

# Simultaneous Estimation of Esomeprazole and Domperidone in Combined Dosage form by HPLC

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**Abstract:** A simple, accurate, reliable and reproducible HPLC method was developed for the simultaneous determination of esomeprazole and domperidone in combined dosage forms. The method employed C18 phenomenex column, acetate buffer: acetonitrile: methanol (55:35:10) as mobile phase and detection was made at 290nm. The retention times were found to be 6.76 and 4.42 min for ESO and DOMPE respectively. The method was validated as per ICH guidelines. The method shows good linearity, accuracy, and precision, limit of detection and limit of quantification. The linearity range was found between 4-19 µg/mL for both ESO and DOMPE with relative standard deviation of 0.022 and 0.076 respectively. The value for LOD was found to be 0.3 µg/mL and 0.4 µg/mL and LOQ was found to be 1.5 µg/mL and 2.5 µg/mL for ESO and DOMPE respectively. The main recovery was found to be  $99.81 \pm 1.27$  and  $100.43 \pm 1.15$  for ESO and DOMPE respectively. The method was suitable for routine analysis of ESO and DOMPE both individually and in combined dosage forms.

**Keywords:** Simultaneous determination; HPLC; Esomeprazole; Domperidone

## 1. Introduction

Esomeprazole [1] (ESO), *S*-isomer of omeprazole inhibits gastric acid secretion and is cost effective in the treatment of gastric oesophageal reflux diseases. It is the first single optical isomer proton pump inhibitor. It provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole. It is chemically bis (5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole)[2].

Domperidone (DOMPE), a dopamine antagonist is usually given along with proton pump inhibitors as ulcers are usually attended with

vomiting. Chemically, it is [5-chloro-1-[1,3-(2,3-dihydro-2-oxo-1*H*-benzimidazole-1yl)prpyl]-4-piperdiny-1,3-dihydro-2*H*-benzimidazole-2-one] [3]. Spectrophotometric and liquid chromatographic methods are available in the literature for estimation of omeprazole and esomeprazole [4-15]. However, no liquid chromatographic method is available for simultaneous analysis of esomeprazole in combination with other drugs. We have reported Q absorption ratio method for simultaneous quantification of esomeprazole and domperidone [17]. Here we report a liquid chromatographic method for simultaneous

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estimation of esomeprazole and domperidone in combined dosage forms. Since combination of esomeprazole and domperidone has been emerging as a successful line of therapy, it is necessary to develop simple and reproducible analytical methods.

## 2. Experimental

### 2.1. Chemicals, Reagents and Apparatus

Esomeprazole was a gift from Torrent Pharmaceuticals Ltd., Ahmedabad, India. Domperidone was procured from Cadila pharmaceuticals Ltd., Gujarat, India. HPLC grade methanol and acetonitrile were purchased from RANCHEM, RFCL Ltd., New Delhi, India. Water from Milli Q (Millipore Bedford, MA) was employed in all experiments. Ammonium acetate and Glacial acetic acid were purchased from MERCK, Pvt Ltd., Mumbai, India.

The HPLC instrumentation comprises of A Shimadzu's HPLC (LC-2010-HT, Shimadzu, Singapore) equipped with UV-Visible Detector, phenomenex, C18, ODS column (250 mm X 4.6 mm; 5 $\mu$ .), Hamilton 20  $\mu$ L syringe, A Citizen analytical balance (Sartorius), An ultra sonic sonicator (Equitron), Borosil volumetric flask of 10, 25, 50, 100 ml capacity, pipettes – 1ml, 5ml, 10ml, beakers, measuring cylinders etc.

### 2.2. Preparation of the mobile phase

Buffer: 1M buffer solution was prepared by dissolving 77.1 g of accurately weighed ammonium acetate and dissolved in 700 mL of water to which 57 mL of glacial acetic acid was added and diluted up to 1000 mL with water. pH was adjusted to  $3.5 \pm 0.05$  with glacial acetic acid.

Acetonitrile and methanol were mixed with buffer to achieve a mobile phase of a mixture of buffer: acetonitrile: methanol (55:35:10). The mobile phase was filtered through a ny-

lon 0.45  $\mu$ m membrane filter and was degassed for 3 min before use.

### 2.3. Chromatographic conditions

The analytical wavelength was set at 290 nm and 20  $\mu$ L of samples were manually injected with Hamilton syringe. The chromatographic separations were accomplished using mobile phase, consisting of buffer (ammonium acetate pH 3.4): acetonitrile: methanol (55:35:10). Mobile phase was pumped in isocratic system at a flow rate of 1.0 mL/min.

