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Simultaneous Method Development and Validation of Esomeprazole and Domperidone in Pure and Pharmaceutical Dosage Forms By RR-HPLC

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A simple, precise RP-HPLC method was developed for the estimation of esomeprazole and domperidone in pure and pharmaceutical dosage forms. The quantification was carried out using a C-18 column 250 × 4.6 mm i.d, 5 µm particle size in isocratic mode, with mobile phase comprising of buffer and acetonitrile in the ratio of 62:38 (v/v) pH 4.5. The flow rate was 1 mL/min and the detection was carried out by UV detector at 220 nm. The retention times were 6.308 and 7.425 min for esomeprazole and domperidone, respectively. The method produced linear response in the concentration range of 200-1000 µg/mL for esomeprazole and 100-500 µg/mL for domperidone. The percentage recovery was found to be 99.98 and 98.95 % for esomeprazole and domperidone, respectively. The method was validated by evaluation of required parameters.

Key Words: Esomeprazole, Domperidone, RP-HPLC, Estimation.

INTRODUCTION

Esomeprazole belongs to gastrointestinal drugs category to suppress the gastric acidity treatment of peptic ulcer by inhibiting the proto pump. Chemically it is known as 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole. Esomeprazole is cost effective in the treatment of gastric oesophageal reflux diseases. Esomeprazole is (S-isomer of omeprazole), the first single optical isomer proton pump inhibitor generally provides better acid control than racemic proton pump inhibitors. It's molecular weight is 713.13.

Domperidone is a unique gastrokinetic and antiemetic drug. It is a peripheral dopamine 2-receptor, antagonist, regulates the motility of gastric and small intestinal smooth muscle and has been shown to have some

effects on the motor function of the oesophagus. It increases the duration of antral and duodenal contractions and also LES resting pressure, thus stimulating gastric emptying both in animals and in man and is also effective in relief of symptoms of reflux oesophagitis. Domperidone is (5-chloro-1-h1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl) propyl] -4-piperidinyl-1,3-dihydro-2H-benzimidazol-2-one). It's molecular weight is 425.92.

The literature survey[1-17] indicates that esomeprazole and domperidone have been determined either individually or simultaneously with some other drugs by UV, HPTLC, HPLC in pure and pharmaceutical dosage forms. There is no method has been reported for simultaneous estimation of esomeprazole and domperidone, thus an attempt was made to develop a simple, precise, accurate and economical HPLC method for the simultaneous estimation of esomeprazole and domperidone in pure and pharmaceutical dosage forms.

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade. Pure standards of esomeprazole and domperidone were obtained as gift samples from MARAL Laboratories Ltd., Chennai. The purities of these standards were 99.85 and 99.76 %, respectively. Acetonitrile, methanol, potassium dihydrogen ortho phosphate and water used were of HPLC grade (Qualigens). Neutraflux (Stedman Pharmaceuticals Ltd) was employed in the study. An isocratic HPLC (Shimadzu Tokyo) with a single pump Lc-10 ATVp equipped with universal injector (Rheodyne) with injection volume 20 µL, ultra violet visible detector (UV-VIS) SPD-10AV_A-Shimadzu series and Shimadzu Class Vp software. A Thermo Hypersil key stone C-18 ODS column 250 × 4.6 mm i.d with 5 µm particles. Detection was carried out by UV detection at 220 nm.

Preparation of standard solution: About 200 mg of pure samples of esomeprazole and 100 mg of domperidone was accurately weighed and transferred to a 100 mL volumetric flask and are dissolved in methanol. Each mL of stock solution contains 1000 mg/mL. 1 mL of this stock solution was diluted to 10 mL with mobile phase to give a concentration of 200 µg/mL of esomeprazole and 100 µg/mL of domperidone.

Chromatographic conditions: Freshly prepared 62:38 (v/v) buffer and acetonitrile were filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1 mL/min. The column temperature was maintained at ambient temperature. The detection was carried out at 220 nm. The injection volume was 20 µL and the total run time was 10 min.

Linearity and calibration: Linearity was assessed by performing single measurement at several analyte concentration varying quantities of

stock solution was diluted with the mobile phase to give a concentration of 200, 400, 600, 800 and 1000 µg/mL of esomeprazole and 100, 200, 300, 400 and 500 µg/mL of domperidone. Injection was made at intervals of 10 min. Linearity of esomeprazole was found to exist between 200 to 1000 µg /mL and the linearity of domperidone were found to exist between 100 to 500 µg/mL.

Preparation of mobile phase solution: Phosphate buffer and acetonitrile in the ratio of 62:38 (v/v) were used as a mobile phase for present study. Phosphate buffer was prepared by taking accurately weighed quantity of 6.804 g of potassium dihydrogen orthophosphate dissolved in HPLC grade water and made up to 1000 mL. The pH of the solution was adjusted to 4.5 by adding 5 % orthophosphoric acid.

Preparation of internal standard solution: Paracetamol was used as internal standard in the present study. About 100 mg of paracetamol was accurately weighed and transferred to 100 mL volumetric flask. It was dissolved in mobile phase and volume was made up to 100 mL so as to give 1000 µg/mL stock solution. From this 1 mL of solution was taken and made up to 10 mL with mobile phase to give concentration about 100 µg/ mL.

