DESIGN, OPTIMIZATION AND VALIDATION OF CHEMOMETRIC ASSISTED SPECTROPHOTO -METRIC METHOD FOR SIMULTANEOUS DETERMINATION OF ESOMEPRAZOLE AND DOMPERIDONE IN COMBINED DOSAGE FORM

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Abstract

Chemometric method – The response surface method was developed for simultaneous spectrophotometric estimation of Esomeprazole and Domperidone in pure form and its pharmaceutical dosage form without any pre-treatment. In the present work, Esomeprazole and Domperidone were dissolved in ethanol and diluted to get a concentration of 10 µg / ml. The solutions were scanned in the UV region in the wavelength range from 200 – 400 nm against ethanol as blank. From the spectrum, it was found that Esomeprazole and Domperidone showed the maximum absorbance at 301nm and 290 nm respectively. Beer's law was obeyed for the drugs in the concentration range of 4-24 µg/ml for Esomeprazole and 3-18 µg/ml for Domperidone. The correlation co-efficient value for the calibration graph was found to be 0.9997 and 0.9998 for Esomeprazole and Domperidone respectively, the accuracy of the method was performed by recovery studies, and the average percentage

recovery was found to be in the range of 99.76±0.7512 and 100.52±1.1023 for Esomeprazole and Domperidone respectively. The amount of the drug recovered from the formulation was very close to the expected value and the % RSD value was found to be 0.7530 and 1.0966 for Esomeprazole and Domperidone respectively. The Percentage purity of Esomeprazole and Domperidone was found to be 100.25±1.0913 and 100.14±0.8103. The developed method was validated statistically by recovery studies as per ICH guidelines. Optical characteristics like slope, intercept, molar absorptivity, correlation coefficient, LOD, and LOQ were calculated. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, rapid, and accurate and can be successfully applied for routine quality control analysis of the simultaneous determination of Esomeprazole and Domperidone in bulk and a combined tablet dosage form.

Keywords:

Chemometry, Central Composite design, UV Method Development, Validation

1. Introduction

Esomeprazole, chemically 6-methoxy-2-[(*S*) - (4-methoxy- 3,5- dimethyl pyridine -2- yl) methylsulfinyl]- 1*H*- benzimidazole, comes under the class of proton pump inhibitor (PPI) and it is the s-isomer of Omeprazole . As a part of a triple-drug regimen for helicobacter pylori infection, Esomeprazole has FDA-approved labeling for use in the treatment of symptomatic gastroesophageal reflux disease(GERD), including healing and maintenance of erosive esophagitis and it is also used to treat duodenal and gastric ulcers, and Zollinger-Ellison syndrome, a condition wherein the stomach produces too much acid. Domperidone, chemically 6-chloro-3-[1-[3-(2-oxo-3*H*-benzimidazol-1-yl) propyl] piperidin-4-yl]-1*H* and vomiting. Dopamine receptors are specifically blocked by the drug domperidone. It accelerates digestion, triggers the production of prolactin, serves as an antiemetic, and is utilized to investigate dopaminergic processes.The structure of Esomeprazole and Domperidone is depicted in Figure 1. This combination of two drugs is useful in the treatment of gastric- related disorders.

Both Esomeprazole and Domperidone are official in the Indian, United States, and British Pharmacopoeia, and their official method of assay is high-performance liquid chromatography (HPLC) [1, 2]. Several analytical methods such as Spectrophotometry [7,8] and HPLC [9] have been reported for the determination of Esomeprazole and Domperidone.

In recent years, the application of chemometrics, particularly multivariate calibration methods, is playing a crucial role in multicomponent analysis[10]. Multivariate calibration methods such as PCR, PLS, and RSM applied to spectral data are being increasingly used for instrumental methods without separation techniques[11]. The purpose of this study was to introduce an alternative analytical procedure based on the chemometric- assisted Spectrophotometric methods for the simultaneous determination of Esomeprazole and Domperidone in pharmaceutical mixtures.

2. Experimental

2.1 Apparatus and Software. Spectrophotometric measurements were performed on a double-beam UV-VIS spectrometer (Perkin Elmer) equipped with 1 cm matched quartz cells, connected to a computer loaded with Lambda 25 software. All absorption spectra were saved in Lambda 25 software and subsequently exported to the Microsoft Excel program for statistical manipulation. Design of Expert 13.0 version software was used for RSM (Response Surface Method) model development and data analysis.

