Title: Chemometric Assisted UV-Spectrophotometric And RP-HPLC Method for Estimation of Domperidone and Esomeprazole in Pharmaceutical Formulation.

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Abstract

The chemometric approaches were used widely as statistical tool for various analytical methods for determination of drugs in their dosage form. The chemometric methods (linear regression and inverse least square) were applied to obtained analytical data to estimate and evaluate the accurate and reliable results. For simultaneous determination of Domperidone and Esomeprazole by UV-Spectroscopy method the absorbance was taken at two wavelengths 284 nm and 301 nm λ max of Domperidone and Esomeprazole respectively. The concentration range of Domperidone and Esomeprazole was 5-30 µg/ml and 6.6-40 µg/ml which obey beer's law. RP-HPLC method was developed for the simultaneous determination of Esomeprazole and Domperidone in the tablet dosage form by using gradient mobile phase consisting of Acetate-buffer: acetonitrile: methanol (55: 35: 10) aid of UV detector at 290 nm. For Esomeprazole and Domperidone, respectively the percentage recoveries were found compared UV spectroscopy method, HPLC method and linear regression applied for HPLC method to determine the p-value from one-way ANOVA and that shows there was no significant difference in assay between the to be 99.758% ± 0.2396% and 99.949% ± 0.2156%. Moreover, Inverse least square applied to ILS applied UV-Spectroscopy method, RP-HPLC method & Linear regression applied for HPLC method at the significant level of 0.05%.

Keywords: Chemometric methods (linear regression and inverse least square), Domperidone, Esomeprazole, UV spectroscopy method, RP-HPLC method.

1. Introduction

Gastroesophageal reflux disorder (GERD) is a chronic gastrointestinal ailment characterised by the emesis of gastric contents into the esophagus [1]. The pooled prevalence of GERD inside the Indian populace is 15.6 (95% CI 11.046 to 20.714) [2]. The primary signs and symptoms are continual heartburn and acid regurgitation. Some humans have GERD without heartburn. As an alternative, they experience pain in the chest, hoarseness in the morning or hassle swallowing [3]. Control of GERD may contain way of life amendment, medical remedy and surgical remedy. Way of life adjustments together with weight loss and/or head of a bed elevation had been shown to improve esophageal pH and/or GERD signs. Medical remedy includes acid suppression which may be accomplished with antacids, histamine-receptor antagonists or proton-pump inhibitors [4]. Domperidone, a famous antiemetic, is an instance of a prokinetic agent. It's far a dopaminergic blocker that will increase lower

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oesophagus sphincter muscle pressure and activates gastric motility [5].

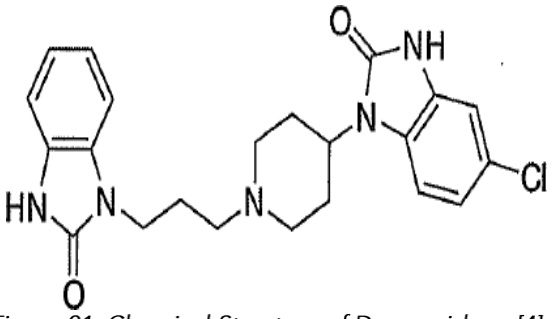

Figure 01: Chemical Structure of Domperidone [4]

Esomeprazole is a proton pump inhibitor (PPI) that works by reducing stomach acid & to alleviate acid-associated indigestion and heartburn [6].

