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Ratio difference and mean centering of ratio spectra spectrophotometric methods for simultaneous determination of domperidone and omprazole in bulk and pharmaceutical formulation

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ABSTRACT

Simple, specific, accurate and precise spectrophotometric methods weredeveloped and validated for simultaneous determination of Domperidone (DP) and Omeprazole(OMP) in bulk powder and pharmaceuticalformulation. The first method was ratio difference (RD) and the second wasmean centering of ratio spectra (MCR). The calibration curve is linear over the concentration range of 4-36 and 2–24µg.ml^{-1} for DP and OMP, respectively. The proposed spectrophotometric methods can analyze both drugs without any prior separation steps. Theselectivity of the adopted methods was tested by analyzing synthetic mixtures of the investigateddrugs, also in their pharmaceutical formulation. The suggested methods were validated according to International Conference of Harmonization (ICH) guidelines and the results revealed that they were precise and reproducible. All the obtained results were statistically compared with those of the reported method,where there was no significant difference.

KEYWORDS

Domperidone;
Omeprazole;
Ratio difference;
Mean centering;
ICH guidlines.

INTRODUCTION

Domperidone (DP), 5- chloro- 1- [1- [3- (2- oxo- 2, 3- dihydro- 1Hbenzimidazol-1- yl) propyl]- piperidin- 4- yl]- 1, 3- dihydro- 2H benzimidazol- 2- one (Figure 1). DP is a peripheral dopamine (D2) and (D3) receptor antagonist. It increases gastrointestinal peristalsisand motility that prevent reflux esophagitis and it is used to prevent nausea and vomiting[1].Omeprazole (OMP),5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (Figure 2)[2]. OMP is a selective and irreversible proton pump inhibitor. It suppresses stomach acid secretion by specific inhibition of the H +/K + ATPase system found at the secretory surface of gastric parietal cells.

Domperidone and Omeprazole are co-formulated in single combined capsule formulation. This combination medication contains a proton pump inhibitor and antidopaminergic agent, prescribed for ulcers, indigestion and acid stomach.

Literature review shows DP is official in BP[1] and Omeprazole is official in Indian Pharmacopoeia (IP)[2], British Pharmacopoeia (BP)[3] and United

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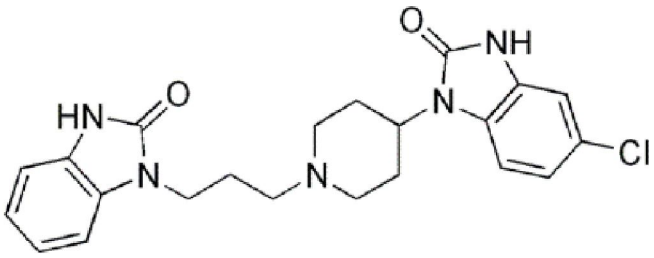

Figure 1 : Structure formula of domperidone

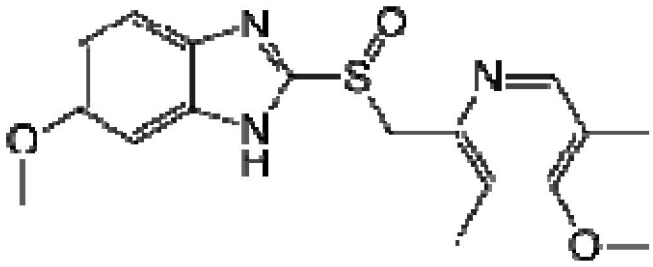

Figure 2 : Stucture formula of omeprazole

State Pharmacopoeia (USP)[4]. The IP, BP and USP describe HPLC method for estimation of omeprazole. There are many reported methods for the determination of either DP, OMP, together or in combination with other drugs in different matrices such as pharmaceutical formulation, human plasma, serum, milk or urine.

Methods for assay of DP include liquid chromatography coupled with ultraviolet(UV)[5-9] or fluorescence[10, 11] detection, liquid chromatographymass spectrometry (LC–MS)[12-15], thin layer chromatography [16, 17], Spectrophotometric and spectrofluorimetric methods[18–24] and Electrochemical method[25].

Spectrophotometric[26], derivative UV spectroscopy[27], spectrofluorimetric[28], voltametric[29], LC-MS[30-31] and HPLC[32-34] methodsused for determination of OMP in pharmaceutical dosage forms as well as in biological fluids.