2.2 Reagents and Samples. Pharmaceutical-grade Esomeprazole and Domperidone were obtained from Mylan Pharmaceuticals (Puducherry). Ethanol (Thomas Baker) was used as solvent throughout the analysis. Commercial sample Sompraz - D 40 (Sun Pharmaceuticals) labeled to contain Esomeprazole 40 mg and Domperidone 30 mg, were purchased from the local pharmacy.

2.3 Preparation of Stock and Working Standard Solutions. Accurately weighed and transferred (40 mg of Esomeprazole and 30 mg of Domperidone) either of the standard drugs into a 100 mL volumetric flask was dissolved in about 30 mL of Ethanol and diluted to volume with the same solution. Suitable aliquots of the stock solutions were diluted with the solvent to obtain the appropriate working standard solutions according to the linear calibration range for each drug.

2.4 Optimization by Response Surface Methodology. A Central Composite design was employed to optimize the responses for simple and precise simultaneous determination of Domperidone and Esomeprazole in bulk as well as formulations. Thirteen experiments were performed by using two independent variables such as Wavelength 1 & Wavelength 2 to optimize the responses Absorbance 1 & Absorbance 2 (Table 1) all the experiments were carried out in a randomized order for minimizing the effects of unrestrained variables, which may responsible for bias in the measurements [3,4,5]. The statistical tools provide the numerical verification of variables and their effects on responses.

2.5 Method validation. The established innovative technique has been authenticated rendering to ICH criteria. The authentication was taking place by diverse strictures such like specificity, linearity, precision, accuracy, quantization's limits, robustness and system suitability[6-8].

2.5.1 Linearity. This was examined in the concentration range of 4-24 µg/ml for Esomeprazole and 3-18 µg/ml for Domperidone. Absorbance values were recorded at λmax of each drug (301nm for Esomeprazole and 290 nm for Domperidone) against Ethanol as blank. The linear dynamic range for each drug was studied by least square linear regression of concentration and the corresponding absorbance.

2.5.2 Accuracy Study. The accuracy of the method was performed at three levels 50%,100% and 150 % of the working concentration of the sample. Calculated amounts of a standard solution of Esomeprazole and Domperidone were spiked into sample solution. Retrieval study was achieved in triplicates by scheming the percent recovery and a couple of RSDs for each medication.

2.5.3 Precision. The preciseness of the established methodology was assessed by activity Intra-day preciseness and Interday preciseness schemes.

2.6 Analysis of the Marketed Formulation. Capsule (Sompraz - D 40) powder equivalent to Esomeprazole 40 mg and Domperidone 30 mg were weighed accurately and transferred into a 100 ml volumetric flask, 30 mL of ethanol was added. The solution was well shaken and ultrasonicated for 15 min. Then the solution was filtered in a Whatman filter paper # 42 filter paper. The residue was washed three times with 10 mL ethanol, and the volume was completed to 100 mL with ethanol. From this solution, 4 ml was pipette out and make up to 10 ml with ethanol to get the final concentration of 16 µg / ml of Esomeprazole and 12 µg / ml of Domperidone. From the measured absorbance, the amount of drugs present in the formulation was estimated by using the simultaneous equation method.

3. Result and Discussion

Table 1. Optimization Method Parameters for Central Composite Experimental Design

		Factor 1	Factor 2	Response 1	Response 2
Std	**Run**	**A: WL 1 nm**	**B: WL2 nm**	**R1 (ABS 1)**	**R2 (ABS 2)**
1	1	288	298	0.5051	0.6059
11	2	290	301	0.4877	0.617
13	3	290	301	0.4877	0.617
3	4	288	304	0.5051	0.6095
12	5	290	301	0.4877	0.617
6	6	292.8	301	0.3939	0.617
7	7	290	296.8	0.4877	0.5955
2	8	292	298	0.4289	0.6059
4	9	292	304	0.4289	0.6095
5	10	287.2	301	0.502	0.617
10	11	290	301	0.4877	0.617
8	12	290	305.2	0.4877	0.6044
9	13	290	301	0.4877	0.617