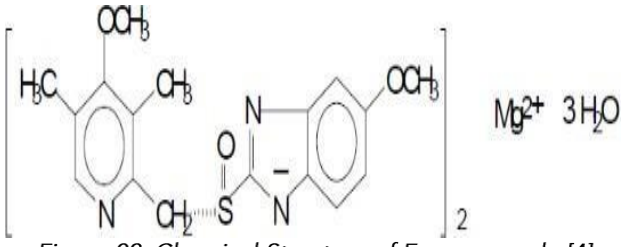

Figure 02: Chemical Structure of Esomeprazole [4]

A Combination of these two drugs is beneficial for the treatment of gastric esophagus reflux disease [7]. A detailed survey of literature revealed the detection of Domperidone and Esomeprazole has been done with UV-spectrophotometry, high-performance liquid chromatography, liquid chromatography-mass spectrometry [8-11]. Moreover, there are no studies have been completed on the chemometric approach of Domperidone and Esomeprazole [12].

Chemometrics is a branch of chemistry that employs formal logic in mathematical, statistical, and other ways to develop or choose the best measurement methods and experiments as well as to analyse chemical data to produce the most pertinent chemical knowledge possible. [13].

In this study, a simple, rapid and economical quantitative method has been evolved for the simultaneous determination of Domperidone and Esomeprazole in tablet dosage form. The method is primarily based on a chemometric manner regarding the inverse UV calibration method without prior separation. In comparison to HPLC, the evolved chemometric UV-method proved to be adequately correct and particular. Moreover, being simple and inexpensive, it is more appealing to use for routine assay of Domeridone-esomeprazole combination in tablet than HPLC which is demanding in terms of running cost and sophistication. [15-19] [14-18].

Experimental work

Instrumentation and software UV-Spectrophotometric of a Shimadzu double beam UV spectrophotometer prepared with UV Probe software (version UV 1800). Chemometric-assisted spectrophotometric measurements had been done with the usage of the software Minitab 21. For the binary mixture of drugs simultaneous equations method is used to determine many equations as variables.

Inverse least square (ILS):

ILS method the mathematical formulation of this method uses the application of Multiple Linear Regression to an inverse equation of the Beer-Lambert law of spectroscopy:

A statistical matrix calibration methodology is the ILS method. ILS employs a Multiple Linear Regression application to invert the Beer's Lambert law equation derived from spectroscopy.

$C = PA$ (or $C = P \times dA/d\lambda$).

Where, C stands for concentration, A stands for obtained absorbance at a given wavelength, $dA/d\lambda$ represent derivative absorbance matrix and p is the calibration coefficient.

The inverse calibration method can be used to apply to a mixtures containing in the following steps:

A1, A2, A3, A4, and A5 are determined by measuring the absorbance of each solution at λ1, λ2, λ3, λ4 and λ5 [22][19].

Two calibration equations were produced by processing the data by using software (Minitab 21). On the basis of inverse least-squares theory, the linear regressions were used to calculate the calibration equations (2 and 3).

$C1 = k1 + \alpha1A1+\alpha2A2+\alpha3A3+\alpha4A4+\alpha5A5$ (2)

$C2 = k2 + \beta1A1+\beta2\ A2+\beta3\ A3+\beta4\ A4+\beta5A5$ (3)

C1 and C2 are concentrations of the components of the binary mixture, k1 and k2 are constants, α1 and β1 are coefficients.

In a similar manner, the samples solutions are examined by measuring their absorbances, A1, A2, A3, A4, and A5, at the respective wavelengths λ1, λ2, λ3, λ4, λ5.

The calibration equations (2 and 3) are substituted with the observed absorbances of the samples to produce C1 and C2 [23][20].

Using C1 and C2, the amount of each ingredient in the tablet formulation was calculated [26][21].

2. Material and Method

Esomeprazole was a gifted from Torrent Pharmaceuticals Ltd., Ahmedabad, India. Domperidone was procured from Cadila pharmaceuticals Ltd., Gujarat, India.

The pharmaceutical formulation of tablets containing Domperidone (15mg) and Esomeprazole (20mg) as brand name Ranidom-RD tablet was obtained from the local drug store [1].

Solvents

Methanol used in UV spectroscopic method was purchased from RANCHEM, RFCL Ltd, New Delhi, India.

