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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE AND DOMPERIDONE BY RP-HPLC IN BULK AND COMBINED DOSAGE FORMS

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ABSTRACT

A new HPLC method was developed and validated for the determination of Esomeprazole and Domperidone in tablet dosage form. The chromatographic separation was achieved on an Intersil C_{18} ODS (4.6 x 250 mm, 5□m) with a mobile phase combination of methanol: water (50:50 V/V) at a flow rate of 1.0 mL/min, and the detection was carried out by using UV detector at 260 nm. The total run time was 10 minutes. The retention time of Esomeprazole and Domperidone were found to be 2.869 min. and 3.942 min. respectively. The performance of the method was validated according to the present ICH guidelines.

KEYWORDS: Esomeprazole and Domperidone in tablet dosage form.

INTRODUCTION

Esomeprazole

Esomeprazole is a Proton Pump Inhibitors and Histamine Antagonist, chemically (S)-5-Methoxy-2-[(4-methoxy-3,5-dimethyl pyridine-2yl) methylsulfinyl}-3H benzimidazole. A highly effective inhibitor of gastric acid secretion used in the therapy of stomach ulcers and Zollinger-Ellison syndrome. The drug inhibits the H^+-K^+-ATP ase (H^+-K^+ exchanging ATP ase) in the proton pump of gastric parietal cells. Esomeprazole is a compound that inhibits gastric acid secretion and is indicated in the treatment of gastroesophageal reflux disease (GERD), the healing of erosive esophagitis, and H.

pylori eradication to reduce the risk of duodenal ulcer recurrence. Esomeprazole belongs to a new class of antisecretory compounds, the substituted benzimidazoles, that do not exhibit anticholinergic or H2 histamine antagonistic properties, but that suppress gastric acid secretion by specific inhibition of the H^+/K^+ ATPase at the secretory surface of the gastric parietal cell. By doing so, it inhibits acid secretion into the gastric lumen. This effect is dose-related and leads to inhibition of both basal and stimulated acid secretion irrespective of the stimulus.

Mechanism of Action: Esomeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H^+/K^+-ATPase in the gastric parietal cell. By acting specifically on the proton pump, Esomeprazole blocks the final step in acid production, thus reducing gastric acidity.

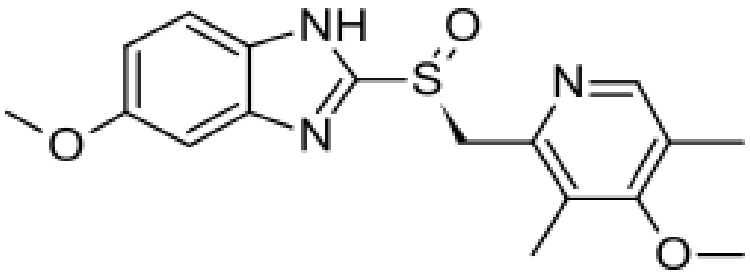

Fig. 1. Structure of Esomeprazole Domperidone

A specific blocker of dopamine receptors. It speeds gastrointestinal peristalsis, causes prolactin release, and is used as antiemetic and tool in the study of dopaminergic mechanisms. Chemically 5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1H-benzo[d] imidazol-1-yl) propyl piperidin-4-yl)-1H- benzo[d]imidazol-2(3H)-one, in Pharmacodynamically, It is a specific blocker of dopamine receptors. It speeds gastrointestinal peristalsis, causes prolactin release, and is used as antiemetic and tool in the study of dopaminergic mechanisms.

Mechanism of Action: Domperidone acts as a gastrointestinal emptying (delayed) adjunct and peristaltic stimulant. The gastroprokinetic properties of Domperidone are related to its peripheral dopamine receptor blocking properties. Domperidone facilitates gastric emptying and decreases small bowel transit time by increasing esophageal and gastric peristalsis and by lowering esophageal sphincter pressure. Antiemetic: The antiemetic properties of Domperidone are related to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level. It has strong affinities for the D2 and D3 dopamine

receptors, which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which - among others - regulates nausea and vomiting.