Simultaneous determination of DP and OMP wasreported by liquid chromatography coupled with ultraviolet(UV)[35-37], thin layer chromatography[38] or spectrophotometric method[39].

The aim of the work, resolving binary mixture of Domperidone and omeprazole using different spectrophotometric methods. These methods show simple and accurate way for the analysis of this binary mixture without the need of sophisticated instruments or expensive solvents.

THEORETICAL BACKGROUND

Ratio difference spectrophotometric method (RD)

Recently, Elzanfaly et al.[40–42] developed a novel simple, rapid and selective method to determine components having overlappingspectra in binary mixtures simultaneously. For any twodrugs X and Y with overlapping spectra, when the spectrum of Xis divided by a divisor of a certain concentration of Y, a ratio spectrumwill result, and a linear relationship between the difference inamplitudes at any two wavelengths and the corresponding concentrationof X will result, while the ratio spectrum of Y will be astraight line of constant amplitude parallel to the x-axis and thedifference in amplitudes of Y at any two wavelengths will be zero.

Mathematically it can be explained as follows: In the ratio spectrum of a lab mixture of X and Y is divided by a divisor Y^1

$$\mathbf{P_1 = P_{1x} + K}$$

$$\mathbf{P_2 = P_{2x} + K}$$

Where P_1 and P_2 are the amplitudes of the mixture spectrum at λ_1 and λ_2, respectively. P_{1X} and P_{2X} are the amplitudes of X at λ_1 and λ_2, respectively. K is the constant resulting from Y/Y^1.

$$\mathbf{\Delta P_{(\lambda 1-\lambda 2)} = P_1 - P_2 = (P_{1x} + K) - (P_{2x} + K) = P_{1x} - P_{2x}}$$

A calibration curve relating the difference in amplitudes in the ratio spectrum at λ_1 and $\lambda_2 \Delta P_{(\lambda 1-\lambda 2)}$ using a certain concentration of Y as a divisor to the corresponding concentration of X will be used for the determination of X in the unknown samples of the binary mixture.

Similarly component Y can be obtained by using certain concentration of X as a divisor.

Mean centering of ratio spectra spectrophotometric method (MCR)

This is a well-established spectrophotometric method in which both binary and ternary mixtures could be determined without previous separation. In this method the ratio spectra are obtained after which the constant is removed by mean centering of the ratio spectra[43].

Consider a mixture of two compounds X and Y. If there is no interaction among the compounds and Beer's law is obeyed for each compound, it can be

written:

$$Am = \alpha xCx + \alpha yCy \quad (1)$$

where Am is the vector of the absorbance of the mixture, αx and αy are the molar absorptivity vectors of X and Y and Cx and Cy are the concentrations of X and Y respectively.

If Eq. (1) is divided by αy corresponding to the spectrum of a standard solution of Y in binary mixture, the first ratio spectrum is obtained in the form of Eq. (2) (for possibility of dividing operation, the zero values of αy should not be used in the divisor):

$$B = Am/\alpha y = \alpha x\ Cx/\alpha y + Cy \quad (2)$$

If Eq. (2) is mean centered (MC), since the mean centering of a constant (Cy) is zero, Eq. (3) would be obtained:

$$MC(B) = MC\ [\alpha xC\alpha/\alpha y] \quad (3)$$

Eq. (3) is the mathematical foundation of binary mixture analysis that permits the determination of concentration of each of the active compounds in the solution (X in these equations) without interfering from the other compound of the binary system (Y in these equations). As Eq. (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 or in the standard binary mixtures. For more sensitivity the amount of MC(B) corresponding to maximum or minimum wavelength should be measured

Calibration graphs for Y could also be constructed as described for X.

EXPERIMENTAL

Chemicals and reagents

Pure standards

Pure Domperidone; kindly supplied by National Organization for Drug Control and Research, (Cairo-Egypt).

Pure Omeprazole was kindly supplied by Pharaonia Pharma, (Cairo-Egypt).

Market samples

Domstal-RD® capsuleslabelled to contain 10 mg domperidone and 20 mg omeprazole per capsule(B.N.:8019414) were supplied from company (Torrent Pharmaceuticals LTD, India).

Solvents

Methanol and acetonitrile of analytical grade (Mtedia, USA)was used in all experiments.

Instrument and software

Instrument

Double-beam Shimadzu (Japan) 1601 PC UV-Visible spectrophotometer with 1 cm quartz matched cuvettes connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used.

