www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(8): 97-101

www.thepharmajournal.com
Received: 15-08-2015
Accepted: 17-09-2015

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RP-HPLC method Development and Validation studies of Ranitidine Hydrochloride and Domperidone in Tablets

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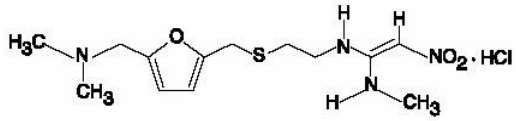
Abstract
This study proposes a liquid chromatography (RP-HPLC) method for quantitative analysis of the most widely prescribed combinations of Ranitidine hydrochloride, Domperidone and Magaldrate in tablet dosage form in the presence of Metronidazole as an internal standard. The chromatography was done on a C18 column (250 mm × 4.6 mm, 5 (m) as stationary phase and Methanol: Potassium dihydrogen o-phosphate (65:35 % v/v, pH 3.0 adjusted to 1% OPA) as mobile phase at a current rate of 1mL/min and measured at 227nm. Retention time was found to be 2.68min, 3.66min and 5.25min for RH, DM and MZ respectively. The assay of RH and DM in the formulation was found to be 100.33% w/w and 99.53% w/w respectively. The linearity range for RH was 3-150 µg/mL and for DM was 0.2-10 µg/mL. The developed method was validated in accordance to ICH guidelines.

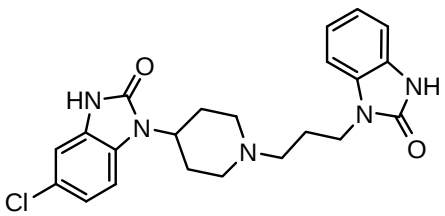
Keywords: Magaldrate (MG), Ranitidine hydrochloride (RH), Domperidone (DM), Metronidazole (MZ), Reverse Phase high performance liquid chromatographic (RP-HPLC), Internal Standard (IS).

Introduction
Ranitidine hydrochloride[1,2,3] is chemically Dimethyl[(5-{[(2-{[(E)-1-(methylamino)-2 nitroethenyl]amino}ethyl)sulfanyl] methyl}furan-2-yl) methyl]amine. The H_2 antagonists are competitive inhibitors of histamine[4] at the parietal cell H_2 receptor. They suppress the normal secretion of acid by parietal cells and the meal-stimulated secretion of acid. They accomplish this by two mechanisms: histamine released by ECL cells in the stomach is blocked from binding on parietal cell H_2 receptors which stimulate acid secretion, and other substances that promote acid secretion (such as gastrin and acetylcholine) have a reduced effect on parietal cells when the H_2 receptors are blocked.



Chemical structure of Ranitidine hydrochloride

Domperidone[1,2,3] is chemically 5-chloro-1-{1-[3-(2-oxo- 2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]piperidin- 4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one. It acts as a gastrointestinal emptying (delayed) adjunct and peristaltic stimulant. The gastroprokinetic properties of Domperidone relate 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.



Chemical structure of Domperidone

Antiemetic[4]: The antiemetic properties of Domperidone relate to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level. It has strong affinities for the D_2 and D_3 dopamine receptors, which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which - among others – regulates nausea and vomiting. Here, we describe a simple, accurate RP HPLC method for simultaneous determination Ranitidine and Domperidone in bulk drugs and pharmaceutical dosage form.

Materials and Methods

Chemicals and solvents

Pure samples of Ranitidine and Domperidone were obtained respectively from Allied Chemicals and Pharmaceuticals Pvt. Ltd, New Delhi.

Pretreatment of Drug Sample

Magaldrate and Ranitidine hydrochloride have the hygroscopic nature. So to avoid weighing error of the drug, the powder mixture was kept in hot air oven at 70˚C for 15min and then cooled at room temperature and kept in desiccators.

Preparation of Standard Solution for selecting a method

Standard RH solution (100µg/mL)

Accurately weighed 10mg of standard RH and transferred into 10 mL volumetric flask, 3-5mL of methanol was added and sonicated for 2 minute to dissolve it in full. Make up the volume with methanol, which gives 1000µg/mL of the standard RH solution. From the first stock pipette out 1mL and diluted to further 10mL volumetric flask and make up the volume with methanol which gives 100 µg/mL.

Standard DM solution (100µg/mL)

Accurately weighed 10mg of standard DM and transferred to 10mL volumetric flask, 3-5mL of methanol was added and sonicated for 2 minute to dissolve it in full. Draw up the volume with methanol, which gives 1000µg/mL of the standard DM solution. From the first stock pipette out 1mL and diluted to further 10mL volumetric flask and make up the volume with methanol which gives 100 µg/mL.