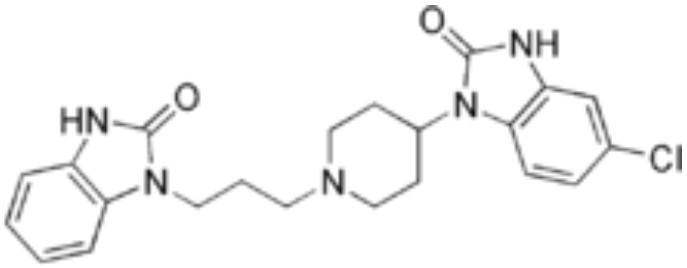

Fig. 1. Structure of Domperidone

METHODS AND MATERIALS

Chemicals

Esomeprazole and Domperidone were obtained as a gift sample, from Casca. HPLC grade methanol, water and acetonitrile and other solvents were obtained from Merck Limited. Waters 2690 pump, Detector 996 PDA, Inertsil-C18 ODS column with Empower-2 chromatogra- phic system. HPLC grade water was obtained by distilling deionizer water produced by a Milli-Q Millipore water system (Milford, MA, USA).

Chromatographic conditions

The HPLC system (LC Waters, Milford, MA, USA) consisted of quaternary gradient system (600 Controller), in-line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters). Data was processed using Empower2 software (Waters, Milford, MA, USA).

Integration of the detector output was performed using the Waters Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45µm membrane filter and degassed by sonication before use. Mobile phase was used as diluents. The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm The run time was set at 6 min and a column temperature was maintained at 30°C. The volume of injection was 5µl, prior to injection of the analyte, the column was equilibrated for 30 with the mobile phase. The eluent was detected at 235 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness as per ICH guidelines.

OPTIMIZED CHROMATOGRAPHIC METHOD

Mobile Phase

Degassed Methanol and Water in the ratio of 50:50 V/V.

Preparation of stock solution

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Esomeprazole and 25.0 mg Domperidone in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20 min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution

The stock solutions equivalent to 20 ppm to 80 ppm with respect to both drugs were prepared in combination of Esomeprazole and Domperidone above, sonicated, and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 25 mg Esomeprazole and 50 mg Domperidone was weighed and dissolved in the 70 mL mobile phase with the aid of ultrasonication for 20 min. The content was diluted to 100 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 25 µg/mL Esomeprazole and 50 µg/mL Piperaquine Phosphate.

RESULTS AND DISCUSSION

The present research work was designed at developing a rapid, sensitive, precise and accurate HPLC method for the simultaneous estimation of Esomeprazole and Domperidone in pharmaceutical dosage forms. In order to affect analysis of the component peaks under isocratic conditions, mixtures of water and methanol in different combinations with different pH were tested as mobile phase on a Waters C8 stationary phase. A binary mixture of water: methanol in the ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing. A flow rate of 1.0 ml/min of the mobile phase was found to be suitable.

Optimized chromatographic conditions

After various trials, the following chromatographic conditions were finally optimized for the simultaneous estimation of Esomeprazole and domperidone in a capsule dosage form. Mobile phase constitutes of Methanol: Water in the ratio of 50:50 v/v. Detection wave length 260 nm flow rate 1.0 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally 120 µg/ml of the standard solution of the individual samples of and domperidone and Esomeprazole mixed standard solutions were injected and the chromatograms were recorded.

Table1: Optimized Chromatographic Conditions

Parameters	**Method**
Stationary phase (column)	Inertsil -ODS C_{18} (250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Water (50:50)
Flow rate (ml/min)	1.0 mL/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	260 nm
Drug RT (min)	2.007 min for Esomeprazole and 3.942 min for Domperidone.

Calculation

The amount of drugs present in each pharmaceutical formulation was calculated by using the standard calibration curves (concentration in ppm was taken on x-axis and peak area on y-axis).

Optimized method

The separation of Esomeprazole and Domperidone with retention time 2.008 min for and 3.912 min respectively for standard. The typical chromatograms of the standard were recorded for the repeatability and were given in Figure No.3.

