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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RANITIDINE HYDROCHLORIDE AND DOMPERIDONE IN COMBINATION

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ABSTRACT

Peptic ulcer has a major threat to the world's population over the past two decades with high morbidity and mortality. Development of many antacids has helped to treat the disease. A fixed dose of combination of Ranitidine Hydrochloride and Domperidone is given to treat the peptic ulcer. A simple, selective and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for estimation of Ranitidine Hydrochloride and Domperidone in synthetic mixture. Chromatographic separation was performed on a HPLC apparatus Cecil CE 4300 having pump CE4100 and reversed-phase Thermo hypersil C18 100Å column (250 mm ×4.6 mm × 5 µm) using an isocratic elution of mobile phase consisting of Aetonitrile: Orthophosphoric acid (pH 3.5 adjusted by 3% triethylamine) (50:50 v/v) at a flow rate of 0.5 ml/min. Wavelength for HPLC method of both the drugs was selected by performing UV. Common absorbance wavelength for Ranitidine Hydrochloride and Domperidone was 296nm. The RP-HPLC method give good resolution for both the drugs. The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and ruggedness. The system suitability parameter, such as theoretical plate, asymmetry, and resolution between standard five replicate were well within the limits.

Keywords: Domperidone, Ranitidine Hydrochloride, RP-HPLC method, Validation Parameters

INTRODUCTION

Ranitidine Hydrochloride is chemically an antacid and used in the treatment of peptic ulcer as H_2 receptor blocker **[1]**. It selectively binds to H_2 receptor and decreases the concentration of HCl in stomach. It is commonly available in tablet dosage form and administered through oral route.

Domperidone is chemicallyan antiemetic drug and dopamine D_2 receptor blocker. It is generally used in case of vomiting **[2]**. It is also used in case of peptic ulcer in combination with Ranitidine Hydrochloride.

Literature survey revealed that UV **[3]** and HPLC **[4]** methods are available for estimation of Ranitidine Hydrochloride. The available methods for estimation of Domperidone are UV **[5]** and HPLC **[6]**. The present work describes a simple, accurate and validated RP-HPLC method for simultaneous estimation of Ranitidine Hydrochloride and Domperidone in a combined dosage form which has not used alcohol in the mobile phase and has less retention time comparatively to other reported methods.

The present research work represents a simple, accurate and validated RP-HPLC method for simultaneous estimation of Ranitidine Hydrochloride and Domperidone in a combined tablet dosage for which is used in the treatment of Peptic Ulcer **[7]**.

MATERIALS AND METHODS

Chemicals and materials

Active Pharmaceutical Ingredients (API) of Ranitidine Hydrochloride and Domperidone were received from Yarrow Chem, Mumbai, methanol, acetonitrile, water, triethylamine and orthophosphoric acid were received from Molychem Limited, Mumbai.

Instrumentation

Chromatography was performed on CECIL CE 4300 chromatographic system equipped with pumped PDA detector. Samples were injected through HAMILTON, an injector valve with fixed loop of 20µl. Data acquisition and integration was performed using software POWER STREAM. Chromatographic conditions are described in the **Table 1**.

Table 1: Chromatographic Condition

Column	**Thermo hypersil C18 column (250mm x 4.6mm x 5µm)**
Detector	**296nm**
Injection volume	**20µl**
Flow rate	**0.5 ml/min**
Mobile phase	**Acetonitrile: Orthophosphoric acid (50: 50 v/v, pH adjusted by 3% triethylamine of AR grade)**

Preparation of standard solution

Accurately weighed 28.33 mg Ranitidine Hydrochloride and 25 mg of Domperidone were transferred to 25 ml volumetric flask. Mixture was diluted with sufficient solvent (Mobile phase, Acetonitrile: Orthophosphoric acid (50: 50 v/v, pH adjusted by 3% triethylamine of AR grade) and sonicated for 10 min then diluted up to the mark with diluent to give concentration of 500µg/ml Ranitidine and 500µg/ml of Domperidone.

Preparation of sample solution

20 tablets were weighed accurately and average weight was calculated. Tablets were triturated with the mortar and pestle. Powder equivalent to 28.33 mg of Ranitidine Hydrochloride and 1.43 mg of Domperidone was weighed. To make the ratio of Ranitidine: Domperidone to 1:1, 23.53 mg API of Domperidone was added by standard addition method and transferred to 25 ml of v olumetric flask, diluent was added and the mixture was sonicated for 30 minutes. Solution was filtered through 0.2µm membrane filter and diluted up to the mark with diluent. It gives the solution of Ranitidine Hydrochloride 500µg/ml and Domperidone 500µg/ml.

RESULTS AND DISCUSSION

Method validation

System suitability studies

The system suitability parameters [8] were evaluated by five replicates analyses of Ranitidine Hydrochloride and Domperidone both at 10µg/ml. The common efficiency, resolution and peak asymmetry were calculated for standard solution. The results of system suitability and system precision were presented in **Table 2**.