Software

Minitab® Release 14.12.0.used for mean centering of data.

Procedures

Standard stock and working solutions

- DP standard working solution: 200 µg/mL in methanol.
- OMP standard working solution: 200 µg/mL in methanol.

Spectral characteristics of DP and OMP

The zero-order (D^0) absorption spectrum ofAliquots of standard working solutions of 20 µg/mLof DP and10 µg/mLof OMP solution were recorded against methanol as a blank overthe range of 200–400 nm (Figure 3).

Construction of calibration curves

Aliquots from working standard solutions equivalent to 40–360µg/mL of DP and 20–240µg/mL of OMP were accurately measured and transferred separately into a set of 10-mL volumetric flasks and completed with methanol. The zero order absorptionspectra of each solution were measured in the range of200–400 nm and stored in the computer.

Ratio difference (RD)

For the determination of DP in presence of

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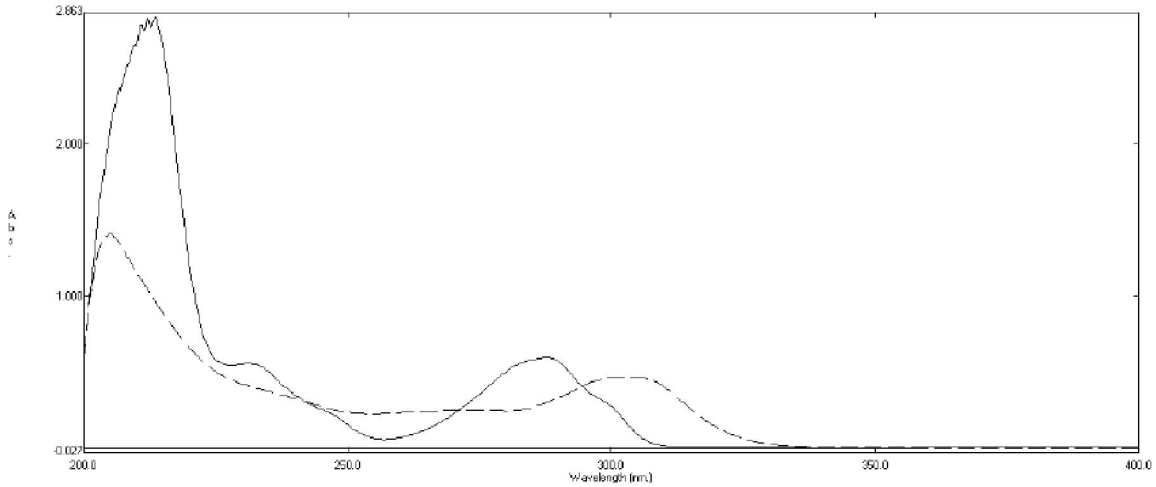

Figure 3 : Zero order spectra for domperidone (—) (20.00 µg/mL), omeprazole (——) (10.00 µg/mL) against methanol

OMP,the stored spectra of DP were divided by theabsorption spectrum of standard solution of OMP(20µg/mL) thenthe difference between (DP/ OMP^1) amplitudes at 283 nm and 257 nm was computed. A calibrationgraph relating the ratio amplitude difference between 283.0 and 257.0 nm to the correspondingconcentrations in µg/mL of DP was constructed.

For the determination of OMP in presence of DP, the stored spectra of OMPwere divided by the absorption spectrum of standard solution of DP (10 µg/mL), smoothed at $\Delta\lambda$ = 2 then the differencebetween (OMP/DP^1) amplitudes at 256.8 nm and 283 nm was computed. A calibration graph relating the ratio amplitude differencebetween 256.8 nm and 283 nm to the corresponding concentrations in µg/mL of OMP wascomputed.

Mean centering of ratio spectra spectrophotometric method (MCR)

The scanned spectra of DP were dividedby the absorption spectrum of standard solution of OMP (10 µg/mL)then spectral data from 220-320 nm exported to Minitab to be mean centered. The samewas applied to OMP spectra as spectra of OMP were divided by the absorption spectrum of standard solution of DP (20 µg/mL) andthe obtained ratio spectra were smoothed with $\Delta\lambda$ = 2 nm then full spectra exported to Minitab to be mean centered. The calibration curvesfor both DP and OMP were constructed by plotting the mean centeredvalues at 283 nm for DP and 314.8 nm forOMPversusthe corresponding concentration in µg/mL.