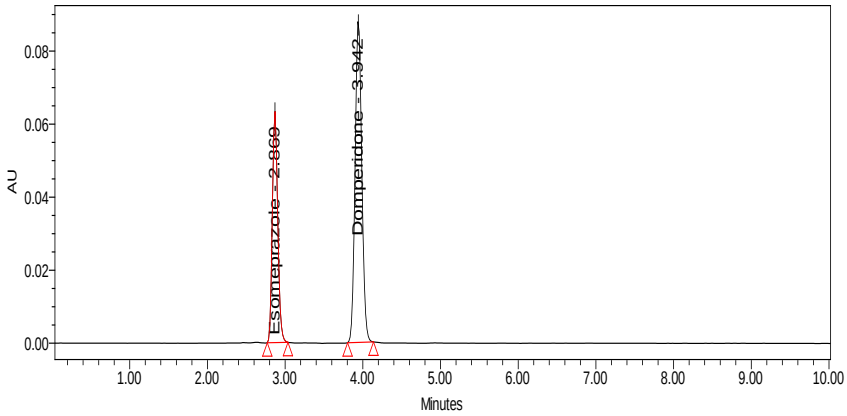

Fig. 3: Chromatogram of standard

The separation of Esomeprazole and Domperidone with retention time 2.007 min and 3.942 min respectively for sample. The typical chromatograms of the standard and sample were recorded for the repeatability and the respective chromatograms were given in Figure No. 4.

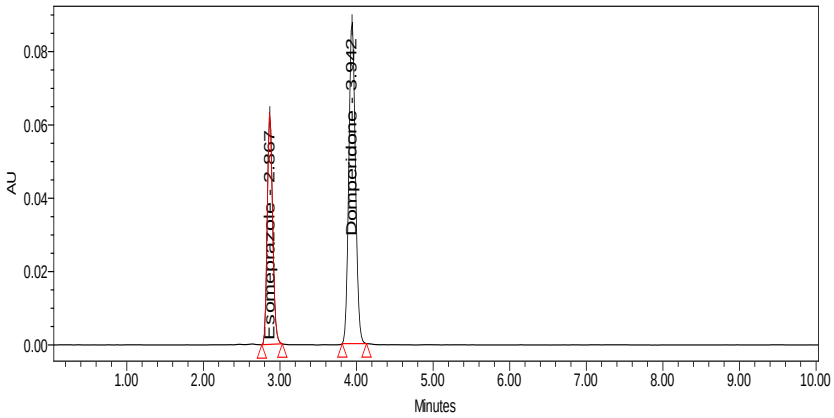

Fig. 4: Chromatogram of sample

Observation: Got chromatogram at RT's of 2.007 min to Esomeprazole and 3.942 min to Domperidone.

Acceptance Criteria: The assay limits for Esomeprazole and Domperidone was 98-102.0% and the results obtained for Esomeprazole and Domperidone was found to be within the limits.

DISCUSSION: In this trail Methanol and water in 50:50 ratio are allowed to pass through Inertsil - C_{18} ODS column at a flow rate of 1.0 mL/min for 10 min and detection is done at 260 nm, peaks were obtained and resolution was good.

METHOD VALIDATION

1) System suitability

A Standard solution was prepared by using Esomeprazole and Domperidone working standards as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Esomeprazole and Domperidone**,** retention times and peak areas.

1. The % RSD for the retention times of principal peak from 5 replicate injections of each standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Esomeprazole and Domperidone peaks is NLT 3000.
4. The Tailing factor (T) for the Esomeprazole and Domperidone peaks is NMT 2.0.

RESULT

The system suitability for Esomeprazole with parameters of mean RT, Peak area, USP Plate count, USP Tailing are 2.8745, 678433.8, 10768.34, and 1.155774 respectively . The SD (Standard deviation) for RT and Peak RT are 0.001817 and 6031.131 respectively. The % RSD for RT and Peak area is 0.05221 and 0.888979.

The system suitability for Domperidone with parameters of mean RT, Peak area, USP Plate count, USP Tailing are 3.9432, 1228593, 9573.997, and 0.892407 respectively . The SD (Standard deviation) for RT and Peak RT are 0.00707and 122124.07 respectively. The % RSD for RT and Peak area is 0.025353 and 1.800764.

