Method Development and Validation for Rabeprazole and Domperidone in Combine Dosage Form by RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Rabeprazole and Domperidone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Rabeprazole and Domperidone by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.170 mins and 7.025 mins. The % purity of Rabeprazole and Domperidone was found to be 99.1% and 98.2% respectively. The system suitability parameters for Rabeprazole and Domperidone such as theoretical plates and tailing factor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Rabeprazole and Domperidone was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125respectively.

Keywords: Rabeprazole and Domperidone, C18 column, RP-HPLC

1. Introduction

Rabeprazole is a proton pump inhibitor that suppresses gastric acid production in the stomach. It has several medical uses: the management of conditions that involve excess gastric acid production (e.g. Zollinger–Ellison syndrome), conditions that are worsened by gastric acid (e.g. ulcerations of the gastrointestinal tract), and conditions involving prolonged exposure to gastric acid (e.g. symptomatic gastro esophageal reflux disease). Rabeprazole's adverse effects tend to be mild but can be serious, including deficiencies in essential nutrients, rare incidences of liver and bone damage, and dangerous rashes.Rabeprazole can theoretically contribute to numerous drug interactions, mediated both through its metabolic properties and its direct effect on acid in the stomach, though its potential for clinically meaningful drug interactions is low Domperidone, sold under the brand name Motilium among others, is a peripherally selective dopamine D2 receptor antagonist that was developed by Janssen Pharmaceutica and is used as an antiemetic, gastroprokinetic agent, and galactagogue. It may be administered orally or rectally, and is available in the form of tablets, orally disintegrating tablets (based on Zydis technology), suspension, and suppositories. The drug is used to relieve nausea and vomiting; to increase the transit of food through the stomach(by increasing gastrointestinal peristalsis); and to promote lactation (breast milk production) by release of prolactin.

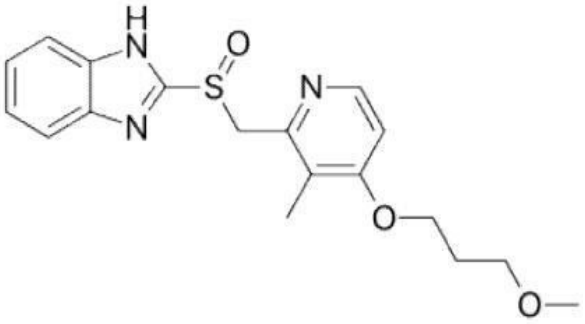


Fig 1: Structure of Rabeprazole

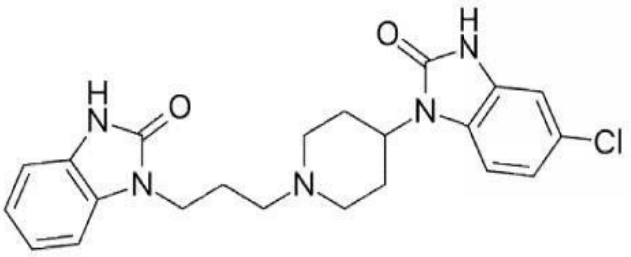


Fig 2: Structure of Domperidone

2. Materials and Methods

Instrumentation

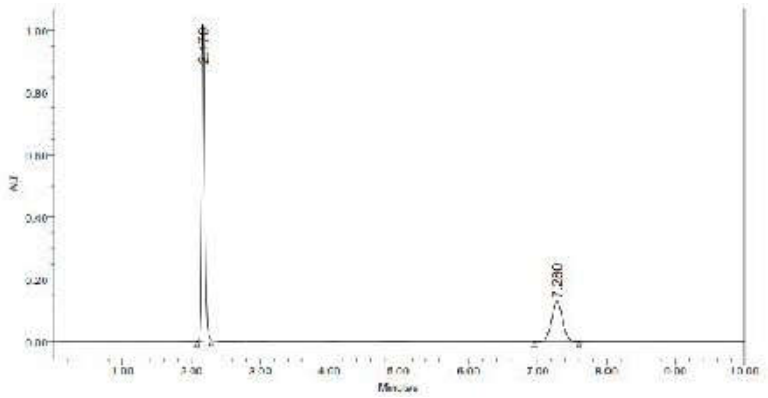
HPLC- Shimadzu, model No. SPD-20MA LC+20AD,
Software- LC-20 Solution U.V double beam spectrometer
UV 3000+ U.V win software Lab India Digital weighing balance (sensitivity 5mg) pH meter Sonicator.