Accuracy

Accuracy of the developed spectrophotometric methods was checked by calculating the % recovery of 6 different samples of DP and OMP using corresponding regression equations.

Precision

Repeatability

Three concentrations of DP(10,20 and 30 µg/mL) and OMP (4, 10 and 20 µg/mL) were analyzed three times, within the same day, using the previously mentions procedures and the mean R% and RSD% were then calculated for each drug by each proposed method.

Intermediate precision

The above mentioned concentrations of DP and OMP were analyzed on three successive days, using the previously mentioned procedures. The mean R% and RSD% were calculated for each drug by each proposed method.

Analysis of laboratory prepared mixtures

For preparation of laboratory mixtures, into a series of 10-mLvolumetric flasks, aliquots equivalent to 40–300µg of DP and20–200 µg of OMP were accurately transferred from their workingstandard solutions with different ratios and the volume was completed with methanol. The spectra ofthe prepared

mixtures were scanned from 200 to 400 nm against methanol as a blank andstored in the computer.

Ratio difference (RD)

The stored spectra of laboratory prepared mixtures were proceed as under the proposed method. The concentration of eachdrug was calculated using the specified regression equation.

Mean centering of ratio spectra spectrophotometric method (MCR)

The stored spectra of laboratory prepared mixtures were proceed as under the proposed method. The concentration of each drug was calculated using the specified regression equation.

Assay of domstal-RD® capsules

Ten capsules were evacuated and contents weighed, powdered then portion of powder equivalent to 25 mg DP and 50 mg OMP is transferred into 100-mL beaker, 30 mL methanol acetonitrile 50:50 v/vadded and sonicated for five minute then contents were filtered into 50-mL volumetric flasks quantitatively and residues were washed with methanol then complete to final volume with methanol, mixed well to get a solution of 0.5 mg/mL DP and 1 mg/mL OMP

Aliquots equivalent to 50 µg of DP and 100 µg of OMP were transferred into to 10-mL volumetric flasks and the volume was completed with methanol to obtain final concentration 5 µg/mL of DP and 10 µg/mL ofOMP.

The proposed methods were applied for the analysis of thestudied drugs in their pharmaceutical formulation using the proceduresas under the proposed method and the concentrations of the cited drugs werecalculated from the corresponding regression equations.

RESULTS AND DISCUSSION

The objective of this work is to establish simple, sensitiveand accurate analytical methods for simultaneous determinationof DP and OMP in their bulk powders and pharmaceuticalformulation with satisfactory precision and accuracy.

By scanning the absorption spectra of DP and OMP in methanol, overlapped spectral bands were observed (Figure 3), so different methods were appliedfor achieving best resolution and quantitative determination ofeach drug without any interference from the other.

Ratio difference spectrophotometric method (RD)

The most striking feature of the ratio difference method is its simplicity, rapidity and accuracy[44]. This is a newly developed method having the ability for solving overlapped spectra without prior separation; meanwhile it doesn't require any sophisticated apparatus or expensive computer programs.

The utilization of ratio difference method is to calculate the unknown concentration of a component of interest present in a mixture containing both the component and an interfering component.

The only requirement in the ratio difference method is the contribution of the two overlapped spectra at the two selected wavelengths λ_1 and λ_2 where the ratio spectrum of the interfering component shows the same amplitude (constant) whereas the component of interest shows significant difference in these two amplitude values at these two selected wavelengths with concentration. Similarly, another two wavelengths are selected for the estimation of the second component. Thus, the overlapped spectra of the cited drugs suggested that a ratio difference method was a suitable method for simultaneous determination of DP and OMP.

Ratio difference method starts by scanning the zero order absorption spectra of the laboratory-prepared mixtures (DP and OMP). For determination of DP, divide the previously scanned ratio spectra by a carefully chosen concentration of standard OMP' (10 µg/mL) as a divisor to produce new ratio spectra which represent DP/OMP' + constant as shown in Figure 4. The amplitudes at 283 nm and 257 nm were selected. The amplitudes at these two wavelengths were subtracted, so the constant OMP/OMP' will be cancelled. The concentration of DP was calculated using the corresponding regression equation (obtained by plotting the difference in the amplitude at 283.0 nm and 257.0 nm of the ratio spectra of DP/OMP' against the corresponding concentrations). Similarly, the two selected wavelengths for the estimation of OMP using stan-