Acceptance criteria

Resolution between two drugs must be not less than 2.

Theoretical plates must be not less than 2000.

Tailing factor must be not less than 0.9 and not more than 2.

Discussion: The resolution of Esomeprazole and Domperidone was within the limits.

The theoretical plate count is also within the limits.

2) Specificity

"Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual procedure may be compensated by other supporting analytical procedure(s)."

With respect to identification, discrimination between closely related compounds likely to be present should be demonstrated by positive and negative samples. In the case of chromatographic assay and impurity tests, available impurities / degradants can be spiked at appropriate levels to the corresponding matrix or else degraded samples can be used. For assay, it can be demonstrated that the result is unaffected by the spiked material. Impurities should be separated individually and/or from other matrix components. Specificity can also be demonstrated by verification of the result with an independent In the case of chromatographic separation, resolution factors should be obtained for critical separation. Tests for peak homogeneity, for example, by diode array detection (DAD) or mass spectrometry (MS) are recommended. The chromatogram of standard is in Fig. 5 and sample in Fig. 6.

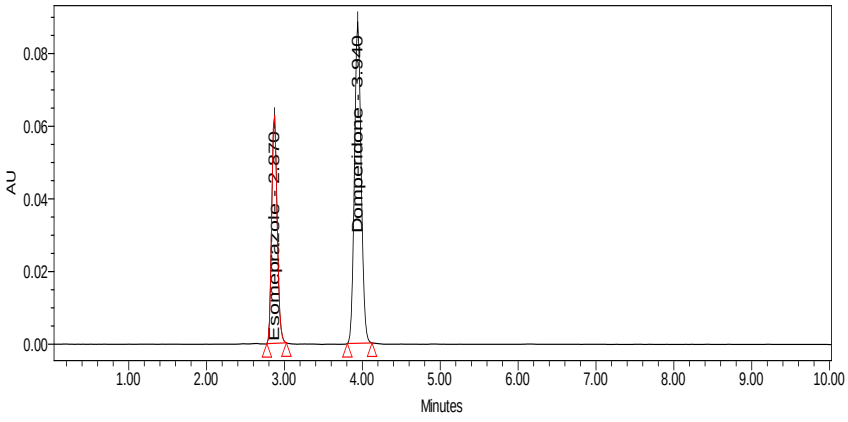

Fig 5: Chromatogram of standard

Observation: Got a peak for standard at an Rt of 2.870 min for Esomeprazole and 3.940 min for Domperidone.

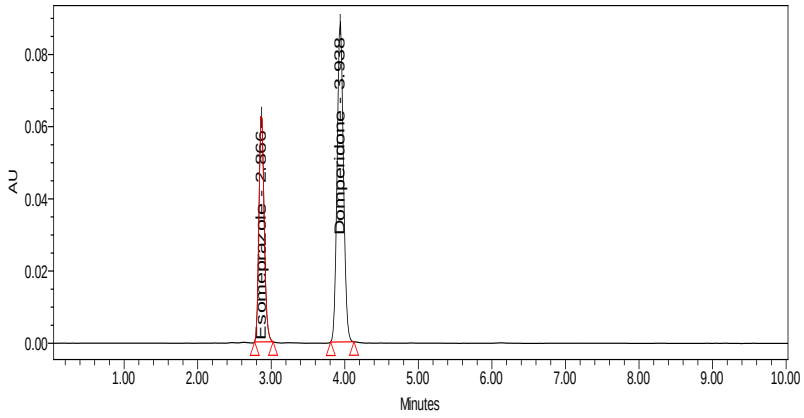

Fig 6: Chromatogram of sample

Observation: Got a peak for sample at an Rt of 2.866 min for Esomeprazole and 3.938 min for Domperidone.

Discussion: The chromatograms for specificity of Esomeprazole and Domperidone were identical with nearly same retention time; specificity was confirmed by peak purity.

3) Precision

"The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels; repeatability, intermediate precision and reproducibility."