Chemicals

Rabeprazole and Domperidone, Ortho phosphoric acid

Chromatographic Conditions:

Column :Zodiac silC18 column (4.6×150mm)5µ
Mobilephaseratio :Methanol: pH 3phosphate buffer (70: 30 % v/v)
Detection wavelength :240 nm
Flowrate : 1.0ml/min
Injectionvolume : 20µl
Columntemperature : Ambient
Auto sampler : Ambient Temperature
Runtime :10min
Retentiontime :2.170 and 7.280mins



Observation:
The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Sample solution preparation:
Accurately weigh and transfer 59.8 mg of Domperidone ml of Domperidone& Rabeprazole the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

Standard solution preparation:
Accurately weigh and transfer 12.5 mg & 8 mg of
Domperidone and Rabeprazole working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.6ml of Domperidone & Rabeprazole the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Method Validation

Specificity:
The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity:

Accurately weigh and transfer 12.5 mg&8 mg of Domperidone and Rabeprazole working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Range:
Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 16µg/ml-80µg/ml and 25µg/ml to 125µg/ml of Rabeprazole and Domperidone respectively.

Accuracy:
Accurately weigh and transfer 12.5 mg &8 mg of Domperidone and Rabeprazole working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

Precision:
Repeatability: Accurately weigh and transfer 12.5 mg &8 mg of Domperidone and Rabeprazole working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by usingdifferent make column of same dimensions.

Limit of detection (LOD):
LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Limit of quantification (LOQ):
LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) accor ding to the formula. Again, the

standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness: As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

System suitability:
Accurately weigh and transfer 12.5 mg &8 mg of Domperidone and Rabeprazole working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

3. Results and Discussions

Linearity:

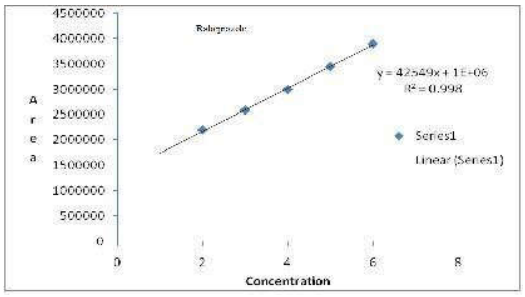


Fig 4: Showing calibration graph for Rabeprazole

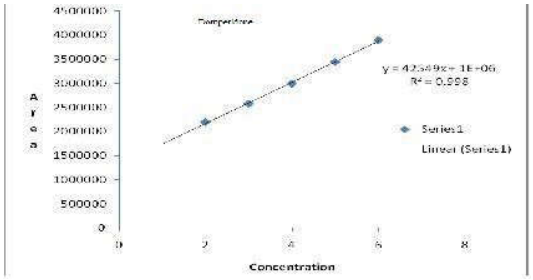


Fig 5: Showing calibration graph for Domperidone

Robustness:

