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A Novel RP-UPLC Method for the Simultaneous Estimation of Domperidone and Rabeprazole in Pharmaceutical Dosage Form

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A B S T R A C T

A simple, accurate, rapid, precise and novel Reverse phase Ultra Pressure liquid chromatographic method has been developed and validated for simultaneous determination of Domperidone and Rabeprazole in pharmaceutical dosage form. The chromatographic separation was carried out on a Symmetry C18 (2.1 x 100mm, 1.7µm, Make: BEH) or equivalent column with a mixture of Buffer (pH: 6.0 adjusted with ortho-phosphoric acid): Acetonitrile (35:65, v/v) as mobile phase; at a flow rate of 0.4 ml/min. The retention times for DOM and RAB were observed to be 2.391 and 4.602 min respectively. Calibration plots were linear (r^2 >0.999) over the concentration range of 48,54,60,66,72 µg/ml for DOM and 32,36,40,44,48 µg/ml for RAB. The method was validated for accuracy, precision, specificity, linearity, robustness, sensitivity, LOD and LOQ. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Domperidone and Rabeprazole in bulk and in its pharmaceutical formulations.

Keywords: Domperidone, Rabeprazole and UPLC

A R T I C L E I N F O

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1. Introduction

Rabeprazole sodium [1,2,3] is chemically known as 2-({[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methane} sulfinyl)-1H-1,3-benzodiazole. It is a proton pump inhibitor and used for the treatment of peptic ulcer or GERD. It is not official in any pharmacopoeia. Domperidone is chemically known as 5-chloro-1-[1-[3-(2, 3-dihydro-2-oxo-1*H*benzimidazol-1-yl) propyl] piperidin-4-yl]-2,3-dihydro-1*H*-benzimidazol-2-one. It is a gastro-kinetic and anti-emetic.It is a peripheral dopamine-2 receptor antagonist. Literature survey revealed have not been reported for estimation of Domperidone and Rabeprazole by UPLC method was reported for its analysis. Hence the objective was to develop a simple, sensitive, accurate and precise method for Simultaneous Determination of Domperidone and Rabeprazole in Pharmaceutical Dosage form.

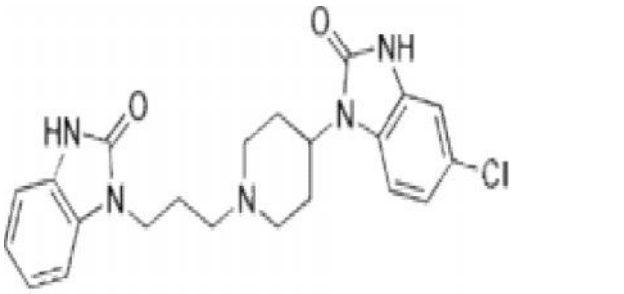

Figure 1: Chemical structure of Domperidone

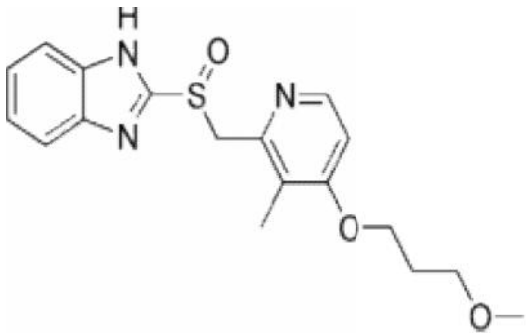

Figure 2: Chemical structure of Rabeprazole

2. Materials and Methods

Chemicals and reagents: Pure samples of Rabeprazole and Domperidone were obtained from Chethana chemicals, Trichur. The commercial samples of razo-D capsules were obtained from Dr. REDDY'S. Water (HPLC) was obtained from a Milli-QRO water purification system. Methanol (HPLC grade) and Potassioum di hydrozen ortho phospate (HPLC grade) were procured from Merck Ltd. (Mumbai, India.)

Chromatographic Conditions:

When several mobile phases were tried, the mobile phase containing Acetonitrile and Buffer (pH 6.0 adjusted with Orthophosphoric acid) in the ratio of 65:35 v/v was considered to be appropriate.

The mobile phase was filtered through 0.45µm membrane filter and ultra sonicated for 10 minutes. The flow rate selected was 0.4 ml/min with wave length of 217 nm. All the determinations were performed at constant column temperature (Ambient) and a injector volumeis 10µl.

Standard Preparation:

Accurately 30 mg and 20 mg of Domperidone & Rabeprazole [36,37] were weighed and transferred into 100 ml volumetric flask, about 20ml of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. From this 5 ml of solution was pipetted out and transferred into 25 ml of volumetric flask and the volume was made up with diluent to give a concentrations of 60 µg/ml and 40 µg/ml of Domperidone and Rabeprazole respectively.