HPLC grade methanol and acetonitrile were purchased from RANCHEM, RFCL Ltd., New Delhi, India. Water from Milli Q (Millipore Bedford, MA) was used in all experiments. Ammonium acetate and Glacial acetic acid were purchased from MERCK, Pvt Ltd., Mumbai, India.

Instrumentation

Shimadzu Groups UV-Visible Spectrophotometer model 1800 was used in the spectrophotometric measurements.
The HPLC instrumentation comprises A Shimadzu's HPLC (LC-2010-HT, Shimadzu,Singapore) equipped with UV-Visible Detector, Phenomenex, C18, ODS column (250 mm X
4.6 mm; 5µ.), auto sampler.

Preparation of standard stock solutions

Standard (STD) Domperidone powder was precisely weighed out at 10mg and transfer into 100ml volumetric flask; and diluted to 100 ml with methanol to prepare a working standard having a concentration of 100µg/ml of Domperidone. The same stock solution was prepared for both the UV spectroscopy method and the HPLC method.
STD Esomeprazole powder was accurately weighed out at 10mg and transfer into 100ml volumetric flask and diluted to 100 ml with methanol to prepare a working standard having a concentration of 100µg/ml of Esomeprazole. The same stock solution was prepared for both the UV spectroscopy method and the HPLC method.

Assay preparation

Contents of 20 tablets having Esomeprazole and Domperidone were weighed accurately. A quantity of powder equivalent to about 15 mg of Domperidone and 20 mg of Esomeprazole was taken into a 100 ml volumetric flask and completely dissolved and filtered through Whatman filter paper No.41. The 15µg/ml dilution was prepared in a 10 ml volumetric flask with a mobile phase for assay preparation from the stock solution. The UV spectra were recorded in the wavelength range of 200-400 nm.

The Calibration Equations

For measurement of the absorbance A1, A2, A3, A4, and A5 of the binary mixtures containing known varying concentrations of Domperidone and Esomeprazole five wavelengths 279, 285, 290, 297 and 304 nm were selected in the overlapping zones toacquire the two calibration equations.
Table a. shows the absorbance's of six mixtures.
By using Minitab 21 software using the data in Table a gave the following calibrationequations.

$C_1=3.0166+172.06A_1+434.588A_2+251.827A_3-2.291A_4+64.593A_5$ $R^2=99.8\%$ (4)

$C_2=3.762+213.8\ A_1+548.0\ A_2+339.1\ A_3-1031\ A_4+83.77\ A_5$ $R^2=99.8\%$ (5)

Where, C1 and C2 represent concentrations of Domperidone and Esomeprazole, respectively[25] [22].

Checking the Reliability of the Calibration Equations:

A series of synthetic binary mixtures of Domperidone and Esomeprazole reference standards were prepared and the amount of each recovered was found from the calibration equations (2 and 3) by using A1, A2, A3, A4, and A5 absorbance (Table b).

The HPLC Method

Chromatographic conditions

The analytical wavelength was set at 290 nm and 20 µL of samples were automatically injected. The chromatographic separations were accomplished by using the mobile phase, consisting of buffer (ammonium acetate pH 3.4): acetonitrile: and methanol (55:35:10). Mobile phase was pumped in the gradient system at a flow rate of 1.0 mL/min.

3. Result

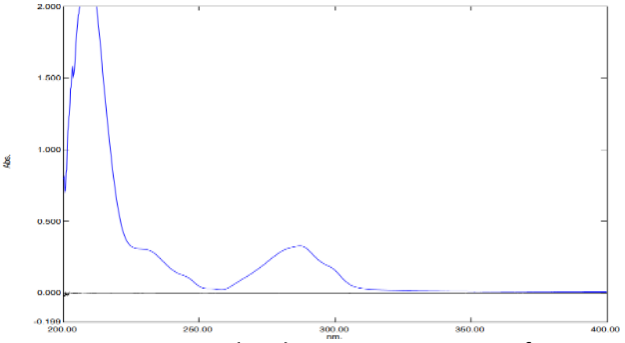

Figure 3. Zero order absorption spectrum of pure Domperidone.

