

## QUANTIFICATION OF DOMPERIDONE, PARACETAMOL, ESOMEPRAZOLE AND LANSOPRAZOLE IN PHARMACEUTICAL DOSAGE FORMS BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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### ABSTRACT

A simple, sensitive, and accurate high performance liquid chromatographic method developed and validated for the simultaneous estimation of domperidone, paracetamol, esomeprazole, and lansoprazole in pharmaceutical dosage forms. The assay involved the use of photo diode array detection at 285 nm for domperidone, 246 nm for paracetamol, 300 nm for esomeprazole, and 283 nm for lansoprazole. The compounds were well separated on a Supelco C18 (250x4.6mm,5 $\mu$ ) reversed-phase column and mobile phase consisting of 0.01 M, pH 7.00, dipotassium hydrogen phosphate buffer - acetonitrile (65:35 v/v) at a flow rate of 1.0 mL min<sup>-1</sup>. The linearity range was 0.5 - 50  $\mu$ g mL<sup>-1</sup> for all the four analytes domperidone, paracetamol, esomeprazole and lansoprazole. Limits of detection (LOD) obtained were 0.1  $\mu$ g mL<sup>-1</sup> for each of the four drugs. The developed analytical method was validated as per ICHQ2 (R1) guidance. Study showed that reversed-phase liquid chromatography is sensitive and selective for the determination of domperidone, paracetamol, esomeprazole, and lansoprazole in pharmaceutical dosage forms.

**Keywords:** Reverse phase liquid chromatography, Domperidone, Paracetamol, Esomeprazole, Lansoprazole.

### INTRODUCTION

Antiemetics are agents used to treat nausea and vomiting. These agents are used in treatment of upper gastrointestinal (GI) motility disorders as in diabetic gastropathy and irritable bowel disease; to reduce emesis caused by anticancer drugs and that in Parkinson's disease. The central neural regulation of vomiting is vested in two separate units in the medulla viz. chemoreceptor trigger zone (CTZ) in the floor of the IV ventricle, that is particularly sensitive to chemical stimuli (dopamine, serotonin) and vomiting center (VC) located in the dorsolateral border of the reticular formation of the medulla. VC receives many excitatory inputs from: nerve endings of vagal sensory fibers in the G-I tract; the labyrinth via the vestibular nuclei (histamine, acetylcholine); intracranial pressure receptors and visceral stimulation (dopamine and serotonin)<sup>1, 2</sup>.

Antiemetic drugs can be combined with other drugs, with the goal of increasing efficacy or decreasing associated drug toxicity (by reducing dosage)<sup>1</sup>. Prokinetic anti-emetics are first-line choices in the treatment of acute migraine attacks when combined with standard analgesics<sup>2</sup>. The medications commonly used to treat gastro esophageal reflux disease (GERD) include acid-suppressive medications, such as the proton-pump inhibitors (PPIs) which are combined with antiemetics. The PPIs lansoprazole and esomeprazole are the most potent acid-inhibitors available<sup>3</sup>.

Domperidone (DOM), 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazole-1-yl)propyl]-4-piperidinyl]-1-3-dihydro-2H-benzimidazole-2-one is a dopamine antagonist and exerts its effect at peripheral D2 receptors in the GI tract; the CTZ, which is outside the blood-brain barrier; and the pituitary<sup>4, 5</sup>. It has antiemetic property similar to metoclopramide and neuroleptic drugs. Unlike these drugs, however, domperidone does not readily cross the blood brain barrier and seldom causes extra pyramidal side effects<sup>6, 7</sup>. It is a potent gastrokinetic agent causing faster gastric emptying. The combination of domperidone with lansoprazole or esomeprazole is used to treat GERD.

Lansoprazole (LAN), 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl] methyl] sulfinyl]-benzimidazole and Esomeprazole (ESO), 5-Methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl] methyl] sulfinyl] benzimidazole are proton pump inhibitors and inhibit basal & stimulated gastric acid secretion by forming active sulfonamide metabolite that irreversibly binds to proton pump & blocks final step in acid secretion<sup>8, 9</sup>.

Paracetamol (PAR), N-(4-Hydroxyphenyl) acetamide is an analgesic and produces analgesia by central inhibition of prostaglandin synthesis<sup>10</sup>. It is used in the treatment of acute migraine attacks in combination with domperidone.