Standard Solution of MZ (20µg/mL)

Weighed accurately 20 mg of standard MZ and transferred to a 10 ml volumetric flask. MZ was dissolved in 5 mL of HPLC grade methanol and the volume was made up to the mark with same solvent to obtain final concentration of 2000 µg/mL of MZ and labelled as 'Stock MZ-A'.

From the 'Stock MZ-A' solution, 0.5mL of aliquot was piped out in a 50 mL volumetric flask and the volume was made up to the mark with mobile phase to obtain a final concentration of 20 µg/mL and labelled as 'Stock MZ-B'.

Preparation of Buffer (10mM PHP)

Accurately weighed 680mg of HPLC grade potassium dihydrogen ortho phosphate and transferred into a 500mL volumetric flask and added 200-300mL double distilled water and sonicated for 5 min to dissolve it fully. Finally, make up the volume up to the mark with double distilled water.

Preparation of 1% OPA

1mL of ortho phosphoric acid was a pipette out to 100mL of volumetric flask and make up the final volume up to the mark with double distilled water and labelled.

Standard solution of RH (100 µg/mL) and DM (100 µg/mL) was prepared separately and injected into the HPLC system. The Standard solutions were analysed using different proportions of Methanol: buffer, different pH,

Selection of Mobile Phase pH

pH of HPLC grade water was adjusted to pH 3, 4, and 4.5 using 1% orthophosphoric acid. Individual solution of RH (100 µg/mL), DM (100 µg/mL) and MZ (100 µg/mL) were prepared and injected into the HPLC system.

Selection of Internal Standard (IS)

Metronidazole (MZ) was selected as an internal standard (IS) based on its pKa value (2.6) and λ_{max} as 277nm. Separation of MZ, RH and DM were performed to develop a new method. As a resolution was found satisfactory, MZ (20 µg/ml) was studied to use as IS for the chromatography procedure in different mobile phase composition having a different pH at different flow rate.

Selection of Flow Rate

Chromatogram of mixed solution of RH (100 µg/mL), DM (100 µg/mL) and MZ (20 µg/mL) was considered at different flow Rate such as 0.5, 1. 1.2 mL/min.

Selection of Analytical Wavelength

The standard solutions of RH (100 µg/mL), DM (100 µg/mL) and MZ (20 µg/mL) in mobile phase were scanned separately in the UV region of 190 to 400 nm and the overlain spectra were recorded.

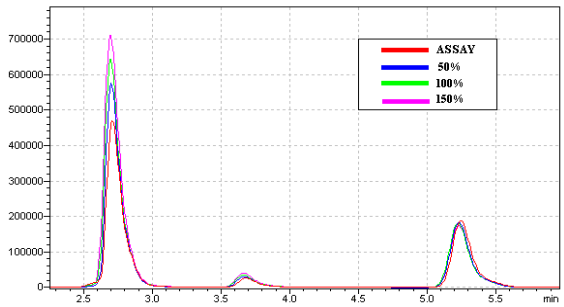
Analysis of Tablet Formulation

Equivalent weight of 180mg of powder drug was taken and transferred in to 50mL of volumetric flask and diluted with methanol and made up the mark up to methanol which gives 1000µg/mL. From this 1mL pipette out and diluted with 10mL and further 1mL in 10mL along with 1mL of standard MZ solution to obtain the final concentration of 100µg/mL.

Validation of Rp-Hplc Method

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision, Specificity, Linearity, Accuracy, Limit of detection and Limit of quantification.

Accuracy - The percentage recovery for RH and DM were carried out at 3 different levels and the results obtained are presented below. Accuracy of the method was done by recovery study. Sample solutions were prepared by spiking at about 50%, 100%, and 150% of specification limit to placebo and analyzed by the proposed HPLC method.