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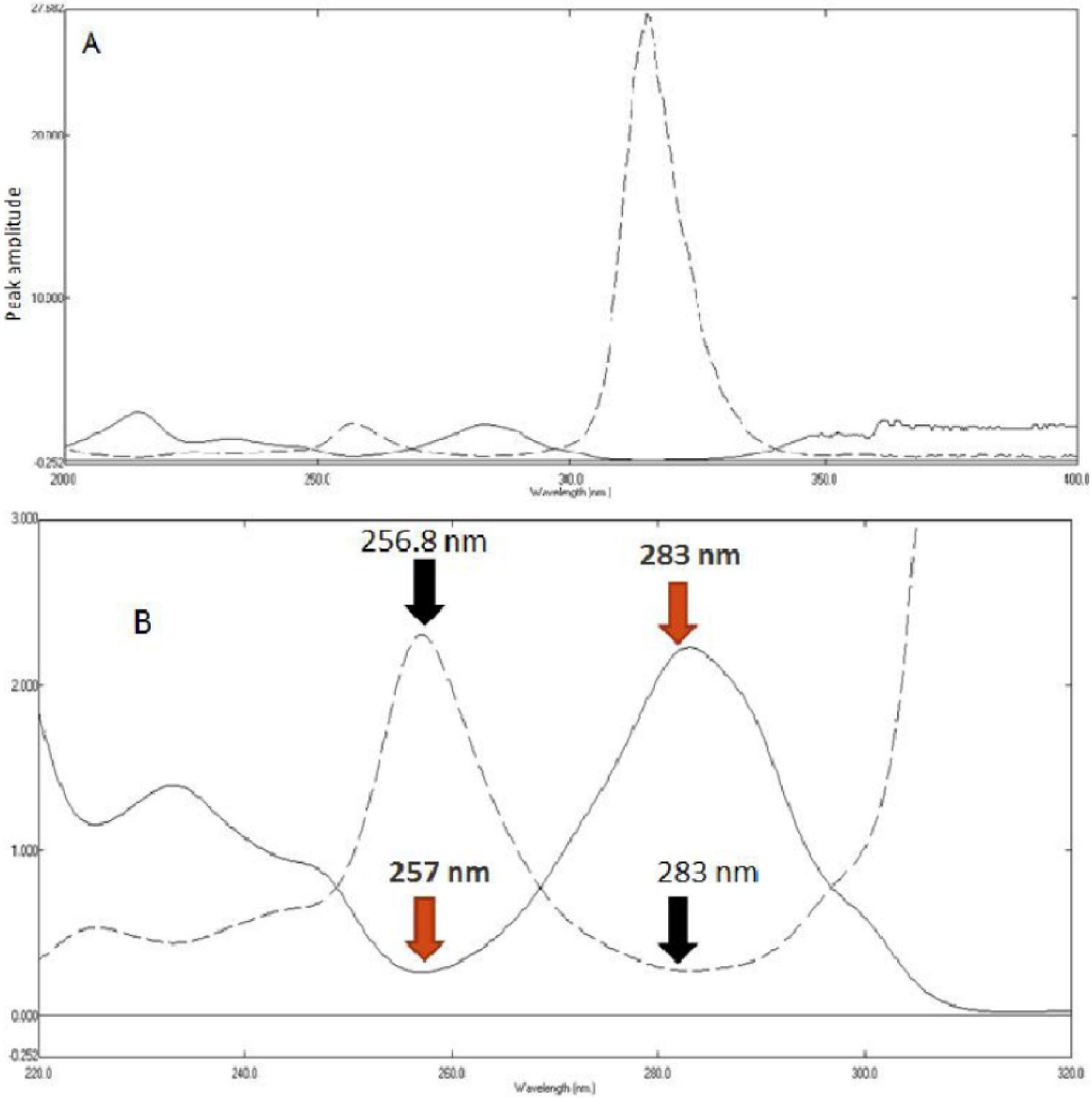

Figure 4 : (A) Ratio spectra of 20.00 µg/mL DP (—) and 6.00 µg/mL OMP (——) using 20 µg/mL OMP and 10 µg/mL DP, respectively, as divisors. (B) is amplified part of A showing wavelengths of interest

dard DP' (20 µg/mL) as a divisor were 256.8 nm and 283 nm as shown in Figure 4(B).