Precision should be obtained preferably using authentic samples. As parameters, the standard deviation (SD), the relative standard deviation (coefficient of variation) and the confidence interval should be calculated for each level of precision.

Repeatability expresses the analytical variability under the same operating conditions over a short interval of time (within-assay, intra-assay). At least nine determinations covering the specified range or six determinations at 100 % test concentration should be performed. Intermediate precision includes the influence of additional random effects within laboratories, according to the intended use of the procedure, for example, different days, analysts or equipment, etc.

Reproducibility, i.e., the precision between laboratories (collaborative or interlaboratory Studies), is not required for submission, but can be taken into account for standardization of analytical procedures.

Calculation

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (% CV) or relative standard deviation (RSD) of the replicate measurements.

$$\% \text{ RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Acceptance Criteria

Chromatograms of standard and sample should be identical with near Retention time.

a) Repeatability

Data of Repeatability (System precision) for Esomeprazole

System precision: Standard solution prepared as per test method and injected five times with concentration of 40 ppm , the Peak area and its % assay is 674753(98.66), 674261(99.30), 675298(101.53), 679221(100.53), 688636(99.98), respectively ,the mean value of this precision was 678433.8 (100.00) and SD 6031.135, 1.107678 and % RSD 0.888979 (1.10).

Data of Repeatability (System precision) for Domperidone

System precision: Standard solution prepared as per test method and injected five times with concentration of 40ppm, the Peak area and its % assay is 1218805 (99.95), 1214014 (100.24), 1215474 (100.06), 1227655 (99.30), 1267019 (100.00), respectively, the mean value of this precision was 1228593 (99.91) and SD 22124.07(0.35819) and % RSD 1.800764(0.35).

Acceptance criteria

The %RSD for sample should be NMT 2.

The %RSD, which is within the limits hence method, is precise.

Discussion: The proposed RP-HPLC method was also validated for system precision and method precision. When the solution of Esomeprazole and Domperidone was repeatedly injected on the same day, the % RSD of the Esomeprazole and Domperidone was found to be 1.10 %, 0.35 %.

4)Accuracy

"The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found".

Accuracy can be demonstrated by the following approaches:

- Inferred from precision, linearity and specificity
- Comparison of the results with those of a well characterized, independent procedure.
- Application to a reference material (for drug substance)
- Recovery of drug substance spiked to placebo or drug product (for drug product)
- Recovery of the impurity spiked to drug substance or drug product (for impurities)

The closeness of agreement between the true value which is accepted either conventional new value or an accepted reference value and the value found.

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Esomeprazole and Domperidone into each volumetric flask for each spike level to get the concentration of Esomeprazole and Domperidone equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Esomeprazole and Domperidone were calculated.

Acceptance Criteria:

The mean % recovery of the Esomeprazole and Domperidone at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

Observation

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

The recovery results indicating that the test method has an acceptable level of accuracy.

The mean % recovery and % RSD for 50% concentration of three injections for Esomeprazole are 100.06 and 0.18. For 100%, 100.04 and 0.0191 and for 150%, 100.02 and 0.035 respectively.

The mean % recovery and % RSD for 50% concentration of three injections for Domiperitone are 99.69333 and 0.92 For 100%, 99.83333 and 0.41 and for 150% 99.97333 and 0.31 respectively.

Acceptance Criteria: The mean recoveries of both the drugs were found to be 100.04%, 99.83%. Under the acceptance criteria within the limit of 98 to 102 %. So the results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Discussion: The % recovery studies are performed by spiking known amount of analyte at three different concentrations (50%, 100%, and 150%). There was a high % recovery of Esomeprazole and Domperidone indicating the proposed method is accurate.

5) Linearity

"The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample".

It may be demonstrated directly on the analyte, or on spiked samples using at least five concentrations over the whole working range. Besides a visual evaluation of the analyte signal as a function of the concentration, appropriate statistical calculations are recommended, such as a linear regression. The parameters slope and intercept, residual sum of squares and the coefficient of correlation should reported. A graphical presentation of the data and the residuals is recommended.