For the simultaneous determination of Domperidone and Esomeprazole in tablet UV- spectrophotometric chemometric method was developed. Figure 3 and Figure 4 depict the ordinary UV-spectra of metabolic solutions of pure Domperidone and Esomeprazole with absorption maxima at 284 nm and 301 nm, respectively.

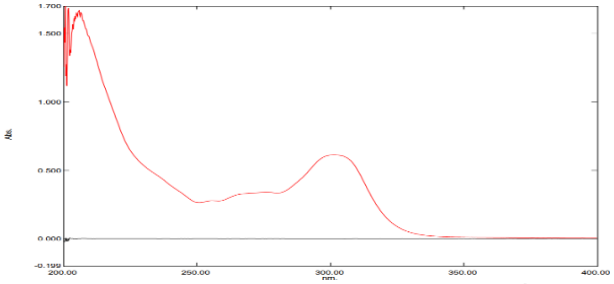

Figure 4. Zero order absorption spectrum of pure Esomeprazole.

The combination of Figure 3 and Figure 4 highlights the need for a selective analytical method for the simultaneous determination of the two components. To resolve the problem developed chemometric method could be advantageous.

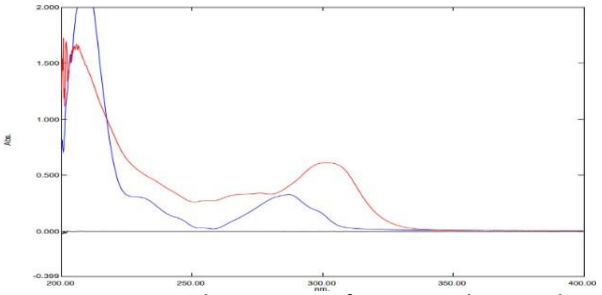

Figure 5. Overlay spectra of Domperidone and Esomeprazole.

Figure 5 represents the UV-absorption spectrum of

the binary mixture.

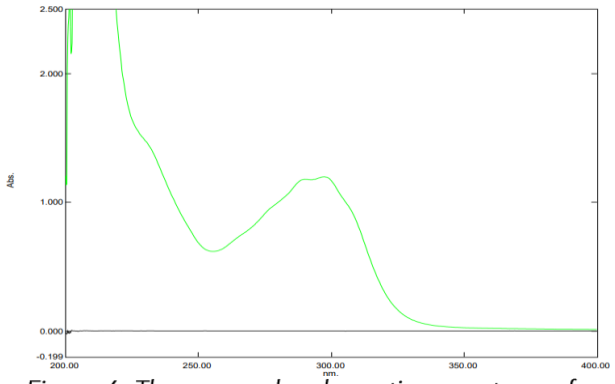

Figure 6. The zero-order absorption spectrum of a mixture of Domperidone andEsomeprazole.

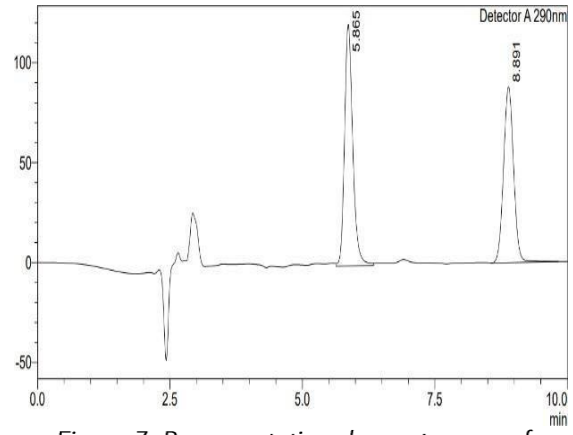

Figure 7. Representative chromatogram of Domperidone and Esomeprazole in thetablet extract.