Preparations of Sample Solutions:

Accurately 276 mg of Domperidone & Rabeprazole were weighed which is equivalent to 30 mg and 20 mg of Domperidone & Rabeprazole and transferred 100 ml volumetric flask, about 15 ml of diluent and sonicated for 20 minutes to dissolve it.

The volume was made up with diluent. The solution was filtered through 0.45µm membrane filter (Stock solution). From this 5 ml of solution was pipetted out and transferred into 25 ml of volumetric flask and the volume was made up with diluent to give a concentrations of 60 µg/ml and 40 µg/ml of Domperidone and Rabeprazole respectively.

Assay

10 µl of standard and sample solutions were injected into an injector of UPLC, and the peak areas of the drugs in standard and sample were compared and assay was calculated.

3. Results and Discussion

System suitability

System suitability tests were carried out on freshly prepared standard solution of DOM and RAB to check the various parameters such as efficiency, retention time, and peak tailing which was found to comply with ICH requirements. The instrumental precisions as determined by six successive injections of the standard solutions give RSD below 2% of Retention Time and Area. The data is given in Table no:1 as shown in figure-3.

Table 1: System suitability parameters of Domperidone & Rabeprazole

Parameters	Domperidone	Rabeprazole
Tailing factor	1.54	1.34
Retention time	2.391	4.60
Theoretical plates per unit	8025	6424

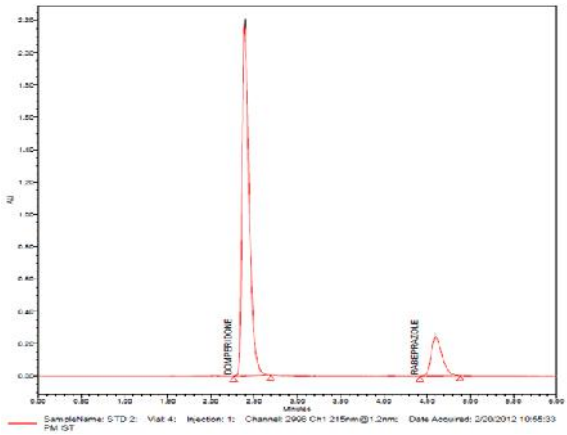

Figure 3: Chromatogram showing system suitability testing of Standard Solution of Domperidone & Rabeprazole

Specificity:

The specificity of the method was confirmed by injecting the placebo and placebo spiked standard and observed that there was no shift in wavelength interference due to placebo. This confirms the specificity of the proposed method**.** The results are reported in Table no: 5. The chromatograms are shown in figure 4. There is no peak in the blank and Placebo solution run at the retention time corresponding to Domperidone & Rabeprazole as in standard run.

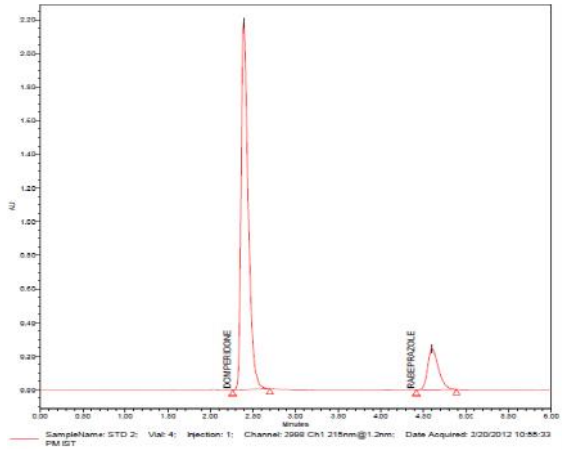

Figure 4: Chromatogram showing Specificity testing of Domperidone & Rabeprazole

Linearity

Linearity is the ability of the method to obtain test results that are directly proportional to the analyte concentration within a given range.

Range:

Range of analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

Procedure :

Preparation of Standard Stock Solution:

Accurately 30 mg and 20 mg of Domperidone & Rabeprazole respectively were weighed and transferred into 100 ml volumetric flask, about 20 ml of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution).

Preparation of sample solutions:

From the above stock solution pipette out 4, 4.5, 5.0, 5.5 and 6.0 ml respectively into individual 25 ml of volumetric flasks and diluted up to the mark with diluent to prepare 48,54,60,66,72 µg/ml of Domperidone and 32,36,40,44,48 ug/ml of Rabeprazole respective.