### 2.4. Preparation of standard stock solutions A. Standard ESO stock solution (100 $\mu$ g/mL)

Standard ESO powder (5 mg) was weighed accurately and transferred in to 50 mL volumetric flask and dissolved in and diluted to 50 mL with methanol to prepare working standard solution having concentration of 100  $\mu$ g/mL.

### B. Standard DOMPE stock solution (100 $\mu$ g/mL)

Standard DOMPE powder (5 mg) was weighed accurately and transferred in to 50 mL volumetric flask and dissolved in and diluted to 50 mL with methanol to prepare working standard solution having concentration of 100  $\mu$ g/mL.

### 2.5. Standard solution of mixture of ESO and DOMPE

Accurately weighed ESO (5 mg) and DOMPE (5 mg) were transferred to a 50 mL volumetric flask and dissolved in and diluted to 50 mL with methanol. The solution (1 mL) was transferred to a 10 mL volumetric flask and diluted to the mark with methanol to obtain final solution with ESO (10  $\mu$ g/mL) and DOMPE (10  $\mu$ g/mL).

## 2.6. Sample solution

Contents of 20 capsules having ESO and DOMPE were emptied and weighed accurately. A quantity of the powder equivalent to about 20 mg of ESO and 30 mg of DOMPE was taken in to 100 mL volumetric flask, completely dissolved and filtered through whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined and diluted to the mark with methanol. One mL of extract was transferred into 10 mL volumetric flask and diluted to the mark with methanol to get an approximate concentration of 20 µg/mL of ESO and 30 µg/mL of DOMPE.

## 3. Method validation

### 3.1. Solution stability

Sample solutions were kept at 25°C and 2-8°C for 24 h and 3 days, respectively. Assay of initial time period was compared with these two time points. The falls in the assay values were evaluated. The difference between assays should not be more than 2 % for formulation, and 0.5% for API.

### 3.2. Linearity (Calibration Curve):

A calibration curve was plotted over a concentration range of 4 - 19 µg/mL for both ESO and DOMPE. Accurately measured standard stock solution of ESO (0.4, 0.7, 1.0, 1.3, 1.6 & 1.9 mL) and standard stock solution of DOMPE (0.4, 0.7, 1.0, 1.3, 1.6 & 1.9 mL) were transferred to a separate series of 10 mL of volumetric flasks and diluted to the mark with methanol. Twenty microlitre of each solution in the concentration was injected under operating chromatographic conditions described above. Calibration curves were constructed for ESO & DOMPE by plotting area versus concentrations. Each reading was average of five determinations.

## 3.3 Precision

### A. Repeatability (Precision on replication)

Method precision of experiment was performed by preparing the standard solution of ESO (5 µg/mL) and DOMPE (5 µg/mL) for six times and analyzed as per the proposed method.

### B. Intermediate precision (Reproducibility)

The Intra-day precision (C.V) was determined for standard solution of ESO and DOMPE (4 - 19 µg/mL) for five times on the same day. The Inter-day precision (C.V) was determined for standard solution of ESO and DOMPE (4-19 µg/mL) for five days.

### 3.4. Accuracy (% Recovery)

The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the capsules (ESO 2 µg/mL and DMPE 3 µg/mL) with three different concentrations of standards (ESO 1,2,3 µg/mL and DOMPE 1,2,3 µg/mL).

### 3.5. Limit of Detection

Limit of detection was calculated using following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times \text{N/S}$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve. It is expressed as signal to noise ratio of **2:1** or **3:1**

### 3.6. Limit of Quantification

Limit of quantification was calculated using following equation as per ICH guidelines.

$$\text{LOQ} = 10 \times \text{N/S}$$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve. It is expressed as signal to noise ratio of **10:1**.

**System suitability:** Number of theoretical plates was determined by employing the formula  $n = 16(t/w)^2$  where  $t$  = retention time and  $w$  = width of the peak. Tailing factor was derived from the formula  $t = w/2t$  where  $w$  = half of the width,  $t$  = retention time.

## 4. Results and discussion

### 4.1. Selection of mobile phase:

Of the various combinations of the mobile phases tried, the one consisting a mixture of buffer: acetonitrile: methanol (55:35:10) at 1 mL/min flow rate, was found to be most suitable. Both ESO and DOMPE resolved well and could be detected at 290 nm. With retention times of 6.76 and 4.42 minutes respectively (Figure 1). Good linearity could be achieved for both ESO and DOMPE between the concentration ranges of 4-9  $\mu\text{g/mL}$  (Figure 2 and 3).