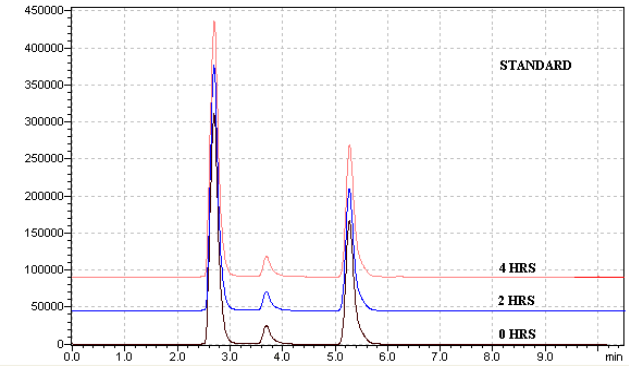
Overlain Chromatograms for Percentage Recovery of RH and DM

Table for Percentage recovery for RH-DM at three different levels

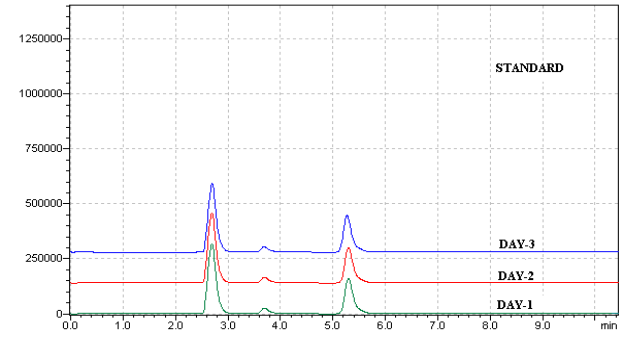
% STD Addⁿ	Area Under Curve (AUC)			CONC OF RH(µg/mL)			%RECOVERY for RH				
	Set I	Set II	Set III	Set I	Set II	Set III	Set I	Set II	Set III	MEAN	% RSD
50	4467071	4423112	4476243	73.88	73.90	74.1	99.8	99.8	100.2	99.9	0.209
100	5364348	5308198	5430326	98.0	98.21	98.2	102.4	103	103.0	103	0.732
150	5917876	5902305	5914414	123.2	123.7	123	103.6	101	101.9	102	1.610
% STD Addⁿ	Area Under Curve (AUC)			CONC OF DM(µg/mL)			%RECOVERY for DM				
	Set I	Set II	Set III	Set I	Set II	Set III	Set I	Set II	Set III	MEAN	% RSD
50	287569	285729	280593	9.80	9.60	9.5	95.14	93.2	94.2	94.18	1.03
100	340794	344933	335468	17.50	17.8	17	101.1	103	103.1	102.4	1.56
150	401296	396650	400765	24.55	24.7	24	101.0	101	102.1	101.6	0.54

Report: From the obtained data, % recovery of Ranitidine hydrochloride and Domperidone were found to be 99% to 102% w/w and 94% to 102% w/w for RH-DM respectively in all the stages of recuperation.

The precision - precision of a method is the extent to which the individual test results of multiple injections on a series of standards. The measured standard deviation can be subdivided into the following categories: Intraday, Interday study, Repeatability, Ruggedness.

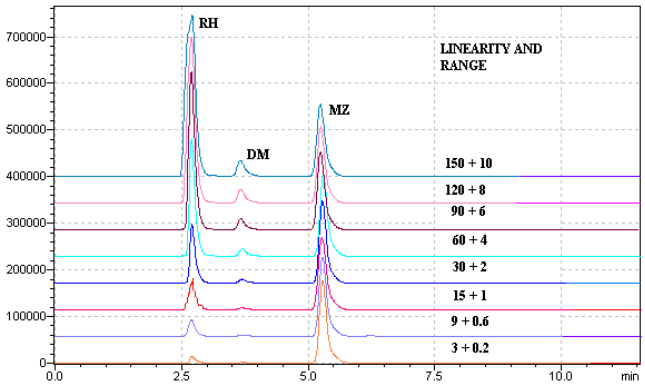


Overlain Chromatograms of Intraday study



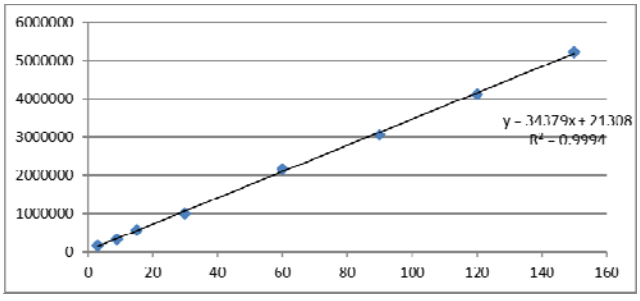
Overlain Chromatograms of Interday study

Linearity and the Range - The linearity and Range was calculated from the Calibration Curve data as specified in Table. The data were plotted for Absorbance v's Concentration and the regression coefficient was calculated.