Table 2. ANOVA Results for Response 1 (ABS 1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0145	5	0.0029	14603.86	< 0.0001	significant
A-WL 1	0.0116	1	0.0116	58680.75	< 0.0001	
B-WL2	0.0000	1	0.0000	0.0000	1.0000	
AB	0.0000	1	0.0000	0.0000	1.0000	
A^2	0.0028	1	0.0028	14130.99	< 0.0001	
B^2	2.959E-07	1	2.959E-07	1.49	0.2616	
Residual	1.390E-06	7	1.985E-07			
Lack of Fit	1.390E-06	3	4.632E-07			
Pure Error	0.0000	4	0.0000			
Cor Total	0.0145	12				

Table 3. ANOVA Results for Response 2 (ABS2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0006	5	0.0001	169.36	< 0.0001	significant
A-WL 1	0.0000	1	0.0000	0.0000	1.0000	
B-WL2	0.0000	1	0.0000	70.95	< 0.0001	
AB	0.0000	1	0.0000	0.0000	1.0000	
A^2	2.611E-07	1	2.611E-07	0.3786	0.5578	
B^2	0.0005	1	0.0005	766.70	< 0.0001	
Residual	4.828E-06	7	6.897E-07			
Lack of Fit	4.828E-06	3	1.609E-06			
Pure Error	0.0000	4	0.0000			
Cor Total	0.0006	12				

Table 4. The Predicted and Observed Response Values

Factor	**Optimized Level**	
A : Wavelength 1	290 nm	
B : Wavelength 2	301 nm	
Response	Predicted	Observed
R1 : Absorbance 1	0.4877	0.4842
R2 : Absorbance 2	0.617	0.6153
Desirability	1	

Table 5. Accuracy Data of Esomeprazole and Domperidone

Parameters	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Amount Recovered
Esomeprazole					
50%	16	8	23.98	7.98	99.75
			23.86	7.86	98.25
			24.05	8.05	100.62
100%	16	16	31.88	15.88	99.25
			31.85	15.85	99.06
			32.09	16.09	100.56
150%	16	24	40.05	24.05	100.20
			39.94	23.94	99.75
			40.11	24.11	100.45
				Average	**99.76**
				SD	**0.7512**
				% RSD	**0.7530**
Domperidone					
50%	12	6	18.03	6.03	100.50
			18.11	6.11	101.83
			17.96	5.96	99.33
100%	12	12	24.28	12.28	102.33
			24.05	12.05	100.41
			24.17	12.17	101.41
150%	12	18	29.87	17.87	99.27
			29.83	17.83	99.05
			30.10	18.10	100.55
				Average	**100.52**
				SD	**1.1023**
				% RSD	**1.0966**

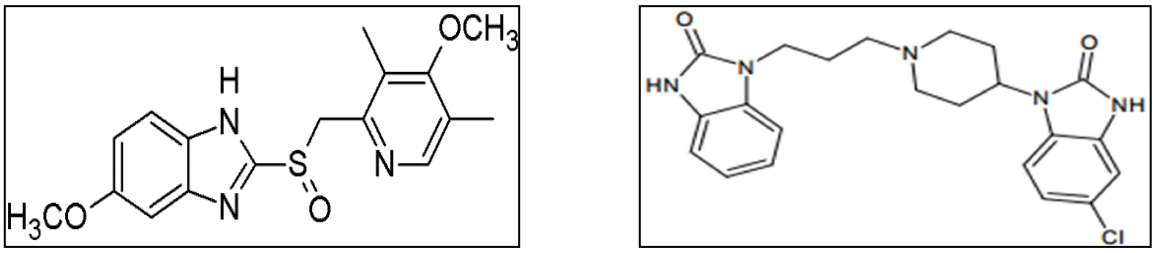
Table 6. Intra-day and Inter-day Precision Data of Esomeprazole and Domperidone