Table a. Absorbance values (A1, A2, A3, A4, and A5) at 279, 285, 290, 297, and 304 nm for Domperidone and Esomeprazole binary mixtures. [n=3]						
Domperidone C1 (µg/ml)	Esomeprazole C2 (µg/ml)	A279	A285	A290	A297	A304
5	6.6	0.239	0.272	0.305	0.314	0.275
10	13.3	0.542	0.617	0.686	0.707	0.620
15	20	0.785	0.908	1.004	1.034	0.914
20	26.6	1.087	1.252	1.378	1.423	1.246
25	33.3	1.330	1.516	1.678	1.729	1.531
30	40	1.562	1.806	1.991	2.050	1.806

Table a. Depicts the absorbance values of six synthetic mixtures used for the computation of the constants and the coefficient of the calibration equations. The value of the coefficient determinations (R2 = 99.8%) suggests required linearity.

Domperidone (µg/ml)		%Recovery	Esomeprazole (µg/ml)		%Recovery
5	4.984	99.680	6.6	6.58	99.815
10	9.998	99.980	13.3	13.29	99.984
15	14.976	99.840	20	19.95	99.753
20	19.995	99.975	26.6	26.57	99.907
25	24.982	99.928	33.3	33.26	99.461
30	29.973	99.910	40	39.78	99.474
Mean % recovery = 99.886 RSD% = 0.112			Mean % recovery = 99.732 RSD% = 0.220		

The data collected in Table b summarize the results obtained when checking the validity of the two calibrating equations. The % recoveries of 99.886% and 99.732% and the RSD% of 0.112 and 0.220 reflect valid results.

Table c. Values of % recoveries of Domperidone and Esomeprazole in tablet formulation by UV spectroscopy. [n=3]					
Claimed Domperidone (µg/mL)	Found Domperidone (µg/mL)	%Recovery	Claimed Esomeprazole (µg/mL)	Found Esomeprazole (µg/mL)	%Recovery
5	4.997	99.953	6.6	6.58	99.767
10	9.994	99.947	13.3	13.29	99.986
15	14.992	99.946	20	19.93	99.687
20	19.989	99.949	26.6	26.56	99.706
25	24.987	99.948	33.3	33.09	99.692
30	29.985	99.952	40	39.88	99.712
Mean % recovery = 99.886 RSD% = 0.239665			Mean % recovery = 99.732 RSD% = 0.215655		

Table c depicts the % recoveries obtained by applying the developed chemometric method to the simultaneous determination of Domperidone and Esomeprazole in the tablet dosage form. The mean % recoveries were 99.949% and 99.758% and the RSD% of 0.239665 and 0.215655 for the Domperidone and Esomeprazole respectively.
The assay results were found within the range of 95

to 105% as per ICH guidelines.

Table d. Peak area obtained from RP-HPLC method. [n=3]					
Domperidone			Esomeprazole		
Conc.	Peak area	%RSD	Conc.	Peak area	%RSD
2	1955074±16418.1	0.844	2.6	1325625±37803.0	2.833
5	2035074±43024.6	2.101	6.5	1428567±46975.3	3.253
8	2144762±25753.0	1.194	10.4	1561729±24185.7	1.577
11		1.003	14.3		2.465
14	2355872±33979.3	1.447	18.2	1732702±27187.1	1.543
17		0.044	22.1		1.557

Table d represent the peak areas which were obtained from RP HPLC method.