Mean centering spectrophotometric method (MCR)

In order to optimize the developed Mean centering spectrophotometric method, the influence of different variables was studied, including divisor concentration and smoothing factor, where the careful choice of the divisor and the working wavelengths were of great importance, so different concentrations of OMP (2, 5, 10, 15 and 20 µg/mL) were tried as divisors for determination of DP, and concentration of 10 µg/mL of OMP was selected as divisor as it gave minimum noise and better selectivity. Also, different concentrations of DP (5, 10,15,20,25 and 30 µg/mL) were tried as divisors for determination of OMP and concentration of 20 µg/mL of DP was selected as a divisor as it gave minimum noise and better selectivity.

Mean-centering of the ratio spectra was obtained in the wavelengthrange of 220–320 nm for DP and 200-400 nm for OMP.

The concentration of DP and OMP was determined by measuring the amplitude at 283.0nm for DP and 314.8 nm for OMP corresponding to a

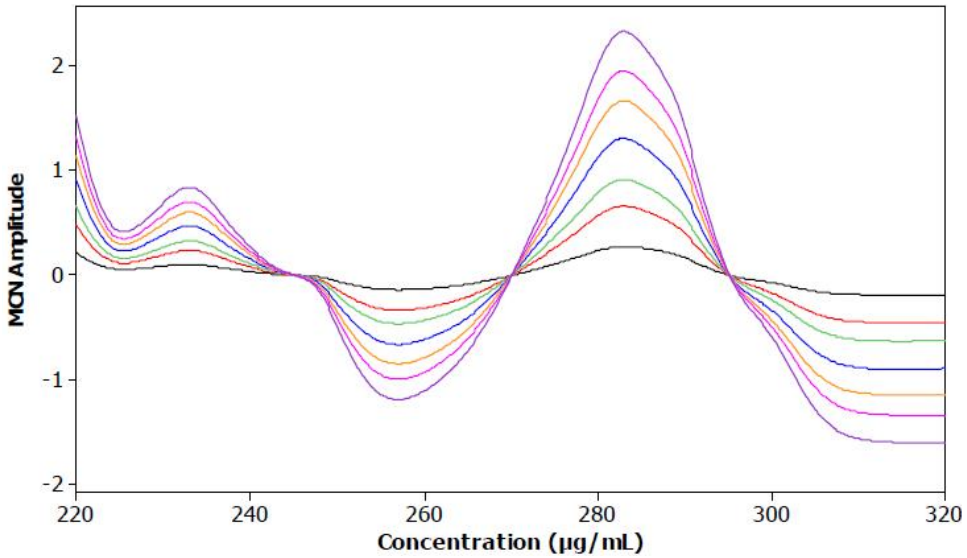

Figure 5 : Mean centered ratio spectra for different concentrations of DP (4.00-36.00 µg/ml) after division by 10.00 µg/ml OMP against methanol

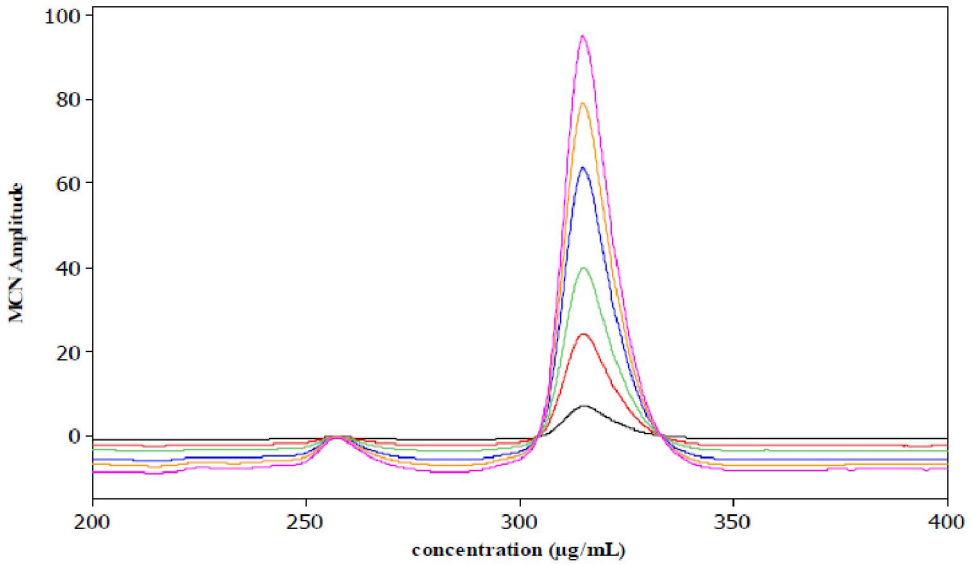

Figure 6 : Mean centered ratio spectra of different concentrations of OMP (2.00-24.00 µg/ml) using 20.00 µg/ml DP as divisor against methanol