Several techniques for example spectrophotometry, HPLC, LC-MS, and HPTLC have been reported in the literature for the determination of DOM<sup>11, 17</sup>, PAR<sup>18, 21</sup>, ESO<sup>22, 25</sup> and LAN<sup>26, 30</sup> in pharmaceuticals and biological samples.

This paper describes the development and validation of RP-HPLC for assay of DOM simultaneously with any of its combination drugs PAR or ESO or LAN by use of normally used column and single mobile phase. With the developed method, only one mobile phase is sufficient for quantification of all mentioned drugs either in combination or in single dosage form as per availability of formulation. Therefore with the method that we have developed, time and cost required for changing different mobile phases could be saved. The low amounts of analytes that we have quantitated indicate that the method is sensitive. With the proposed method, all the four drugs could be eluted within 15 minutes, indicating that the method is rapid.

### MATERIALS AND METHODS

Di potassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>), acetonitrile (purity not less than 99.80%) and methanol and were used as received from Merck India Ltd. (Mumbai, India). Milli-Q water used was of Millipore, Mumbai and orthophosphoric acid was from SD fine Chemicals Ltd, Mumbai. Domperidone was obtained from Mahrshee Laboratories Pvt. Ltd., Panoli; Paracetamol was obtained from GlaxoSmithKline, Mumbai; Esomeprazole magnesium trihydrate was obtained from Glenmark Pharmaceuticals Ltd., Nasik and Lansoprazole was obtained from Zydus Cadila Ahmedabad; (All 99.0 - 99.5 % quality). All other chemicals and reagents used were of analytical reagent grade.

### Solution Preparation

#### Stock and Working Standard Solutions for DOM, PAR, ESO and LAN

Primary Stock solutions were prepared by weighing 10 mg of all the four drugs domperidone, paracetamol, esomeprazole, and lansoprazole in 10-mL volumetric flasks, dissolving in methanol and diluting to volume with the same solvent. Of these solutions, 1.0 mL was further diluted to 10 mL with methanol to obtain working

standard solutions with the drugs ( $100 \mu\text{g mL}^{-1}$ ). Further dilutions from these solutions were made with the mobile phase. Solutions were freshly prepared before use.

#### Preparation of Phosphate Buffer Solution

Anhydrous  $\text{K}_2\text{HPO}_4$  (1.74 g) was dissolved in milli-Q water, diluted to 1,000 mL with the same solvent, and adjusted to pH  $7.00 \pm 0.05$  with 85% orthophosphoric acid.

#### Preparation of the Sample Solutions

DOM and PAR (Brand Name: Grenil containing DOM -20 mg, PAR -500 mg)

Ten tablets of DOM and PAR available as a combination dosage form were weighed and powdered. A quantity of powder equivalent to 1 mg of domperidone and 25 mg of paracetamol was weighed and transferred to a 100 ml volumetric flask. A small quantity of methanol was added and sonicated to dissolve the drugs and volume was made upto the mark with methanol. This gave a concentration of  $10 \mu\text{g/ml}$  of domperidone and  $250 \mu\text{g/ml}$  of paracetamol. From this solution, 1 ml was diluted to 10 ml with the mobile phase to give a solution with a final concentration of  $1 \mu\text{g/ml}$  of domperidone and  $25 \mu\text{g/ml}$  of paracetamol.

DOM and ESO (Brand name: Esoz-D 40 containing DOM -30 mg, ESO -40 mg)

Contents of ten capsules were weighed. A quantity of capsule contents equivalent to 15 mg of domperidone and 20 mg of esomeprazole was weighed and transferred to a 100 ml volumetric flask. A small quantity of methanol was added and sonicated to dissolve the drugs. The volume was made upto the mark with methanol. This gave a concentration of  $150 \mu\text{g/ml}$  of domperidone and  $200 \mu\text{g/ml}$  of esomeprazole. From this solution, 1 ml was diluted to 10 ml with the mobile phase to give a solution with a final concentration of  $15 \mu\text{g/ml}$  of domperidone and  $20 \mu\text{g/ml}$  of esomeprazole.

#### Equipment

An HPLC instrument of LC-10ADVP series (Shimadzu Corporation, Japan) consisting of a LC-20AD solvent delivery system, SPD-M10AVP photo diode array detector, SIL-10ADVP autoinjector and Supelco  $\text{C}_{18}$  column ( $250 \times 4.6 \text{ mm i.d.}$ ,  $5 \mu$ ) was used.