Parameter	Intra-day			Parameter	Inter-day	
	Concentration (µg/ml)	Absorbance *	% Amount Found*		Absorbance *	% Amount Found*
Esomeprazole						
0 Hours	16	0.5679	101.37	Day - I	0.5784	99.67
3 Hours		0.5732	101.75	Day - II	0.5641	101.35
6 Hours		0.5591	101.32	Day - III	0.5395	100.66
		SD	**0.1920**		**SD**	**0.6894**
		% RSD	**0.1892**		**% RSD**	**0.6856**
Domperidone						
0 Hours	12	0.4395	101.17	Day - I	0.4482	100.02
3 Hours		0.4683	101.25	Day - II	0.4627	99.62
6 Hours		0.4700	101.01	Day - III	0.4952	100.43
			SD	**0.0997**	**SD**	**0.3306**
			% RSD	**0.9865**	**% RSD**	**0.3306**

*Mean of six determinations

Table 7. LOD and LOQ Data of Esomeprazole and Domperidone

S.No	Esomeprazole		Domperidone	
	Slope	Y-Intercept	Slope	Y-Intercept
1	0.0348	0.0038	0.0357	-0.0003
2	0.0345	0.0054	0.0355	-0.0024
3	0.0352	0.0034	0.0358	-0.0023
4	0.0352	0.0029	0.0359	-0.0051
5	0.0354	0.0014	0.0356	-0.0010
6	0.0346	0.0082	0.0356	0.0005
Average	**0.0349**		**0.0357**	
SD		**0.002351**		**0.002009**
	LOD (µg/ml)	**0.2217**		**0.1856**
	LOQ (µg/ml)	**0.6719**		**0.5624**



(a) **(b)**

Figure 1. Structure of Esomeprazole (a) and Domperidone (b)

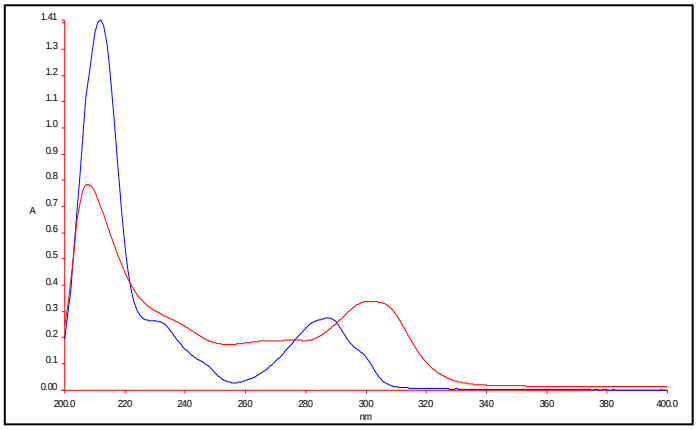


Figure 2. Overlay UV Spectrums of Esomeprazole and Domperidone

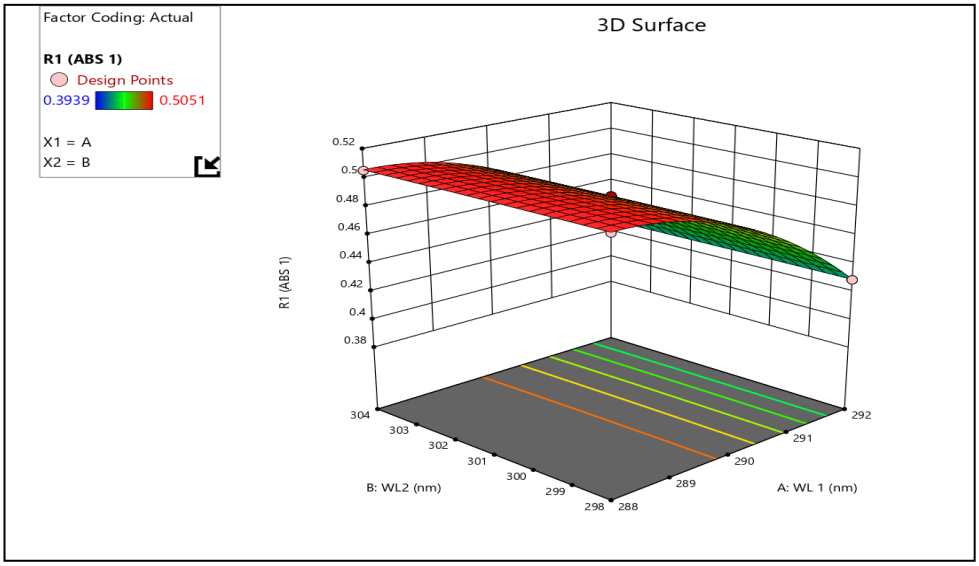


Figure 3. Interaction & 3D Image Showing the Influence of Independent Variables on A& B on Absorbance 1