maximumwavelength for each drug respectively (Figures 5 and 6). For determination of the concentration of DP and OMP in laboratory-prepared mixtures and samples of a pharmaceutical formulation, the same procedure was used.

For all the proposed methods, the statistical parameters of theregression equations and the concentration ranges are shown in (TABLE 1), the table shows that the proposed methods were appliedfor the determination of pure drugs and satisfactory results wereobtained.

The proposed method was successfully applied to the analysis of DP and RT in their laboratory prepared mixtures (TABLE 2).

Application of the methods in assay of pharmaceutical formulation

The proposed UV methods were applied for the determination of DP and RT in their combined pharmaceutical formulation Domstal-RD®and the results are shown in (TABLE 3a). The good percentage recoveries confirm the suitability of the proposed methods for the routine determination of these components in their combined formulation.

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TABLE 1 : Regression parameters and results of determination of pure samples of DP and OMP by the proposed methods

Parameter	DP		OMP	
	RD	MCR	RD	MCR
Linearity (µg/ml)	4-36	4-36	1-45	1-45
Slope	0.0968	0.0642	0.3388	3.9675
Intercept	0.0233	0.0153	-0.0436	0.0505
Correlation coefficient (r)	0.9999	0.9998	0.9999	0.9998
Accuracy (Mean ± SD)	100.07 ± 0.440	100.29 ± 0.714	100.17 ± 0.840	100.25 ± 0.968
Selectivity[a] (Mean ± SD)	99.90 ± 0.729	99.83 ± 0.522	100.06 ± 0.767	100.26 ± 0.564
Precision				
RSD%[b]	0.610	0.343	0.438	0.425
RSD%[c]	0.674	0.555	0.560	0.751
LOD[d] (µg/mL)	0.459	0.494	0.258	0.424
LOQ[d] (µg/ mL)	1.392	1.496	0.781	1.285

[a]Selectivity of analysis of laboratory prepared mixtures (n=7); RSD%[b]&RSD%[c]: the intra-day and inter-day respectively (n = 3) relative standard deviation of three different concentrations of DP and OMP; [d]Limit of detection and limit of quantitation

TABLE 2 : Determination of DP and OMP in laboratory prepared mixtures by the proposed spectrophotometric methods

Concentration (µg/ml)		DP		OMP	
Amount taken (µg/ml)		Recovery%[a]		Recovery%[a]	
DP	OMP	RD	MCR	RD	MCR
30	12	100.87	100.28	99.45	100.55
20	4	100.42	99.27	98.83	100.60
12	6	99.66	99.38	100.03	99.77
14	14	99.07	99.78	100.93	100.37
8	16	100.62	100.71	99.82	101.15
4	20	99.17	99.88	100.51	99.80
4	10	99.48	99.47	100.86	99.58
Mean		99.90	99.83	100.06	100.26
S.D.		0.729	0.522	0.767	0.564
RSD%		0.730	0.523	0.766	

[a] Average of three determinations.

TABLE 3.a : Determination of DP and OMP in Domstal-RD® tablets by the proposed spectrophotometric methods

Pharmaceutical formulation	Claimed	%Found[a] ± S.D	
	DP	RD	MCR
Domstal-RD® capsules B.N.:8019414	10 mg	98.82 ±1.246	98.82 ± 1.312
	OMP	RD	MCR
	20 mg	99.01 ± 0.513	98.66 ± 0.550

[a] Average of three determinations.