Results obtained by applying the RP-HPLC method showed that the concentrations of ESO and DOMPE can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of ESO and DOMPE in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The market formulation was found to contain  $101.85 \pm 1.07$  and  $100.13 \pm 1.05$  % of the labeled amount of ESO and DOMPE respectively in one formulation and  $101.45 \pm 1.60$  and  $101.80 \pm 1.00$  % of the labeled amount of ESO and DOMPE respectively in another formulation (Table 3).

### 4.2. Validation:

**Linearity and range-**The six-point calibration curves that were constructed were linear over the selected concentration range for both ESO and DOMPE ranging between 4 - 19  $\mu\text{g/mL}$ . The linearity of the calibration graphs and adherence of the system to Beer's law were validated by the high value of the correlation coefficient 0.999 and 0.993 and the intercept values of 6.63 and 8.05 for ESO and DOMPE respectively (Table 1).

**Accuracy-**Good recoveries were obtained in the method for both the compounds which were found to be  $99.81 \pm 1.27$  and  $100.43 \pm 1.15$  for ESO and DOMPE respectively (Table 2).

**Precision-**The % R.S.D were found to be 0.31 - 1.05 and 0.42 - 1.87 for intra-day and 0.36 - 1.60 and 0.39 - 1.75 for inter-day variations (Table 1).

**Limit of Detection and Limit of Quantitation-**The value for LOD was found to be 0.3  $\mu\text{g/mL}$  and 0.4  $\mu\text{g/mL}$  and LOQ was found to be 1.5  $\mu\text{g/mL}$  and 2.5  $\mu\text{g/mL}$  for ESO and DOMPE respectively (Table 1).

**System suitability testing for HPLC-** The number of theoretical plates, tailing factor and retention times was well within the accepted values in the method. The values are presented in (Table 4).

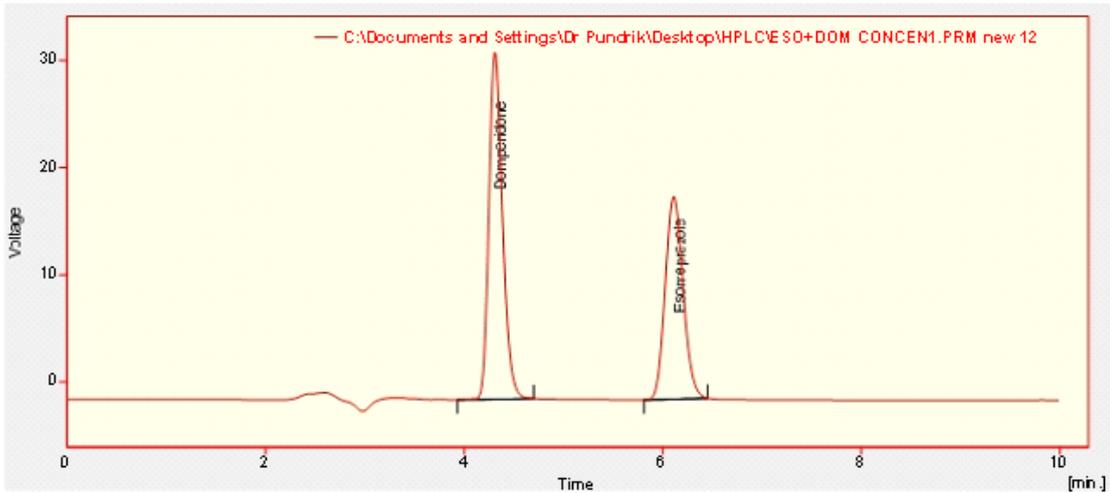


Figure 1. Chromatogram of Esomeprazole and Domperidone  
Retention time: 4.42 min for DOMPERIDONE and 6.76 min for ESOMEPRAZOLE

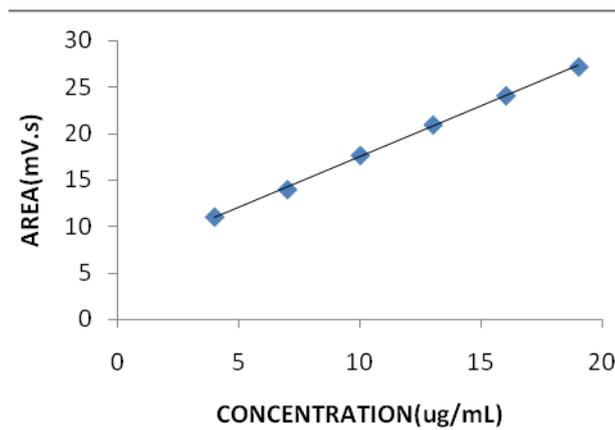


Figure 2. Calibration curve of Esomeprazole  
Slope: 1.087 Intercept: 6.630 Correlation coefficient: 0.999