Linearity and Range

Linearity **[9]** and Range **[10]** of both drugs were found in the range of 10-50 µg/ml. The results are presented in **Table 3**.

Accuracy

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method **[11]**. Accuracy was determined at three different levels – at 80%, 100%, and 120% of the target concentration in triplicate. The results are presented in **Table 4**.

Precision

Intraday precision and interday precision

The precision of the developed method was assessed by analysing samples of the same batch with two combined solutions of Ranitidine and Domperidone in the concentration of 20, 30 and 40 µg/ml both the drugs in three replicates (n=3) each on same day **[12]**. The percentage of RSD value of the results corresponding to the peak area was expressed for intra-day precision. The precision of the developed method was assessed by analysing samples with three standard solutions of Ranitidine

and Domperidone similarly like above concentration respectively in three replicates (n=3) each on different day. The results are presented in **Table 5**. The results obtained were within 2% RSD.

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

The LOD & LOQ **[13]** were found to be 3.14 and 9.51 µg/ml for Ranitidine Hydrochloride, 9.51 and 11.37 µg/ml for Domperidone respectively.

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.

Robustness

As defined by The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters **[14]**. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by ±0.1 ml/min variation in flow rate of mobile phase, and ±0.1 variation in detection wavelength. These results are presented in **Table 6**.

Assay

Tablet assay results are shown in **Table 7**.

Retention time for Ranitidine Hydrochloride and Domperidone was 6.33 and 9.33 mins respectively.

Chromatogram for both the drugs is mentioned in **Figure 2**.

Table 2: System Suitability Parameters

Parameters	Observed values		IP Specification
Retention time (min)	6.33 ± 0.20 min	9.33 ± 0.30 min	-
%RSD	0.005	0.015	-
Theoretical Plates	9390	12381	Not less than
Asymmetry Factor	0.87	0.90	Not greater
Resolution	5.12	5.12	>2

Observed values for system suitability test *(n=5)

Table 3: Result of Linearity Study of Ranitidine and Domperidone

Concentration µg/ml	Peak area of Ranitidine Mean* ± SD (n=5)	Peak area of Domperidone Mean* ± SD (n=5)
10	0676828 ± 4136.18	0307113 ± 04920.50
20	1375258 ± 19353.48	0621995 ± 09195.82
30	2346876 ± 32977.26	1038542 ± 04557.64
40	3233856 ± 55385.49	1483522 ± 16852.94
50	3849613 ± 45677.23	1860021 ± 45677.23

***Average of five determinations**

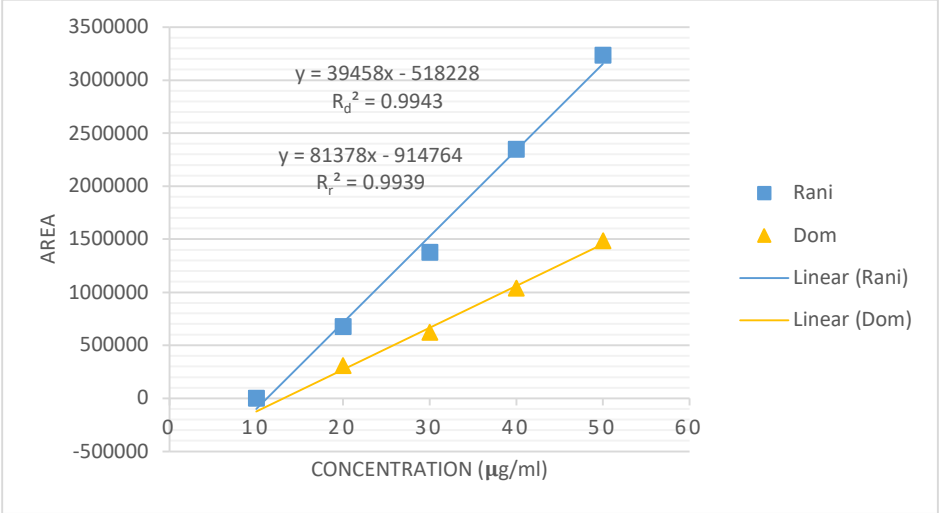

Figure 1: Calibration curve of Ranitidine Hydrochloride and Domperidone

Table 4: Recovery/Accuracy Data

Drug	Accuracy level	Amount taken (µg)	Amount found (µg)	Total amount taken (µg)	Total amount found (µg)	%Recovery
Ranitidine	Pre analysed	20	00	20	19.23	
	80%	20	16	36	36.08	98.8
	100%	20	20	40	40.49	101.1
	120%	20	24	44	44.37	100.4
Domperidone	Pre analysed	20	00	20	19.66	
	80%	20	16	36	35.86	101.2
	100%	20	20	40	39.51	99.2
	120%	20	24	44	44.14	102