#### Chromatographic Condition

Chromatographic separation was performed on a Supelco  $\text{C}_{18}$  reversed phase column ( $250 \times 4.6 \text{ mm i.d.}$ ,  $5 \mu$ ). The mobile phase was dipotassium hydrogen phosphate buffer-acetonitrile, 65:35 (v/v). Flow-rate was  $1.0 \text{ mL min}^{-1}$ . The column was kept at  $25.0 \pm 0.1 \text{ }^\circ\text{C}$  during the analysis; the detection wavelengths were DOM: 285 nm, PAR: 246 nm, ESO: 300 nm and LAN: 283 nm. The injection volume was  $50 \mu\text{L}$ .

#### Method Validation

##### Linearity

A series of solutions ranging from concentration 0.5, 1.0, 2.0, 5.0, 10, 20, 30 and  $50 \mu\text{g/ml}$  of standard mixture of the four drugs were prepared in the mobile phase. Fifty microlitre of each solution was injected under the chromatographic conditions described above. Each solution was injected three times over a week. Calibration

curves were constructed by plotting peak areas versus concentrations of DOM, PAR, ESO and LAN to get the regression equations, and coefficient of determination ( $r^2$ ).

#### Limits of Detection and Quantitation

The limits of detection (LOD) and quantitation (LOQ) were calculated in accordance with the  $3.3 \text{ sd } S^{-1}$  and  $10 \text{ sd } S^{-1}$  criteria, respectively, where sd is the standard deviation of the blank responses (for six replicates) and S is the slope of calibration curve, determined from linearity studies.

#### Accuracy

The accuracy of the RP-HPLC method was determined by calculating recoveries of domperidone, paracetamol, esomeprazole, and lansoprazole by the standard addition method. Known amount of standard drug mixture (8, 10 and  $12 \mu\text{g/ml}$ ) was spiked in triplicate to the test sample mixture ( $10 \mu\text{g/ml}$ ). The amount of each drug was estimated by applying these values to the regression equation of calibration curve.

#### Precision

The repeatability and intermediate precision of the proposed method were determined by estimating the corresponding responses six times on the same day and on three different days over a week for  $10 \mu\text{g/ml}$  of mixture of DOM, PAR, ESO and LAN.

#### Robustness

Small deliberate changes in the chromatographic conditions like mobile phase composition (buffer pH), flow-rate, detection wavelength and column temperature were done. Obtained results were compared with original chromatographic conditions. Because the stability of standard solutions can also affect the robustness of analytical methods, the stability of the standard solutions of the drug substances was tested for one month. Standard solutions were stored under refrigeration at  $4^\circ\text{C}$  and the content of these solutions was compared with that of a freshly prepared solution.

#### System-Suitability Test

The system suitability tests are used to ensure that the method can generate results of acceptable accuracy and precision. The criteria used in this test were column efficiency, peak asymmetry, peak resolution, repeatability, as RSD of peak area for 6 replicate injections and capacity factor.

#### Analysis of Pharmaceutical Dosage Form (Tablets/Capsules)

All tablets were purchased from local market for quantification of DOM, PAR and ESO by HPLC as described above. Concentrations of the drugs present in the sample solution were determined by fitting the responses into the regression equation for each drug.

## RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and peak symmetry for DOM, PAR, ESO and LAN were obtained with mobile phase consisting of 0.01 M, pH  $7.00 \pm 0.05$ , dipotassium hydrogen phosphate buffer: Acetonitrile (65:35 v/v). Quantification was achieved with photo diode array detection at the wavelengths mentioned previously. Better resolution of the peaks with clear base line separation was found (Fig. 1,2 &3).

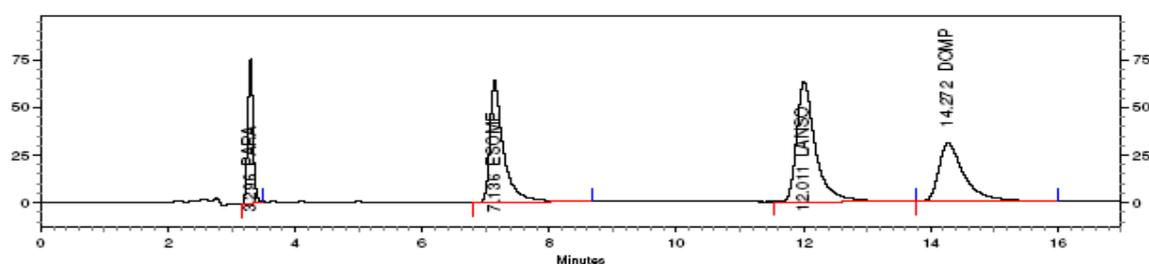


Fig. 1: Chromatogram of PAR (3.29 min), ESO (7.13 min), LAN (12.01 min) and DOM (14.27 min)