Statistical analysis

TABLES 4 showed statistical comparison of the results obtainedby the proposed methods and reported chromatographicmethod[36]. The calculated *t* and F values were less than the theoretical ones indicating that there was no significant difference between the proposed and the reported methods with

TABLE 3.b : Results of application of standard addition technique

Amount taken dosage form (µg/ml)	Amount added (µg/ml)	Recovery%[a]	
DP		**RD**	**MCR**
5	5	100.14	99.47
	10	99.58	99.81
	15	100.64	99.91
	20	101.17	100.32
	30	99.31	99.37
Mean ± SD		100.17 ± 0.760	99.78 ± 0.339
OMP		**RD**	**MCR**
10	2	100.09	99.05
	4	100.96	101.54
	6	98.55	99.71
	8	98.19	99.95
	10	99.45	100.22
Mean ± SD		99.450 ± 1.130	100.16 ± 0.836

[a] **Average of three determinations**

respect to accuracy and precision (TABLE 4).

METHOD VALIDATION

Validation was done according to ICH recommendations[45].

Linearity

The linearity of the methods was evaluated by analyzing sevenconcentrations of DP and six concentrations of OMP rangingfrom 4–360µg/mL and 2–24µg/mL, respectively. Each concentrationwas repeated three times. The assay was performed accordingto the experimental conditions previously mentioned. The linearequations were summarized in (TABLE 1).

Accuracy

The accuracy of the proposed methods results was checked by applying the methods for determination of different samples of DP and OMP. The concentrations were obtained from the correspondingregression equations. The mean of percentage recoveries and standard deviations of the proposed methods were summarized in (TABLE 1). Accuracy of the methods was further assured by the use of the standardaddition technique, it was performed by addition of knownamounts of pure DP and OMP to known concentrations of the pharmaceuticalformulation the resulting mixtures were assayed, andthe results obtained were compared with the expected results (TABLE 3.b). The good recoveries of standard addition technique suggestedgood accuracy of the proposed methods.

Range

The calibration range was established through considerations ofthe practical range necessary according to adherence to Beer's lawand the concentration of DP and OMP present in the pharmaceutical formulation to give accurate precise and linear results (TABLE1).

Selectivity

Selectivity of the methods was achieved by the analysis of different laboratory prepared mixtures of DP and OMP within thelinearity range. Satisfactory results were shown in (TABLE 2).

Detection and quantitation limits

They are calculated from the standard deviation (r) of theresponse and the slope of the calibration curve (S) in accordanceto the following equations: LOD = 3.3 (r/S) and LOQ = 10 (r/S).

Results presented in TABLE 1, indicated that the method is sensitivefor determination of the studied drugs.

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TABLE 4 : Statistical analysis of the proposed methods and the reported method of DP and OMP in their pure powder form

Parameter	Domperidone (DP)		
	RD	MCR	Reported method **
Mean	100.07	100.29	99.52
SD	0.440	0.714	0.544
n	6	6	5
Variance	0.193	0.509	0.296
t-Test(2.262)*	1.850	1.965	-
F(5.192)*	1.530	1.721	-
	Omeprazole (OMP)		
	RD	MCR	Reported method **
Mean	100.17	100.25	99.46
SD	0.840	0.968	0.832
n	6	6	5
Variance	0.705	0.937	0.693
t-Test(2.262)*	1.393	1.426	-
F(6.256)*	2.270	1.354	-

***Values in parenthesis are the theoretical values of *t* and F at P = 0.05; **HPLC method for the assay of OMP and DP tablets using (25 cm x 4.6 mm, 5 micron) C-18 column withacetonitrile: 0.05M ammonium acetate buffer (pH- 4) in the ratio of (85:15) in isocratic mode at flow rate 1.0 ml/min and UV detection at 280 nm[36].**

Precision

Repeatability and intermediate precision

They were determined using three concentrations (10, 20 and 30 µg/mL) ofDP and (4, 10 and 20 µg/mL) of OMP which were analyzed three timesintra-daily and inter-daily on three different days using the proposedmethods. The relative standard deviations were calculated (TABLE 1).

Stability

DP and OMP working solutions in methanol showed no spectrophotometricchanges up to 3 weeks when stored at 4 ºC covered with aluminum foil.

CONCLUSION

In this work simple, accurate, and specific spectrophotometric methodswere applied for the simultaneous analysis of binary mixture of DP and OMP.

The proposed methods were very simple with minimum manipulationsteps, very sensitive, precise, do not need any sophisticatedapparatus and could be easily applied in qualitycontrol laboratories as they are having equal accuracy and precision compared to the reported chromatographic method forthe simultaneous determination of DP and OMP. So that the proposedmethods could be successfully applied for the routine analysisof the studied drugs either in their pure bulk powders and indosage form in quality control laboratories without any preliminaryseparation step.

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