 μg/mL of RPZ and 0.1–1.5 μg/mL of DOM respectively when both of the proposed methods were applied. The high value of the correlation coefficients (r-value >0.999); with small value of intercept indicate the good linearity of the calibration graphs over the working concentration ranges. Statistical analysis of the data [24] gave small values of the standard deviation of the residuals ($S_{y/x}$), of slope (S_b) and of intercept (S_a) (Table 2). Thus, indicating low scattering of the points around the calibration curves.

Limit of quantitation and limit of detection

Detection limit (LOD) is the lowest concentration of the drug that can be detected, but not necessarily quantitated, under the stated experimental conditions. The limit of detection is generally quoted as the concentration yielding a signal-to-noise ratio of 3:1 [1] and is confirmed by analyzing a number of samples near this value using the following equation:

$$\text{The signal-to-noise ratio } s = H/h$$

Where H = height of the peak corresponding to the drug.

H = absolute value of the largest noise fluctuation from the baseline of the peak of a blank solution.

While the limit of quantification (LOQ); is the lowest concentration of the analyte that can be determined with acceptable precision and accuracy. It is quoted as the concentration yielding a signal-to-noise ratio of 10: 1 and is confirmed by analyzing a number of samples near this value [1]. The calculated values are listed in Table 2.

Accuracy

The accuracy of an analytical method is defined as the closeness of the results obtained by this method to the true values. To test the validity of the proposed methods, they were applied to the determination of pure samples of the concerned drugs over the working concentration ranges. The high percentages recoveries and small values of S.D. indicated the accuracy of the proposed methods. The accuracy of the proposed methods was also evaluated by studying the accuracy as percent relative error (% Error) and precision as

Table 4 Application of the proposed methods for the analysis of the studied drugs in laboratory prepared mixtures

	Taken (μg/mL)		%Found		Comparison method [21], %Found	
	RPZ	DOM	RPZ	DOM	RPZ	DOM
HPLC method						
	0.3	0.2	99.25	100.05	100.25	100.75
	0.6	0.4	99.78	99.56	99.65	100.66
	0.9	0.6	99.35	100.45	99.42	99.82
	1.2	0.8	100.45	100.39		
	1.5	1.0	100.36	99.66		
$\bar{X}$ ± SD			99.84±0.49	100.02±0.36	99.77±0.35	100.41±0.42
t test			0.53	*0.48		
F test			1.96	*1.36		
%RSD			0.49	0.36		
%Error			0.22	0.16		
TDSFS method						
	0.3	0.2	99.25	99.85	100.25	100.75
	0.6	0.4	100.14	99.14	99.65	100.66
	0.9	0.6	100.35	100.47	99.42	99.82
	1.2	0.8	100.08	100.67		
	1.5	1.0	99.54	100.45		
$\bar{X}$ ± SD			99.87±0.41	100.12±0.56	99.77±0.35	100.41±0.42
t test			0.39	0.65		
F test			1.37	1.78		
%RSD			0.41	0.56		
%Error			0.18	0.25		

*2.13, 4.32 are tabulated t and F values at P=0.05 [24]

Table 5 Precision data for the determination of the studied drugs in pure form by the proposed methods

HPLC method			
RPZ pure form	(0.15 μg/mL)	(1.0 μg/mL)	(2.0 μg/mL)
Intra-day precision, %found	100.25	99.58	99.45
	100.87	99.64	100.75
	100.09	99.12	100.38
$\bar{X}$ ± SD	100.41±0.34	99.45±0.23	100.19±0.55
Inter-day precision, %found	99.84	100.58	99.25
	99.12	100.05	100.78
	100.98	99.12	101.58
$\bar{X}$ ± SD	99.98±0.77	99.92±0.61	100.54±0.97
DOM pure form	(0.1 μg/mL)	(0.75 μg/mL)	(1.5 μg/mL)
Intra-day precision, %found	100.25	100.12	100.05
	100.39	100.85	100.87
	99.56	99.78	99.92
$\bar{X}$ ± SD	100.07±0.36	100.25±0.45	100.28±0.42
Inter-day precision, %found	99.12	100.05	100.78
	100.98	99.12	101.58
	99.56	100.34	99.58
$\bar{X}$ ± SD	99.89±0.79	99.84±0.52	100.47±0.82
TDSFS method			
RPZ pure form	(0.15 μg/mL)	(1.0 μg/mL)	(2.0 μg/mL)
Intra-day precision, %found	100.48	99.85	99.32
	99.68	99.32	99.55
	99.12	100.48	100.12
$\bar{X}$ ± SD	99.76±0.56	99.88±0.47	99.66±0.34
Inter-day precision, %found	100.82	99.32	101.85
	101.36	99.16	100.97
	99.42	101.25	100.27
$\bar{X}$ ± SD	100.53±0.82	99.91±0.95	101.03±0.65
DOM pure form	(0.1 μg/mL)	(0.75 μg/mL)	(1.5 μg/mL)
Intra-day precision, %found	99.86	99.42	99.36
	99.12	99.32	99.45
	100.34	100.54	100.34
$\bar{X}$ ± SD	99.77±0.51	99.76±0.55	99.72±0.44
Inter-day precision, %found	99.62	99.89	99.65
	101.25	100.75	99.12
	101.45	100.32	100.47
X ± SD	100.77±0.82	100.32±0.35	99.75±0.56