Preparation of Calibration curve for RH and DM with MZ as Internal standard

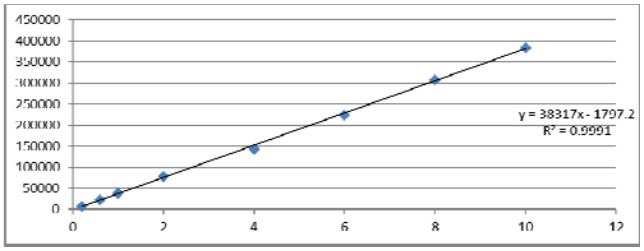
Overlain Chromatograms of Serial Dilutions of RH (3-150 µg/mL), DM (0.2-10 µg/mL) and MZ (20µg/mL) in MEOH: PHP (65:35 % v/v, pH 3) at a Flow Rate of 1 ml/min, at 227 nm



Calibration Curve of RH of RP-HPLC Method

Table for Linear Regression Analysis

Parameters	RH at 227 nm
Linearity Range (µg/ml)	3-150
Slope	33778
Intercept	12000.00
Correlation Coefficient (r^2)	0.999
LOD (µg/mL)	1.17
LOQ (µg/mL)	3.55

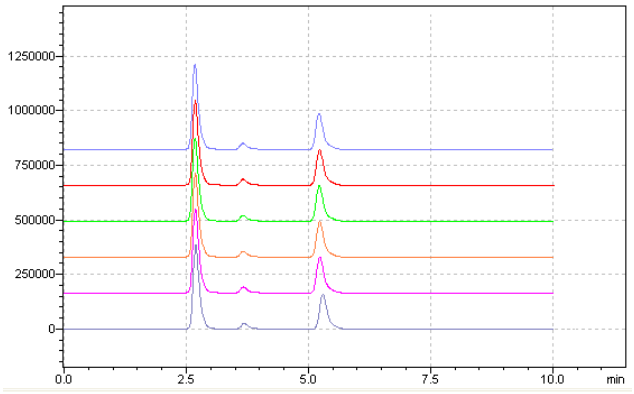


Calibration Curve of DM of RP-HPLC Method

Table for Linear Regression Analysis

Parameters	DM at 227 nm
Linearity Range (µg/ml)	0.2-10
Slope	33476
Intercept	1020.39
Correlation Coefficient (r^2)	0.999
LOD (µg/mL)	0.0893
LOQ (µg/mL)	0.2707

Repeatability- The system precision was checked by using standard RH-DM, and IS, to ensure that the analytical system is working properly. The area ratio of drug/IS of six consecutive injections is measured and % RSD was calculated.



Overlain Chromatograms for Repeatability

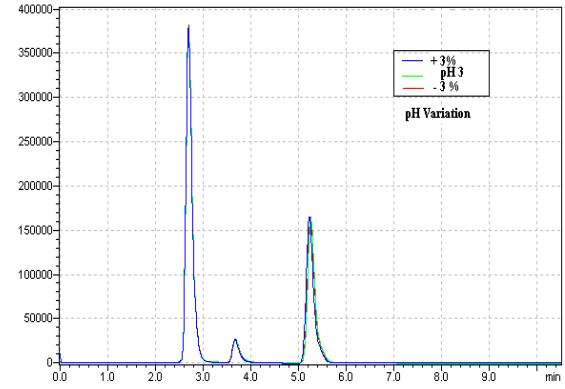
Limit of Detection (LOD) and Limit of Quantification (LOQ)- Detection limit and Quantitation limit were determined from the standard deviation of y-intercept of six calibration curves and average slope of six calibration curves.

LOD=3.3 X Standard deviation of the intercept/Mean of the slope.

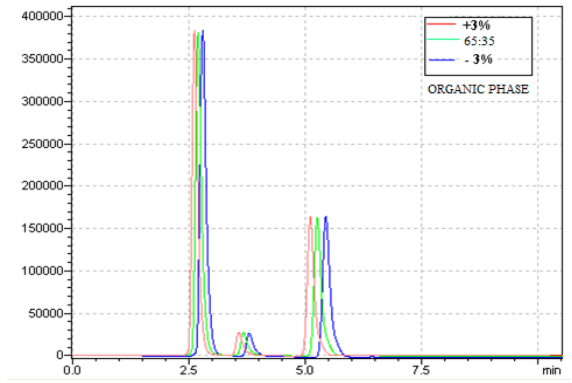
The LOQ=10 X Standard deviation of the intercept/Mean of the slope

Report: LOD and LOQ for RH found to be as 1.17 and 3.55µg/mL and for DM 0.089 and 0.270 µg/mL respectively.