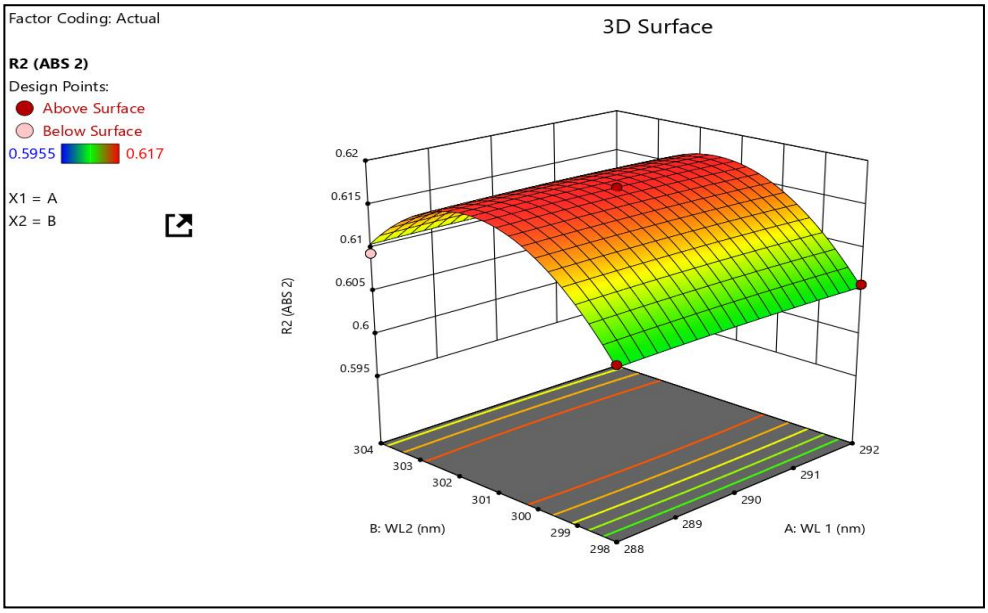


Figure 4. Interaction & 3D Image Showing the Influence of Independent Variables on A& B on Absorbance 2

3.1 Response Surface Methodology. The low and high level of independent variables for the method optimisation was selected based on overlay UV absorption spectra (Figure 2). The calibration and prediction sets were designed in 13 experiments and performed by using two independent variables such as wavelength 1 & wavelength 2 to optimize the responses (ABS 1 & ABS 2).

*3.2. Effect of Independent Variables on Response 1 (Absorbance 1).*The effect of independent variables on Absorbance 1 (Domperidone) was expressed by the 3D response, contour plot, and polynomial equation. 2nd order quadratic polynomial for response R1 (Absorbance 1) is given as follows;

R1 (Absorbance 1) = +0.4877 -0.0382 A +0.0000 B +0.0000 AB -0.0201 A^2 -0.0002 B^2

In this polynomial equation, A, and A^2 are the significant model terms as they have P-values less than 0.05. Thirteen experiments were performed to optimize the Absorbance of Domperidone (A1). The R 1(ABS 1) results varied from 0.3939 to 0.5051. The independent variable A (wavelength 1) had a negative effect on Absorbance1 and independent variable B (wavelength 2) had no impact, the regression coefficients of variables A & B were found to be 0.0382, 0.0000, respectively. Thus, it indicates, that Absorbance 1 decreases with an increase of A. However, wavelength 2 showed no notable influence on Absorbance 1. The Positive regression coefficient for AB was observed & was found to be 0.0000and it indicates, that response 1 (Absorbance 1) had nil effect with the simultaneous increase in AB.

3.3 Effect of Independent Variables on Response 2 (Absorbance2). The effect of independent variables on Absorbance 2 (Esomeprazole) was expressed by the 3D response, contour plot, and polynomial equation (Figure 3). 2nd order quadratic polynomial for response R2 (Absorbance 2) is given as follows.