Mix well and filter through 0.45 µm filter. Inject 10 µl of blank solution and each linearity level standard solutions into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

The calibration curve for Domperidone and Rabeprazole as shown in Table-2 and Table-3 respectively was drawn by plotting the mean peak area versus concentration, yielded coefficient of regression r^2=0.999 for DOM (48 to 72 µg/ml) and r^2 =0.999 for RAB (32 to 48 µg/ml) over a concentration range the representative linear regression equation for Domperidone Y=125346.224 and Rabeprazole Y= 21209.695 as shown in table-2 and Figure 5 & 6.

Table-3: Linearity Results for Domperidone

Parameters	Domperidone	Rabeprazole
Linear Dynamic Range	48 to 72 µg/ml	32-48 µg/ml
Correlation Co-efficient	0.999	0.999
Slope (m)	125346.224	21209.695

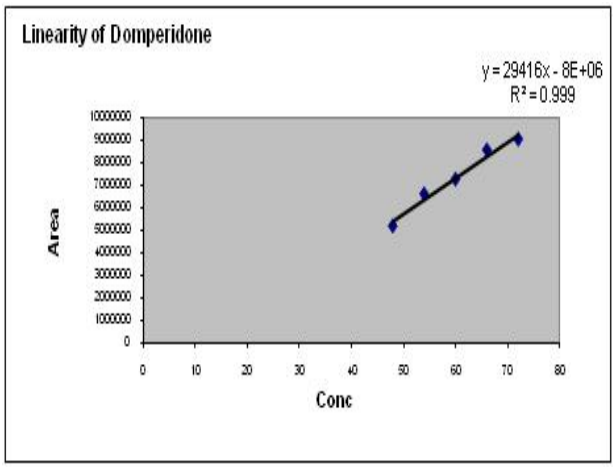

Figure 5: Linearity plot of Domperidone

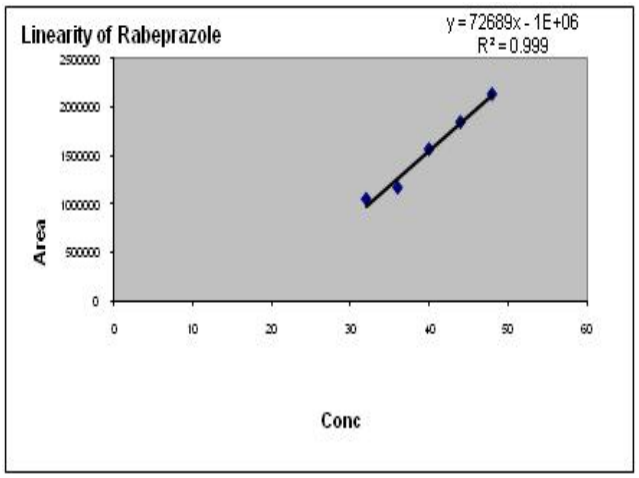

Figure 6: linearity plot of Rabeprazole

Precision:

a) System precision for DOM and RAB

The system precision was evaluated by calculating the RSD for retention time and peak area of DOM and RAB for six replicate injections of standard solution. The result are given in Table- 3 &4 as shown in the figure-7. Indicates that the precision of the system is within the limit. (Acceptance criteria: % RSD not more than 2%).

Table no 3: system precision data for domperidone

	Vial	Sample Name	Inj	Name	R_T	Area	Height	Tailing	Plate count
1	4	Sys pre	1	DOM	2.391	12514807	2167483	1.54	8025
2	4	Sys pre	2	DOM	2.390	12523307	2178594	1.56	8983
3	4	Sys pre	3	DOM	2.390	12530112	2149379	1.58	8976
4	4	Sys pre	4	DOM	2.392	12529742	2156771	1.57	8985
5	4	Sys pre	5	DOM	2.389	12553265	2187683	1.61	7967
6	4	Sys pre	6	DOM	2.389	12604652	2215866	1.58	7017
Mean					2.390	12548216	2177659		
Std. dev.					0.0011	33541	26444		
% RSD					0.04	0.3	1.2		

Table no 4: System Precision Data for Rabeprazole

	Vial	Sample Name	Inj	Name	R_T	Area	Height	Tailing	Plate Count
1	4	Sys pre	1	RAB	4.602	2125112	242632	1.34	6424
2	4	Sys pre	2	RAB	4.601	2115417	242744	1.34	6420
3	4	Sys pre	3	RAB	4.603	2120834	242667	1.35	6496
4	4	Sys pre	4	RAB	4.606	2117467	242714	1.33	6550
5	4	Sys pre	5	RAB	4.605	2122476	242831	1.34	6513
6	4	Sys pre	6	RAB	4.604	2127408	243986	1.34	6332
Mean					4.603	2120720	242988		
Std. dev.					0.0018	4651	561		
% RSD					0.04	0.2	0.2		