Domperidone

Table e. Results of assay of Domperidone by the developed chemometric UV- Spectrophotometric and the linear regression applied RP-HPLC methods.		
Parameter	Developed chemometric UV-Spectroscopicmethod	Linear regression appliedRP-HPLC method
	15	15
Recoveredamount	15.09	15.01
P-Value	0.100471(NS)	
*NS = not significant		

Esomeprazole

Table f. Results of assay of Esomeprazole by the developed chemometric UV- Spectrophotometric and the linear regression applied RP-HPLC methods.		
Parameter	Developed chemometric UV-Spectroscopicmethod	Linear regressionapplied RP-HPLC method
Claimed amount	20	20
Recovered amount	19.91	19.98
P-Value	0.091786(NS)	
*NS = Not significant		

The results assembled in Table e and Table f show the comparison of the developed chemometric UV-Spectroscopic method and RP-HPLC methods by linear regression of assay. The calculated P-Value at a 0.05% significance level is greater than the tabulated counterparts suggesting a non-significant difference between the chemometric UV-Spectroscopic method and RP-HPLC method by linear regression.

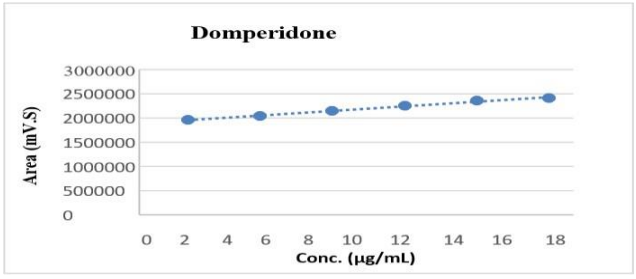

Figure 8. Calibration curve of Domperidone (RP-HPLC method).

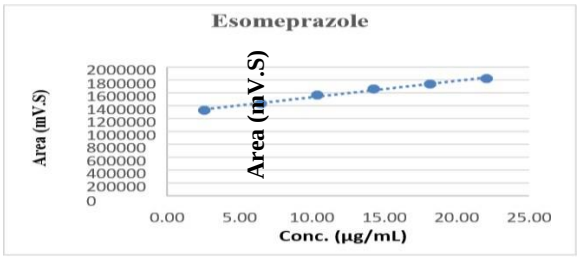

Figure 9. Calibration curve of Esomeprazole (RP-HPLC method).

The linear regression method was applied on obtained results of the RP-HPLC method and % RSD was calculated.

4. Discussion

Domperidone and Esomeprazole UV spectra shown in Figure 3 to 5 were considered to overlap with the absorption maxima at 284 nm and 301 nm. The inverse calibration method can be used to determine two components simultaneously in cases of such extreme spectral overlaps. As a result, the absorbance of a binary synthetic mixture with known variable concentrations was determined for each mixture at the five chosen wavelengths in the overlapping spectrum. Using Minitab 21, multiple linear regressions produced two calibration equations (4 and 5).

C1=3.0166+172.06A1+434.588A2+251.827A3-802.291A4+64.593A5 (4)

R2=99.8%

C2=3.9217+223.687 A1+564.964 A2+327.375 A3-1042.98 A4+83.9716 A5 (5)

R2=99.8%

Where, C1 and C2 stand for the dosages (in µg/mL) of the drugs Domperidone and Esomeprazole, respectively and A1-A5 represented the absorbance of the binary mixtures at the chosen wavelengths. Excellent linearity was revealed by the R2 and coefficient of determination. The percentage recoveries found in Table b which demonstrated the reliability of the two calibration equations (4 and 5).

Adequate percentage recoveries were attained when the Chemometrics technique was used for the simultaneous assay of Domperidone and Esomeprazole in tablet dosage form, as indicated in Table c. Peak area obtained from RP- HPLC method of Domperidone and Esomeprazole which Depicted in Table d. The results obtained from the RP-HPLC method R2 value obtained were 0.99. Table e and Table f represented the assay results and p-value obtained from applying t-test to developed chemometric UV-Spectroscopic method and RP-HPLC method by linear regression [15-19][14-18].

The inverse least square method and the linear regression method were applied for the estimation of Domperidone and Esomeprazole in a combined dosage form using UV spectroscopy and RP-HPLC method respectively. The method can be used effectively for the simultaneous determination of Domperidone and Esomeprazole which can be helpful for industrial purposes.

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