R2 (Absorbance 2) = +0.6170 +0.0000 A +0.0025 B +0.0000 AB-0.0002 A^2-0.0087 B^2

In this polynomial equation, B, and B^2 is the significant model terms as they have P-values less than 0.05. Thirteen experiments were performed to optimize the Absorbance of Esomeprazole (A2). The R 2(ABS 2) results varied from 0.5955 to 0.617. The independent variable B (wavelength 2) had a positive effect on Absorbance 2 and independent variable A (wavelength 1) had no impact, the regression coefficients of variables A & B were found to be 0.0000,0.0025 respectively. Thus, it indicates, that Absorbance 2 increases with an increase of B. However, wavelength1showed no notable influence on Absorbance 2. The

Positive regression coefficient for AB was observed & was found to be 0.0000 and it indicates, that response 2 (Absorbance 2) had nil effect with the simultaneous increase in AB. Adjusted R1 and R2 were found to be within acceptable bounds (R2> 0.9), indicating that the experimental model fits polynomial equations well. The model is significant for the quantification process since the appropriate precision value of all responses was determined to be larger than 4, indicating a sufficient signal. The coefficient of variation (C.V.) represents the model's repeatability for all responses within the limit (percent C.V< 10). The 3D plot demonstrated the effects of variables on distinct responses. The graphical interpretation of the interactions can be done using three-dimensional (3D) plots of the model (Figure 4). As a result, the 3D graphs of the model were accustomed to assess the outcomes. The responses were mapped against two experimental factors while the other factors are held constant at their central level. The response surface plot was obtained for the maximum desirability function (D = 1), and the ANOVA findings indicate that obtained mathematical model is excellent (Tables 2 & 3). The maximum desirability value was obtained at wavelength 1 (290 nm), wavelength 2 (301 nm). As can be seen, the predicted and observed experimental values were very similar (Table 4)

3.4 Accuracy. The reliability and validity of the proposed method were examined by the standard addition technique at 50,100 and 150 % of the test concentration were analyzed and the percent recoveries ranged from 99.76±0.7530 & 100.52±1.0966 for Esomeprazole and Domperidone (Table 5). These findings confirmed that the excipients in pharmaceutical products do not interfere with the determination of Esomeprazole and Domperidone.

3.5 Precision. The intermediate precision of the method was confirmed by intra-day and inter-day analysis i.e. the analysis was repeated three times on the same day and on three successive days. The percentage relative standard deviation (% RSD) values for intra-day and inter-day studies were ≤ 2.0 percent of Esomeprazole and Domperidone (Table 6).

3.6 Limit of Detection and Limit of Quantification. The limit of detection (LOD) and limit of quantification (LOQ) of Esomeprazole and Domperidone were determined by using the standard deviation of the response and slope approach as defined in ICH guidelines (Table 7).

3.7 Analysis of Marketed Formulation. The proposed method was applied for the assay of Esomeprazole and Domperidone in pharmaceutical formulation. The findings of assay results were found to be in good agreement with the concentration taken for the formulations. This revealed that the matrices and /or excipients did not interfere with the quantifications.

The developed technique was much easier than HPLC methods stipulated in pharmacopeia[1, 2] and other existing analytical methods[7, 8, 9]. The method employed ethanol as a solvent and the procedure does not involve any sample pre-treatment.

4. Conclusion

UV Spectroscopic method is typically employed in research laboratories or industrial product laboratories for quality control or analysis of commercial products. When interferences are present, this approach may not be sufficient for sample analysis in some circumstances. As a result, new analytical applications and approaches based on the simultaneous use of classical analytical methods and sophisticated mathematical algorithms to deliver precise and reliable experimental results are required for quantitative analysis of drugs. For the simultaneous estimation of Esomeprazole and Domperidone, a new UV assay method was developed and validated. The chemometric (Analytical Quality by Design) technique was successfully applied for the optimization of spectroscopic method response parameters. The uses of analytical Quality-by-Design tools lead to a deeper understanding of how critical variables influence method performance and result in more robust and reliable methods. For optimizing the method parameters and examining the interaction of multiple variables, the RSM (Central Composite Design) was used. The UV method developed was found to be simple, accurate, precise, & robust. All of the validation parameters yielded positive findings. The study proved that chemometrics can be effectively coupled with UV spectroscopy to produce a more robust method (i-e) with no need for frequent validation. Hence, the validated UV method could be readily adapted for the simultaneous estimation of Esomeprazole and Domperidone in bulk and formulations.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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