percent relative standard deviation (% RSD), and the results are shown in Table 3. The proposed methods were also applied to the simultaneous determination of the studied drugs in their laboratory prepared mixtures (Table 4) containing different concentrations of RPZ and DOM standard solutions in the ratio of 2:3 as present in their combined tablets. The concentrations of the drugs in the synthetic mixtures were calculated according to the linear regression equations of the calibration graphs. The results obtained were compared with those obtained using the comparison method [21].

Precision

The intra-day precision was evaluated through replicate analysis of three different concentrations of the drugs in pure form on three successive times. The inter-day precision was also evaluated through replicate analysis of three concentrations

Table 6 Determination of studied drugs in laboratory prepared tablets using the proposed methods

	Taken (μg/mL)		%Found		Comparison method [21], %Found	
	RPZ	DOM	RPZ	DOM	RPZ	DOM
HPLC method						
	0.3	0.2	99.52	99.89	99.52	99.41
	0.6	0.4	99.05	100.75	100.46	100.06
	0.9	0.6	100.45	100.12	100.67	100.75
	1.2	0.8	100.78	99.32		
	1.5	1.0	99.06	99.45		
$\bar{X}$±SD			99.77±0.72	99.91±0.51	100.22±0.51	100.07±0.55
t test			0.32	*0.51		
F test			1.99	*1.16		
%RSD			0.72	0.51		
%Error			0.32	0.23		
TDSFS method						
	0.3	0.2	99.35	100.95	99.52	99.41
	0.6	0.4	100.85	100.75	100.46	100.06
	0.9	0.6	100.06	99.26	100.67	100.75
	1.2	0.8	99.42	100.03		
	1.5	1.0	99.45	99.76		
$\bar{X}$±SD			99.83±0.57	100.15±0.63	100.22±0.51	100.07±0.55
t test			0.65	0.44		
F test			1.25	1.31		
%RSD			0.57	0.63		
%Error			0.25	0.28		

*2.13, 4.32 are tabulated t and F values at P=0.05 [24]

for a period of 3 successive days. The results of intraday and inter day precision are summarized in Table 5. The small values of SD indicate high precision of the proposed methods.

Specificity

The specificity of the proposed methods was proven by its ability to determine the drugs in their laboratory prepared tablets confirming that, there was no interference from common excipients and additives. These matrix components did not show any interfering peaks with the studied drugs in either method.

Solution and mobile phase stability

The stability of the stock solutions was determined by quantitation of the drugs at different time intervals and comparison to freshly prepared standard solutions. No significant change was observed in standard solution response relative to freshly prepared standards. Similarly, the stability of the mobile phase was checked. The results obtained in both cases proved that the sample solutions and mobile phase used during the assay were stable up to 14 and 6 days, respectively.

Applications

Analysis of laboratory prepared tablets

The proposed methods were successfully applied to the assay of the investigated drugs in their laboratory prepared tablets. The average percent recoveries of different concentrations were based on the average of three replicate determinations (Table 6).

Application of proposed methods for analysis of the studied drugs in spiked human plasma.

Many experimental trials were attempted to determine RPZ and DOM in spiked human plasma. Although many reported extraction techniques were tried, the percent recoveries obtained were not satisfactory, this could be attributed to the interference encountered by amino groups present in plasma proteins and amino acids, which might have possibly, react with NBD-Cl giving unacceptable results.

Conclusion

Two simple, sensitive, rapid, economic and validated methods have been developed for the simultaneous determination of rabeprazole sodium and domperidone. The proposed methods were successfully applied for the analysis of the studied drugs in their laboratory prepared mixtures, and laboratory prepared tablets; the obtained results were favorably compared with those obtained by the comparison method. Furthermore, detailed validation procedure was also conducted. The simplicity and low cost of the proposed methods allows their application for routine work in quality control laboratories.

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