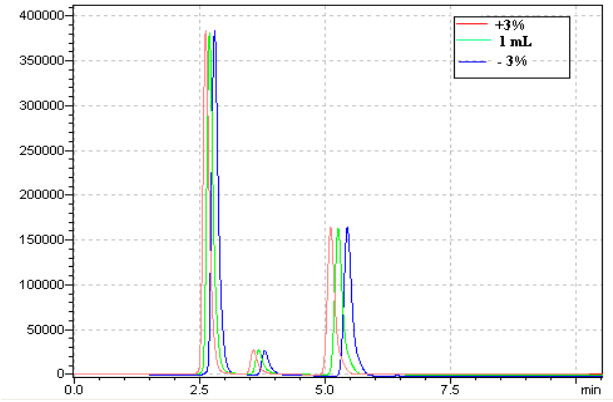
Robustness: The robustness of the method are determined by deliberate change in the physical parameters like change in pH of the mobile phase and flow rate. On changing the parameter's assay value is calculated and was compared with the standard to know whether the change in the parameters will show any deviations in the method.



Chromatogram for Robustness studies (pH variation) of RH-DM



Chromatogram for Robustness studies (Organic phase) of RH-DM



Chromatogram for Robustness studies (Flow rate) of RH-DM

System suitability parameters: System suitability was carried out using standard solution and injected six consecutive times. The following parameters like retention time, Ratio of AUC, Theoretical plates, tailing factor and resolution factor was determined and System suitability of the method showed good theoretical plates above 2000, tailing factor less than 2.

Summary of the Validation Parameters for Hplc

Parameters	RH	DM
Linearity (R^2)	0.999	0.999
Range (µg/mL)	3-150	0.2-10
Regression Equation (y = mx+c)	34379x+21308	38317x-1797
LOD (µg/mL)	1.17	0.089
LOQ (µg/mL)	3.55	0.270
% Assay(n=6)	100.33	99.53
Accuracy (n=3)	99.96	94.18
Intra Day Precision (%RSD)	0.610	0.921
Inter Day Precision (%RSD)	1.201	0.238
Repeatability (±SD)	0.006	0.009
Robustness (%RSD)	< 3 %	< 3 %

Discussion

Several attempts were made to select the optimum mobile phase in its pH and strength to get a better resolution peak of RH and DM. The mobile phase of methanol and PHP buffer 65:35, adjusted to pH 3 with 1% OPA, flow rate 1mL/min, 227nm as detection wavelength. The standard solutions of RH (100 µg/mL), DM (100 µg/mL) and MZ (20 µg/mL) in mobile phase were scanned in the UV region of 190 to 400 nm and the overlain spectra were recorded. It was observed that all the three drugs show the absorbance at 230, 285, 320 nm. So, the wavelength of detection used was 227 nm. RH and DM were found to be linear in the concentration range of 3-150 µg/mL and 0.2-10 µg/mL, respectively. Amount of RH and DM present in the marketed formulation were determined and the result complies with the IP specification. Amount of RH and DM were found in the range from 100.33%w/w and 99.53%w/w respectively. This method was validated in accordance to ICH guidelines. In Accuracy studies, Percentage recoveries of RH and DM were found to be 103.1%w/w and 102.4%w/w respectively. The precision of the method was determined by % RSD found among intra-day precision, inter-day precision, repeatability. It was found to be less than 3%. Reproducibility was determined by preparing and measuring the mixed standard solutions of RH (100 µg/mL), DM (6.6 µg/mL) and MZ (20 µg/mL) by Analyst 1 and Analyst 2, separately. There was no significant difference between the results of two analysts. LOD and LOQ of RH were found to be 1.17 and 3.55 µg/mL and for DM were found to be 0.089 and 0.27 µg/mL, respectively. In Robustness studies, the effects of change in the pH of mobile phase, flow rate and organic phase on the retention time were studied. Mixed standard solutions of RH (100 µg/mL), DM (6.6 µg/mL) with MZ (20 µg/mL) were developed and analysed at different pH (2.91, 3.00, 3.09) of the mobile phase, at different flow rates (0.97, 1.00, 1.03 ml/min) and organic phase (67:33 and 63:37). Percentage RSD of retention time of each peak in both variables was found to be less than 3 %. System suitability parameter like Retention time, Theoretical plate, Tailing factor, HETP were calculated and was found to be within the limits.

Conclusion

RP-HPLC method for simultaneous estimation of Ranitidine hydrochloride and Domperidone was developed with Metronidazole as an internal standard whereas Magaldrate was unable to determine by this method. All the three validated according to ICH guidelines. Results of the assay and validation studies were found to be satisfactory.

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