Linearity of test method

It is an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration of analyte in the sample.

A Series of solutions are prepared using Esomeprazole and Domperidone working standards at concentration levels from 20 ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance Criteria

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%

The concentrations from 0 to 80 ppm with increment of 10 , the respective average area were 0, 632546, 658296, 694400, 730308, 916282, 9402046, 9788277 and its slope value 18600, Y-intercept 276.2 and correlation coefficient is 1 for Esomeprazole. For Domperitone 0, 1202965, 1254371, 1295856, 1297167, 1308577, 1359903, 1411306 and its slope value 5140, Y-intercept 114.7 and correlation coefficient is 1.

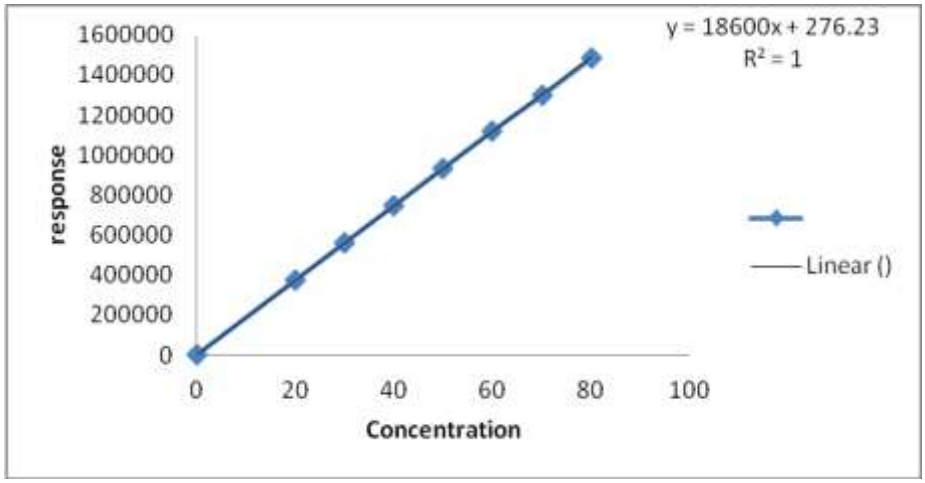

Fig 7: Linearity Plot (Concentration Vs Response) of Esomeprazole.

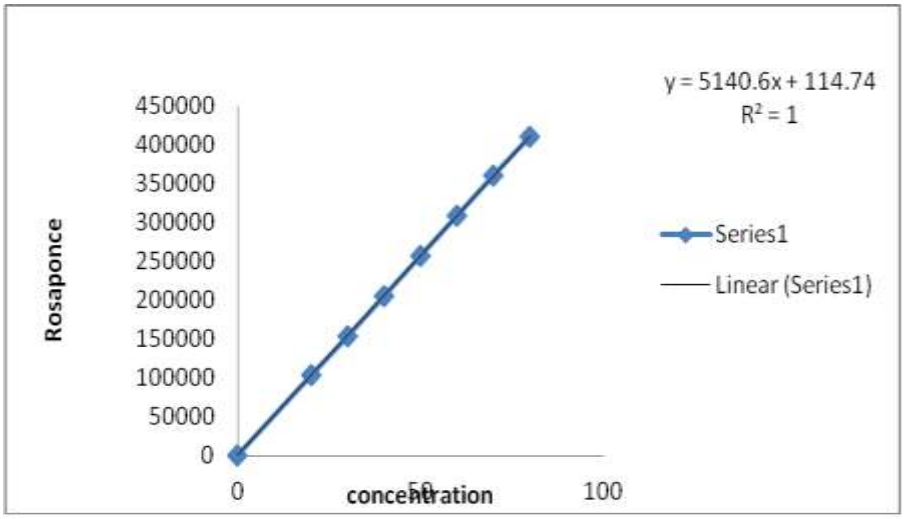

Fig 8: Linearity Plot (Concentration Vs Response) of Domperidone.

Acceptance criteria: Correlation coefficient (R^2) should not be less than 0.998. The correlation coefficient obtained was 0.998, which is in the acceptance limit.