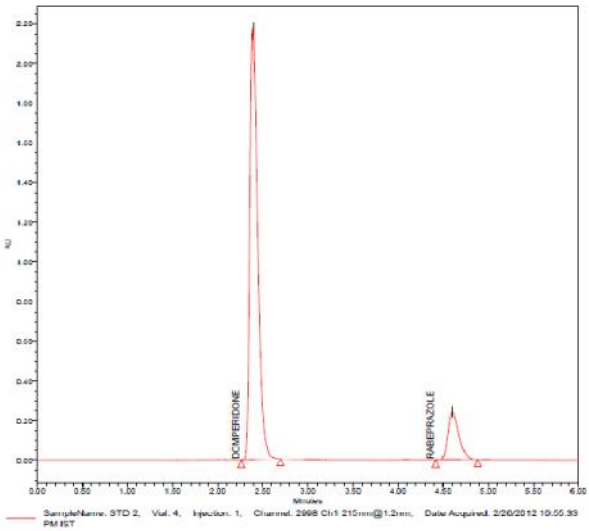

Figure 7: Chromatogram showing System Precision - preparation - 1 solution of Domperidone & Rabeprazole

b) Method precision for DOM and RAB. The method precision was determined by preparing a sample solution of a single batch of DOM and RAB Tablet six times and analyzing as per the proposed method. The result shown in Table-5 & 6. Indicates that the proposed method is precise. (Acceptance criteria: % RSD not more than 2%).

Table 5: Results for method precison of domperidone

	Vial	Sample Name	Inj	Name	RT	Area	Height
1	5	Precision	1	DOM	2.388	12364527	2152405
2	6	Precision	1	DOM	2.389	12392671	2125908
3	7	Precision	1	DOM	2.390	12358502	2149131
4	8	Precision	1	DOM	2.388	12356161	2167628
5	9	Precision	1	DOM	2.388	12343124	2155891
6	10	Precision	1	DOM	2.390	12391131	2148274
Mean					2.388	12367686	2149973
Std. dev.					0.0009	20022	13677
% RSD					0.04	0.2	0.6

Table 5: Results for method precison of rabepraole

	Vial	Sample Name	Inj	Name	RT	Area	Height
1	5	Precision	1	RAB	4.602	2105505	240853
2	6	Precision	1	RAB	4.605	2122970	241220
3	7	Precision	1	RAB	4.605	2103293	239093
4	8	Precision	1	RAB	4.601	2110710	239115
5	9	Precision	1	RAB	4.607	2106755	237151
6	10	Precision	1	RAB	4.607	2110541	240072
Mean					4.607	2109962	239584
Std. dev.					0.002	6996	1477
% RSD					0.05	0.3	0.6

Accuracy (recovery study) for DOM and RAB

The validation of the proposed method was further verified by recovery studies. Acceptance criteria is 98 - 102 % w/v. The results are reported in Table no: 6

Table 6: Accuracy data for Domperidone and Rabiprazole

Parameters	Domperidone	Rabeprazole
% recovery		
50 %	99 % w/v	99 % w/v
100 %	98 % w/v	100 % w/v
150 %	99 % w/v	103 % w/v

This serves as a good index of the accuracy and reproducibility of the proposed method. A known amount of DOM and RAB were spiked to placebo at 50%, 100% and 150% of specification in triplicate and analyzed determine the accuracy of the method.

Limit of detection (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD can be calculated as: LOD = 3.3 σ / S Where, σ =Standard deviation of the response (y-intercept) S= Slope of the calibration curve. The limit of detection (LOD) was found to be 0.00002 µg/ml for DOM and 0.000148 µg/ml for RAB respectively.

Limit of quantification (LOQ)

The limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ can be calculated as: LOQ = 10 σ / S Where, σ = Standard deviation of the response (y- intercept) S= Slope of the calibration curve whereas the limit of quantification (LOQ) was found to be 0.00008 µg/ml for DOM and 0.000496 µg/ml for RAB respectively.

Robustness

Robustness was determined by carrying out the assay during which flow rate and temperature were altered slightly. The results are reported in Table- 7.

Table 7: Robustness data for Domperidone and rabeprazole

Parameters	% RSD Domperidone	% RSD Rabeprazole
Flow rate	0.2	0.2
Detection wave length	0.24	0.26

% RSD values for robustness indicated that the method is robust and does not show variations in the results on slight variations in flow rate and detection wavelength

4. Conclusion

A convenient, rapid, precise and Novel RP-UPLC method has been developed for estimation of Domperidone and Rabeprazole in bulk and in its dosage form. Hence the suggested Reverse phase UPLC method was validated as per ICH guidelines and it can be used for routine analysis of Domperidone and rabiprazole in pharmaceutical dosage forms.

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