Table 5: Precision

	Intraday Precision			Interday Precision		
Drug	Conc (µg/ml)	Peak Area Mean* ± SD	%RSD	Conc (µg/ml)	Peak Area Mean* ± SD	%RSD
RANI	20	1376203.23 ± 01838.23	0.13	20	6365585.40 ± 25061.23	0.39
	30	2330962.60 ± 20132.09	0.86	30	0999906.73 ± 2026.425	0.20
	40	3163594.90 ± 01863.98	0.58	40	1458241.18 ± 13672.96	0.93
DOM	20	1278092.56 ± 24701.76	1.93	20	0615325.46 ± 05950.59	0.96
	30	2263573.63 ± 40687.30	1.79	30	1037192.45 ± 09371.14	0.90
	40	3055418.73 ± 36631.61	1.19	40	1374973.43 ± 23259.64	1.69

Table 6: Robustness studies								
Conditions	**% RSD**		**% Assay Mean***		**% Difference in % assay**		**Retention Time in min**	
	RANI	**DOM**	**RANI**	**DOM**	**RANI**	**DOM**	**RANI**	**DOM**
Change in mobile phase composition (± 2ml in organic phase)								
Normal condition (50: 50)	**0.85**	**0.66**	**99.2**	**99.1**	**-**	**-**	**6.33**	**9.33**
Change in organic phase (+2ml)	**1.42**	**0.70**	**98.9**	**100.3**	**0.3**	**1.2**	**6.27**	**9.36**
Change in organic phase (-2ml)	**1.17**	**1.40**	**99.3**	**100.8**	**0.1**	**1.7**	**6.30**	**9.29**
Change in the detection wavelength (± 2mm)								
Normal condition 296nm	**0.85**	**0.66**	**99.2**	**99.1**	**-**	**-**	**6.33**	**9.33**
Change in wave length (+10mm)	**1.20**	**0.98**	**98.5**	**99.6**	**0.7**	**0.5**	**6.29**	**9.38**
Change in wave length (-10mm)	**1.08**	**1.02**	**99.5**	**99.6**	**0.3**	**0.5**	**6.25**	**9.36**
Change in flow rate (± 0.2 ml/min)								
Normal condition 0.5 ml/min	**0.85**	**0.66**	**99.2**	**99.1**	**-**	**-**	**6.33**	**9.33**
Change in flow rate (+2ml/min)	**1.38**	**0.96**	**98.5**	**99.05**	**0.7**	**0.05**	**6.37**	**9.30**

***Average of three determinations**

Table 7: Assay results						
Sr no	**Amt of RANI in Sample (µg)**	**Amt of DOM in sample (µg)**	**Amt of RANI found (µg)**	**Amt of DOM found (µg)**	**%Mean Recovery (n=3) RANI**	**%Mean Recovery (n=3) DOM**
1	**20**	**20**	**19.89**	**19.97**	**099.4 ± 24701.76**	**099.8 ± 5950.59**
2	**20**	**20**	**19.92**	**20.04**	**099.6 ± 24678.89**	**100.2 ± 5976.40**
3	**20**	**20**	**19.83**	**20.12**	**099.1 ± 24699.93**	**100.6 ± 5962.57**

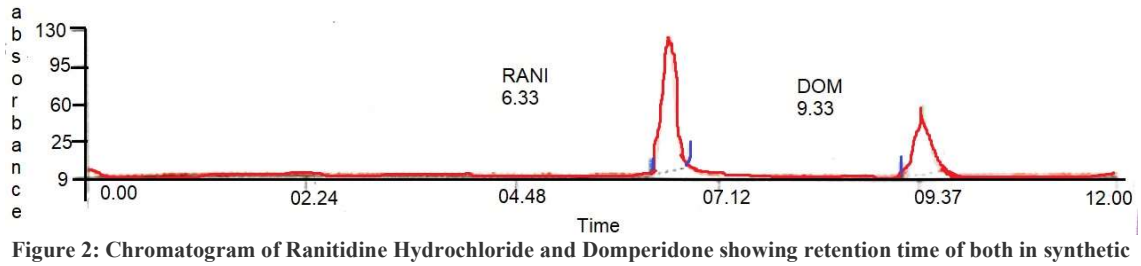

Figure 2: Chromatogram of Ranitidine Hydrochloride and Domperidone showing retention time of both in synthetic mixture

CONCLUSION

A rational and valid attempt has been made for the development of Ranitidine Hydrochloride and Domperidone in synthetic mixture. The accountability of the proposed method has been established by evaluating validation parameters as per ICH guidelines. The developed RP-HPLC methods are simple, economical, precise, and accurate for the simultaneous determination of Ranitidine Hydrochloride and Domperidone in synthetic mixture.

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