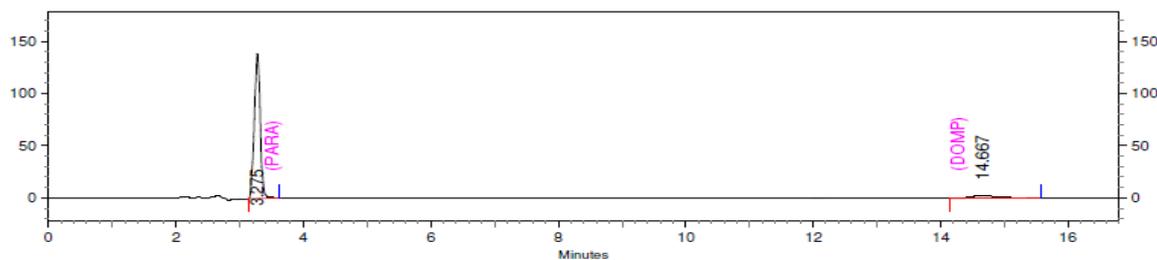


Fig. 2: Chromatogram of PAR (3.27 min) and DOM (14.66 min) from tablets

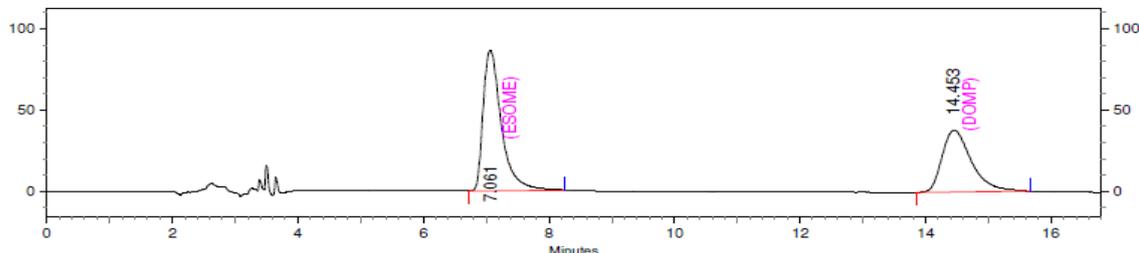


Fig. 3: Chromatogram of ESO (7.06 min) and DOM (14.45 min) from capsules

Table 1: Results from Regression equation of Calibration Curve

Drug Name	Coefficient of Determination ( $R^2$ ) $\pm$ SD	Slope $\pm$ SD	Intercept $\pm$ SD
Domperidone	0.9999 $\pm$ 0.0006	86923 $\pm$ 1999.70	34371 $\pm$ 19282.80
Paracetamol	1.0000 $\pm$ 0.0006	270183 $\pm$ 6076.17	21119 $\pm$ 76730.28
Esomeprazole	0.9998 $\pm$ 0.0016	138586 $\pm$ 4988.64	49896 $\pm$ 7811.41
Lansoprazole	0.9999 $\pm$ 0.0003	126245 $\pm$ 1846.26	2509.3 $\pm$ 17190.26

### Linearity

The linear correlation between the peak area and concentration was checked for each component. Data for eight solutions of different concentration of each drug (0.5, 1.0, 2.0, 5.0, 10, 20, 30 and 50  $\mu\text{g/ml}$ ) were collected and analyzed. For all the compounds the correlation between the peak area and drug concentration were described by linear regression equations with high values of coefficient of determination ( $r^2$ ). The slopes and intercepts were obtained from the regression equations. All results are listed in Tables 1.

### Limit of Detection and Limit of Quantification

LOD for DOM, PAR, ESO and LAN was found to be  $0.1\mu\text{g mL}^{-1}$  and LOQ for all four drugs was found to be  $0.5\mu\text{g mL}^{-1}$  by this method (Table 2).