System suitability parameters: System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system are adequate for the analysis. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution of esomeprazole and domperidone. In addition to this standard deviation of esomeprazole and domperidone standards were evaluated by injecting a mixed standard of both esomeprazole and domperidone (200 and 100 µg/mL) and paracetamol 100 µg/mL as internal standard six times at 12 min interval and the values were recorded. The limit of detection (LOD) and limit of quantitation (LOQ) of the method was determined by injecting progressively low concentrations of the standard solutions on the system using optimized chromatographic conditions. All the above parameters are shown in Table-1.

Assay procedure: 20 Tablets were weighed, powdered and an accurately weighed sample of powdered tablets equivalent to 20 mg of esomeprazole and 10 mg of domperidone was taken in 10 mL volumetric flask and dissolved in methanol and extracted by sonication to ensure complete solubility of the drug. The mixture was then made up to 10 mL with methanol, thoroughly mixed and filtered through a 0.45 µm membrane filter. An aliquot of this filtrate was transferred to a 10 mL volumetric flask along with appropriate volume of internal standard solution and made up to volume with mobile phase to give required concentration of 200 µg/mL of esomeprazole and 100 µg/mL of domperidone and 100 µg/mL of

paracetamol. Then the solution was injected five times in to the column. All the determinations was conducted five times. From the peak areas, the drug content in the tablets was quantified using the regression equation obtained from the pure samples.

The amount of drug present in tablet formulation was calculated as follows:

$$\text{Amount of drug in each tablet} = \frac{\text{Peak area of test}}{\text{Peak area of standard}} \times \frac{\text{Standard dilution factor}}{\text{Sample dilution factor}} \times \text{Average weight of tablet}$$

RESULTS AND DISCUSSION

In order to develop simultaneous estimation of two components under isocratic conditions, the mixture of methanol or acetonitrile with buffer in different ratios were assayed as the mobile phase. A mixture of water and acetonitrile in different ratios were also tried for the assay of combined dosage forms. Finally a mixture of acetonitrile-potassium dihydrogen orthophosphate (buffer) in the ratio of 38:62 v/v, proved to be the effective mixture than the other mixture used for the separation. Then the flow rates tested includes 0.5, 0.8, 1.0, 1.25 and 1.5 mL. Among these flow rates 1.0 mL was selected for the assay because of better resolution of the peaks.

The mentioned chromatographic conditions revealed to provide better resolution between esomeprazole, domperidone and internal standard in a reasonable time of 3.292, 6.308 and 7.425 min, respectively.

System suitability test was applied to freshly prepared standard stock solutions of esomeprazole and domperidone, to check the parameters like tailing factor, resolution factor, theoretical plates, limit of detection and limit of quantitation as shown in Table-1.

TABLE-1
SYSTEM SUITABILITY PARAMETERS

Parameter	Esomeprazole	Domperidone
Tailing factor	0.96	1.32
Resolution factor	2.44	2.44
Theoretical plates	4969	5476
Relative standard deviation	0.541	0.582
Limit of detection (LOD) (µg/mL)	0.236	0.185
Limit of quantitation (LOQ) µg/mL)	0.428	0.632

The developed method was studied for precision. The precision of the method was done by repeatability studies. The precision was studied in terms of intra-day and inter-day changes in peak areas of drug solution on the same day and on 3 different days over a period of 3 weeks. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table-2.

TABLE-2
PRECISION OF METHOD

Drug	Theoretical concentration (µg/mL)	Intra-day concentration measured* (µg/mL)		Inter-day concentration measured* (µg/mL)	
		Mean(a)	RSD (%)	Mean(b)	RSD (%)
Esomeprazole	200	200.12	0.26	200.89	0.35
	400	400.06	0.74	400.76	0.62
	600	600.18	0.89	600.09	0.42
Domperidone	100	100.24	0.68	100.15	0.72
	200	200.13	0.97	200.33	0.63
	300	300.04	0.78	300.06	0.94

*Mean of five different standards for each concentration.

The proposed method was performed in the pharmaceutical dosage form. In the assay, no interfering peaks were found indicating that the tablet excipients did not interfere with the estimation. The amount estimated is given in Table-3.

TABLE-3
ASSAY OF COMBINED TABLET DOSAGE FORM

Drug	Sample No.	Label claim (mg/tablet)	Amount estimated (mg/tablet)	Label claim (%)
Esomeprazole	1	20	19.90	99.05
	2	20	19.89	99.45
	3	20	19.94	99.70
	4	20	20.14	100.10
	5	20	20.06	100.30
Domperidone	1	10	9.95	99.50
	2	10	9.84	98.40
	3	10	9.97	99.70
	4	10	10.05	100.50
	5	10	9.96	99.60

The accuracy of the proposed RP-HPLC method was expressed in terms of recovery. The recovery studies was carried out and given in terms of percentage recovery and given in Table-4.

TABLE-4
RECOVERY STUDIES

Drug	Amount added (µg)	Amount recovered (µg)	Recovery (%)
Esomeprazole	20	19.95	99.75
	40	39.68	99.24
	60	59.89	99.81
Domperidone	10	9.89	98.90
	20	19.94	99.70
	30	29.93	99.76

The proposed method was found to be simple, precise, specific and highly accurate and requires less time consumption for analysis and can be employed for the routine analysis.

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