Discussion: The method was linear in the concentration ranges 20-80 µg mL-1 and 20-80 µg mL^{-1} for Esomeprazole and Domperidone. The correlation coefficient was found to be 1.

6) Ruggedness

"The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different days, etc. Ruggedness is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst". The degree of reproducibility is then evaluated by comparison of the results obtained under varied conditions with those under standard conditions.

a) System to system variability

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Acceptance Criteria

The % relative standard deviation of Esomeprazole and Domperidone from the six sample preparations should be not more than 2.0%

The % assay of Esomeprazole and Domperidone should be between 98.0%-102.0%.

Observation

The % RSD was found within the limit. Ref tables:

Result

The system variability of Esomeprazole of peak area and its % assay of six samples were 634360 (98.65), 634098(98.63), 635696(96.86), 633289(98.52), 634147(98.63), 633495(98.55) and its mean value is 634180.8(98.64) as well as % RSD 0.019(0.12).

The system variability of domperitone of peak area and its % assay of six samples were 1203625 (99.98), 1202225 (99.30), 1202840 (98.60), 1204283 (99.30), 1202735 (98.55), 1203110 (98.73) and its mean value is 1203136.3 (99.07667) as well as % RSD 1.35 (0.56).

7) Robustness

According to ICH Q2A "the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage".

Furthermore, it is stated in ICH Q2B "The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used."

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation were made to evaluate the impact on the method.

a) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0 mL/min and 1.2 mL/min. The system suitability parameters were evaluated and found to be within the limits for 1.0 mL/min and 1.2mL/min flow.

Esomeprazole and Domperidone and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0mL/min.

Acceptance Criteria

The Tailing Factor of Esomeprazole and Domperidone standards should be NMT 2.0 for Variation in Flow.

Observation: The tailing factor for Esomeprazole and Domperidone was found to be within the limits. As shown in table.

b) Effect of variation of temperature

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The

system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºC.

Similarly sample solution was chromatographed at 25ºC temperature. Esomeprazole and Domperidone were resolved from all other peaks and the retention times were comparable with those.

Acceptance Criteria: The Tailing Factor of Esomeprazole and Domperidone standard and sample solutions should be NMT 2.0 for Variation in temperature.

Observation: The tailing factor for Esomeprazole and Domperidone is found to be within the limits.

Table 3: Data for Effect of variation in flow rate (Esomeprazole)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	620286	1.322089	**Flow 1.0 ml**	634322	1.604878	**Flow 1.2 ml**	602077	1.285372
	619282	1.331920		635792	1.584354		601854	1.319385
	621337	1.296438		634360	1.543805		602403	1.292055
	620456	1.315454		635696	1.568590		603421	1.304561
	620765	1.326551		633147	1.559986		602465	1.294621
Avg	620425	1.31849	**Avg**	634663.4	1.572323	**Avg**	602444	1.299199
SD	754.0018	0.013728	**SD**	1100.917	0.023367	**SD**	599.8833	0.013223
%RSD	0.086	1.04	**%RSD**	0.184	1.48	**%RSD**	0.09	1.01

TABLE 4: Data for Effect of variation in flow rate (Domperidone)

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	1273707	1.362089	**Flow 1.0 ml**	1206349	1.280574	**Flow 1.2 ml**	1266195	1.285372
	1273211	1.352617		1205267	1.279932		1265885	1.299385
	1273948	1.376926		1205625	1.261721		1266303	1.308063
	1273465	1.345752		1205840	1.276089		1267243	1.274662
	1273862	1.374925		1205735	1.250640		1265762	1.267630
Avg	1273638.6	1.362462	**Avg**	1205763.2	1.269791	**Avg**	166277.6	1.287022
SD	3301.369	0.013609	**SD**	392.1635	0.01314	**SD**	582.9758	0.016786
%RSD	1.041	0.99	**%RSD**	0.19	1.03	**%RSD**	0.35	1.3

Acceptance criteria

Percentage RSD should be below 2.

The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 1, which is within the acceptance criteria. Hence the method is robust.