### Accuracy

The recovery experiments were carried out by the standard addition method. The percentage recoveries obtained were  $100.20 \pm 0.70$ ,  $100.46 \pm 0.75$ ,  $99.74 \pm 0.87$  and  $99.65 \pm 0.80$  for DOM, PAR, ESO and LAN respectively (Table 2). The recovery of the method was good.

### Precision

The low % RSD values of repeatability (0.53 - 0.95) and intermediate precision (0.85 - 1.92) implied that the reproducibility of the proposed method was good (Table 2).

### Robustness

The method was found to be robust, although small deliberate changes in method conditions did have a negligible effect on the

chromatographic behavior of the solutes. The results indicate that flow-rate had no large effect on the chromatographic behavior of domperidone, paracetamol, esomeprazole, and lansoprazole. Changing the pH ( $\pm 0.2$ ) showed that when the pH of phosphate buffer was reduced to 6.8, the retention time of Domperidone decreased by 1.21 min and this resulted in poor resolution (0.77) between Lansoprazole and Domperidone. Thus when the method is to be used for the separation of Domperidone and Lansoprazole, the pH should be controlled to 7.0 only. A minor increase or decrease of the in flow-rate ( $\pm 0.1$ ) also did not cause any change in the tailing of the peak of each drug. Alteration of the detection wavelength ( $\pm 5\text{ nm}$ ) caused no variation of peak areas. Change in column temperature  $\pm 5^\circ\text{C}$  did not significantly affect the chromatographic behavior of the drugs. The stability of the standard solutions of the four drugs was tested for one month. The standard solutions were stored under refrigeration at  $4^\circ\text{C}$  and the content of these solutions was compared regularly with that of a freshly prepared solution. No significant changes in drug concentrations were observed.

### System-Suitability Test

The percentage of relative standard deviation (% RSD) for DOM, PAR, ESO and LAN were found to be 0.83, 0.53, 0.91 and 0.95 respectively using this method (Table 2, 3). All the results were within the acceptable range.

### Assay of the Dosage Forms (Tablets/Capsules)

The proposed validated method was successfully applied to determine DOM, PAR and ESO in pharmaceutical dosage forms, results obtained for the drugs were comparable with the corresponding labeled amounts (Table 4).

Table 2: Summary of Validation Parameters of the proposed method

Parameters	Domperidone	Paracetamol	Esomeprazole	Lansoprazole
Linearity ( $\text{ng mL}^{-1}$ )	500 - 50,000	500-50,000	500 - 50,000	500 - 50,000
LOD ( $\text{ng mL}^{-1}$ )	100	100	100	100
LOQ ( $\text{ng mL}^{-1}$ )	500	500	500	500
Accuracy (%) $\pm$ SD	$100.20 \pm 0.70$	$100.46 \pm 0.75$	$99.74 \pm 0.87$	$99.65 \pm 0.80$
Repeatability, (n = 6) %RSD	0.83	0.53	0.91	0.95
Intermediate Precision, (n = 6) %RSD	1.2	0.85	1	1.92

Table 3: Summary of System Suitability Parameters

Parameters	Domperidone	Paracetamol	Esomeprazole	Lansoprazole
Retention Time (min)	14.27	3.29	7.13	12.01
Theoretical plates/meter	28561	21627	26237	36665
Tailing factor	1.49	1.01	1.65	1.37
Capacity factor	56.09	12.18	27.54	47.04

Table 4: Results from Assay of Tablets / Capsules use of RP-HPLC Method

% Assay mean $\pm$ SD			
Combined dosage form 1		Combined dosage form 2	
Domperidone	Paracetamol	Domperidone	Esomeprazole
99.96 $\pm$ 0.32	98.14 $\pm$ 0.57	100.63 $\pm$ 0.16	99.30 $\pm$ 0.55

## CONCLUSION

A Novel simple, sensitive, accurate, and precise RP HPLC method for assaying domperidone, paracetamol, esomeprazole, and lansoprazole in dosage forms has been developed and validated. With the proposed method all the four drugs eluted within 15 minutes, indicating that the method is rapid. The proposed method uses a mobile phase consisting of 0.01 M, pH 7.00  $\pm$ 0.05, dipotassium hydrogen phosphate buffer: Acetonitrile (65:35 v/v) for the separation of the drugs. This method uses a common mobile phase for the separation of four different drugs in combination and in single dosage form. This method could be used in the quality control and other pharmaceutical industries. The developed method could save the analysis time, cost and other analytical problems like changing the columns and mobile phases.

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