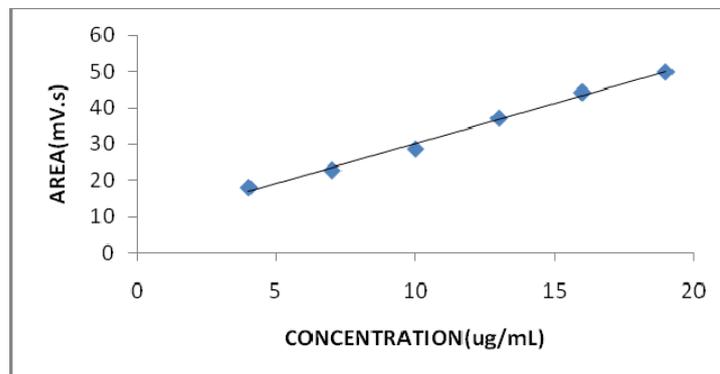


Figure 3. Calibration curve of Domperidone  
Slope: 2.209 Intercept: 8.054 Correlation coefficient: 0.993

Table 1. Method validation parameters obtained by applying the proposed methods for determination of ESOMEPRAZOLE and DOMPERIDONE in binary mixtures

Parameters	ESOMEPRAZOLE	DOMPERIDONE
Calibration range	4 - 19 µg/mL	4 - 19 µg/mL
Limit of Detection	0.3 µg/mL	0.4 µg/mL
Limit of Quantitation	1.5 µg/mL	2.5 µg/mL
Slope	1.087	2.209
Intercept	6.630	8.054
Mean	19.15	33.46
Standard deviation	0.022	0.076
Correlation coefficient	0.999	0.993
Intraday RSD, %	0.31 - 1.05	0.42 - 1.87
Interday RSD, %	0.36 - 1.60	0.39 - 1.75

Table 2. Data of Table-2 Data of recovery study of ESOMEPRAZOLE and DOMPERIDONE by HPLC method

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery ± S.D (n=3)
ESOMEPRAZOLE	4	2	6.08	101.33 ± 1.22
	4	4	7.98	99.75 ± 0.87
	4	6	9.83	98.36 ± 1.74
DOMPERIDONE	6	3	9.10	101.11 ± 1.32
	6	6	11.90	99.16 ± 0.75
	6	9	15.15	101.01 ± 1.37

Table 3. Application of the proposed method to the pharmaceutical dosage forms

Formulation	ESOMEPRAZOLE			DOMPERIDONE		
	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)
Brand I	20	20.37	101.85 ± 1.07	30	30.04	100.13 ± 1.05
Brand II	20	20.29	101.45 ± 1.60	30	30.54	101.80 ± 1.00

Table 4. System Suitability Parameters

System Suitability Parameters	ESOMEPRAZOLE	DOMPERIDONE
Retention Time	6.76	4.42
Tailing factor	1.18	1.26
Theoretical plate	3460	2531

Zarparkar and Kanyawar [15] reported a HPLC method for omeprazole and domperidone. While the mobile phase used by them consists acetonitrile and phosphate buffer. We employed acetate buffer, acetonitrile and methanol. The detection of peaks in our method was done at 290nm for both ESO and DOMPE, which is closer to their individual  $\lambda_{\max}$  values (301nm and 284nm for ESO and DOMPE respectively). However, in the above reported method detection was made at 220nm. Linearity range in the above method was found to be 20-60  $\mu\text{g/mL}$  for both omeprazole and domperidone, whereas in our method linearity was found to be 4-19  $\mu\text{g/mL}$  for both ESO and DOMPE. Hence, the proposed HPLC method is more sensitive than the above HPLC method. Our results of HPLC analysis are similar to the results reported by as using Q absorption method.

## 5. Conclusion

Thus it can be concluded that esomeprazole and domperidone can be quantified simultaneously by the proposed HPLC method using an isocratic mobile phase consisting of buffer: methanol: acetonitrile (55: 35: 10) using a UV detector at 290 nm. The proposed method is simple, sensitive, rapid, accurate and precise. It can be applied successfully for the estimation of esomeprazole and domperidone in bulk and its pharmaceutical formulations.

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