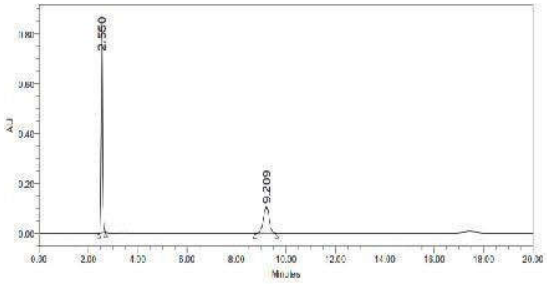


Fig 6: Chromatogram showing more flow rate0.8ml/min

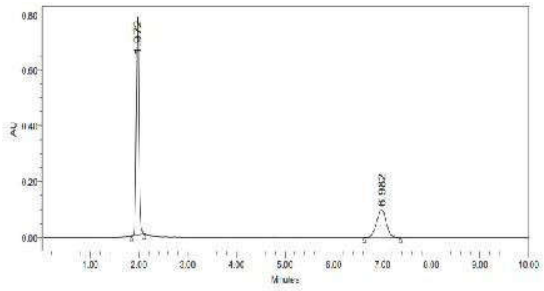


Fig 7: Chromatogram showing less flow rate 1.2ml/min

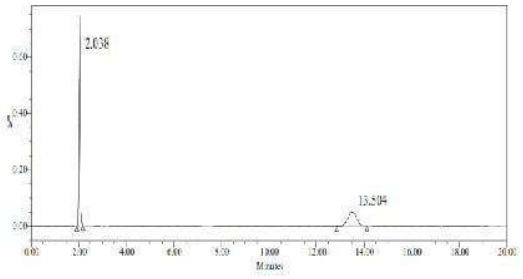


Fig 8: Chromatogram showing less organic phase ratio

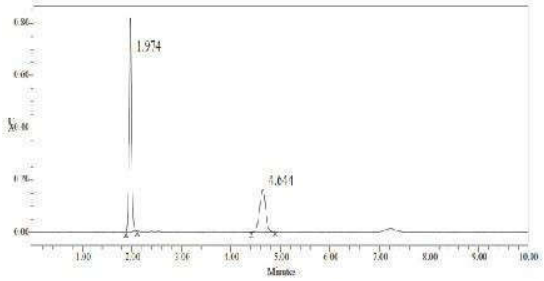


Fig 9: Chromatogram showing more organic phase ratio

Table No 1: Linearity Results forRabeprazole

S.No	Linearity Level	Concentration	Area
1	I	16ppm	1027461
2	II	32ppm	1233566
3	III	48ppm	1437030
4	IV	64ppm	1644336
5	V	80ppm	1880590
Correlation Coefficient			**0.999**

Table No 2: Linearity Results forDomperidone

S.No	Linearity Level	Concentration	Area
1	I	25ppm	2201022
2	II	50ppm	2585033
3	III	75ppm	2996553
4	IV	100ppm	3446224
5	V	125ppm	3897922
Correlation Coefficient			0.999

Table No 3: Showing accuracy results for Rabeprazole

%Concentration (at specificationLevel)	Area	Amount Added (mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	765624	4.25	4.30	101.2%	101.4%
100%	1508055	8.25	8.48	101.5%	
150%	2204983	12.2	12.39	101.6%	

Table No 4: Showing accuracy results for Domperidone

%Concentration (at specificationLevel)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	1726242	7.05	7.1	101.9%	101.7%
100%	3187170	13.1	13.2	101.3%	
150%	4521881	18.5	18.8	101.8%	

Table No 5: Showing results for Limit of Detection

	Peak Name	RT	Area	Height	USP PlateCount	USP Tailing
1	Rabeprazole	2.184	430	154	12989.3	1.2
2	Domperidone	7.503	1754	153	9945.7	1.0

Table No 6: Showing results for Limit of Quantitation

	Peak Name	RT	Area	Height	USP PlateCount	USP Tailing
1	Rabeprazole	2.184	1452	519	12989.3	1.2
2	Domperidone	7.503	5927	517	9945.7	1.0

Table No 7: Showing% RSD results for Rabeprazole and Domperidone

Injection	Area	
	Rabeprazole	Domperidone
Injection-1	1475698	3045768
Injection-2	1461561	3030853
Injection-3	1481379	3063519
Injection-4	1467049	3065127
Injection-5	1472628	3099001
Average	1471663	3060854
Standard Deviation	7664.08	25535.28
%RSD	0.52	0.83

Table No 8: Showing results for intermediate precision of Rabeprazole and Domperidone

Injection	Area	
	Rabeprazole	Domperidone
Injection-1	1419430	3098177
Injection-2	1437396	3075703
Injection-3	1461998	3135114
Injection-4	1484335	3173644
Injection-5	1486671	3179888
Injection-6	1488969	3184696
Average	1463133.2	3141203.7
Standard Deviation	29136.557	46085.033
%RSD	1.99	1.46

Table No 9: Showing system suitability results for Rabeprazole

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	7515.5	0.9
2	1.0	10026.7	1.0
3	1.1	5948.0	1.0

Table No 10: Showing system suitability results forDomperidone

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	8573.5	1.0
2	1.0	12458.5	1.2
3	1.1	6114.5	1.1

Table No 11 : Showing system suitability results forRabeprazole

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6953.5	1.0
2	*Actual	10026.7	1.0
3	10% more	6048.5	1.0

Table No 12 : Showing system suitability results for Domperidone

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	7079.0	1.0
2	*Actual	12458.5	1.2
3	10% more	6228.5	1.1

4. Conclusion

A new method was established for simultaneous estimation of Rabeprazole and Domperidone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Rabeprazole and Domperidone by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.170 mins and 7.025 mins. The % purity of Rabeprazole and Domperidone was found to be 99.1% and 98.2% respectively. The system suitability parameters for Rabeprazole and Domperidone such as theoreticalplatesand tailing fa ctor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Rabeprazole and Domperidone was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatability.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.Hence the suggested RP-HPLC method can be used for routine analysis of Rabeprazole and Domperidone in API and Pharmaceutical dosageform.

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