Discussion: The results of robustness for effect of variation in flow rate and organic phase are presented in **Table: 3 and 4.**

8) Limit of detection and limit of quantification (LOD and LOQ)

"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy."

Various approaches can be applied

Visual definition

Calculation from the signal-to-noise ratio (LOD and LOQ correspond to 3 or 2 and 10 times the noise level, respectively)

Calculation from the standard deviation of the blank

Calculation from the calibration line at low concentrations

LOD; LOQ ¼ F_SD b (2.6-1)

F: factor of 3.3 and 10 for LOD and LOQ, respectively

SD: standard deviation of the blank, standard deviation of the ordinate intercept, or residual standard deviation of the linear regression.

b: slope of the regression line

The estimated limits should be verified by analyzing a suitable number of samples containing the analyte at the corresponding concentrations. The LOD or LOQ and the procedure used for determination, as well as relevant chromatograms, should be reported.

The quantification limit is the lowest level of analyte that can be accurately and precisely measured. This limit is required only for impurity methods and is determined by reducing the analyte concentration until a level is reached where the precision of the method is unacceptable. If not determined experimentally, the quantification limit is often calculated as the analyte concentration that gives S / N = 10. An example of quantification limit criteria is that the limit will be defined as the lowest concentration level for which an RSD 20 % is obtained when an intra-assay precision study is performed.

Esomeprazole

From the linearity plot the LOD and LOQ are calcula ted

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3 \times 5988.87}{18600} = 1.06$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10 \times 5988.87}{18600} = 3.21$$

Domperidone

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3 \times 771.5483}{5140} = 0.49$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10 \times 771.5483}{5140} = 1.50$$

Acceptance criteria

Resolution between two drugs must be not less than 2.

It was found from above data that all the system suitability parameters were within the limit.

Discussion: The Limit of detection (LOD) was 1.06 and 0.49 µg mL-1 and Limit of Quantization (LOQ) was 3.21 µg mL-1 and 1.50 µg mL-1 for Esomeprazole and Domperidone, respectively.

SUMMARY AND CONCLUSION

Summary

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 260 nm for Esomeprazole and 238 nm for Domperidone. Common wavelength will be 260 nm and the peaks purity was excellent. Injection volume was selected to be 20 µL which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 mL/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 50:50 Methanol : Water was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

The present recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Limit of Detection was found to be 1.06 µg/mL for Esomeprazole and 0.49 µg/mL for Domperidone. Linearity study was, correlation coefficient and curve fitting was found to be 0.999. The analytical method was found linearity over the range of 20-80 ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Table 5: Summary for RP-HPLC Method

S. NO	PARAMETER	ACCEPTANCECRITERIA	RESULTS OBTAINED
1	System Suitability	Theoretical Plates-NLT 2000	Esomeprazole-10768 Domperidone-9573
		Tailing factor-NMT 2	Esomeprazole-1.15 Domperidone-0.89
		Retention time NLT 2	Esomeprazole-2.87 Domperidone-3.94
		% RSD of Esomeprazole- NMT 2 % RSD of Domperidone- NMT 2	Esomeprazole-0.05 Domperidone-0.02
2	Precision	% RSD of Esomeprazole- NMT 2 % RSD of Domperidone- NMT 2	Esomeprazole-0.88 Domperidone-1.80
3	Method Precision	% RSD of Esomeprazole- NMT 2 % RSD of Domperidone- NMT 2	Esomeprazole-0.08 Domperidone-0.13
4	ID Precision	% RSD of Esomeprazole- NMT 2 % RSD of Domperidone- NMT 2	Esomeprazole-1.18 Domperidone-1.24
5	Linearity	Correlation coefficient NLT 0.999	Esomeprazole-0.999 Domperidone-0.999

6	Accuracy	Percentage Recovery 98-102%	Esomeprazole-100.4 Domperidone-99.7
7	Limit of detection		Esomeprazole-1.06 µg/mL Domperidone-0.49 µg/mL
8	Limit of quantitation		Esomeprazole-3.21 µg/mL Domperidone-1.50 µg/mL

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Esomeprazole and Domperidone in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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