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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RANITIDINE HYDROCHLORIDE AND DOMPERIDONE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

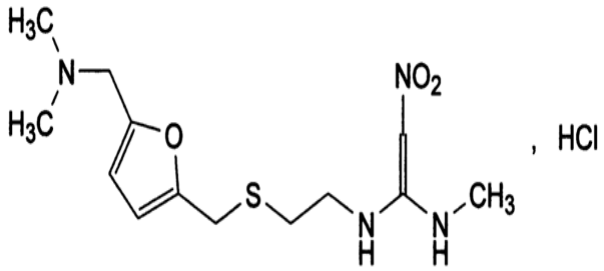
A simple reverse phase HPLC method was developed for the simultaneous estimation of Ranitidine hydrochloride and Domperidone present in combined tablet dosage forms. Efficient chromatographic separation was achieved on Phenomenax C_{18} stationary phase (250 X 4.6 mm i. d., 5µ particle size) with simple mobile phase combination of phosphate buffer: acetonitrile: methanol 40: 30: 30 (V/V/V) in an isocratic mode at a flow rate of 1.5 $mLmin^{-1}$ at 210 nm. The retention times were 2.417 and 7.375 (±0.5) min for Ranitidine hydrochloride and Domperidone respectively. The proposed method has been applied successfully for the simultaneous analysis of Ranitidine hydrochloride and Domperidone in combined tablet dosage form with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of Ranitidine hydrochloride and Domperidone in pharmaceutical dosage form.

INTRODUCTION: It is necessary to find the content of each drug either in pure or single, combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment. The scope of developing and validating analytical methods is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.

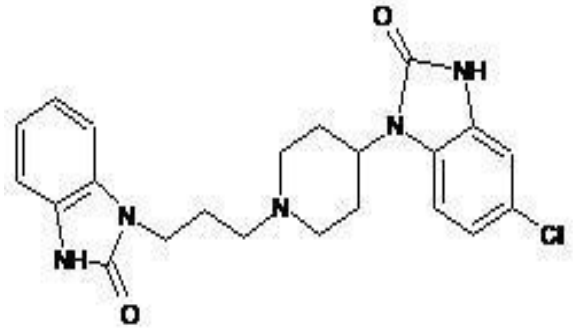
Ranitidine hydrochloride (fig. 1) N- [2- [[[5- [(Dimethylamino) methyl] furan- 2- yl] sulphanyl] ethyl] – N'- methyl - 2- nitroethene - 1, 1- diamine hydrochloride (MW=350.87), is used as H_2-receptor antagonist and is also used in management of ulceration. It is official in I. P. [1] and U. S. P. [2].

Domperidone (Fig. 1), 5- chloro- 1- [1- [3- (2- oxo- 2, 3- dihydro- 1H-benzimidazol- 1- yl) propyl]- piperidin- 4- yl]- 1, 3- dihydro- 2H benzimidazol- 2- one (MW=425.9) acts by selectively antagonizing the peripheral dopaminergic D2 receptors in the gastrointestinal wall, thereby enhancing gastrointestinal peristalsis and motility and increasing lower esophageal sphincter tone.

This increased gastrointestinal motility can facilitates the movement of acid contents further down in the intestine preventing reflux esophagitis and thereby controlling nausea and vomiting [4]. It is official in B. P. [3]. A survey of literature reveals that HPLC method is not available for simultaneous estimation of the drugs like Ranitidine hydrochloride and Domperidone in combined tablet dosage form. Even though very few methods such as UV [5-12], HPLC [13-17] and HPTLC [18] are available for estimation of above drugs either in single or combination with other drugs, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selectivity and simplicity etc.



RANITIDINE HYDROCHLORIDE



DOMPERIDONE

FIG. 1: CHEMICAL STRUCTURE OF ANALYTE

EXPERIMENTAL:

Chemicals and Reagents: Working standards of Ranitidine hydrochloride (98 %) and Domperidone (98 %) were obtained by Wockhardt Research Centre (Mumbai, India) and Sigma Aldrich respectively. Tablets containing Ranitidine-150 mg and Domperidone-10 mg were purchased from local market. Acetonitrile and methanol were of HPLC grade and dipotassium hydrogen phosphate and Potassium dihydrogen ortho- phosphate was of

analytical reagent grade supplied by M/S Qualigens fine chemical. Sodium hydroxide was of analytical reagent grade from Ranbaxy laboratories Ltd... HPLC grade water was obtained from Milli-Q (in house) Millipore (India) Pvt. Ltd. and was used to prepare all solutions.

HPLC Instrumentation and Conditions: Chromatographic measurements were performed on an isocratic HPLC of Shimadzu (Tokyo, Japan) prominence 20A series Consisting of DGU-20A_3 degasser, CBM-20A communications bus module, LC – 20AT liquid pump, 7725i Rheodyne manual injector with 20µl sample injection loop, SPD-20M photo diode array detector and SPD 20A UV-visible absorbance detector. The output signal was monitored and integrated by Shimadzu CLASS-VP software. The HPLC system was used in an air-conditioned laboratory atmosphere (22±2 ◦ C).

Preparation of standard solutions: Standard solutions of Domperidone and Ranitidine hydrochloride were prepared in mobile phase. Accurately weighed and transferred 10 mg Domperidone reference standard to 10 ml volumetric flask (A) the volume was made up to the mark with mobile phase. Take 1 ml from (A) into a 10 ml volumetric flask (B) and added accurately weighed 16.8 mg Ranitidine hydrochloride reference standard, the volume was made up to the mark with the mobile phase. Appropriate volume from this solution was further diluted to get desired concentration levels according to the requirement. Final solution filtered through a 0.2 µm membrane filter and 20 µl of this solution was injected for HPLC analysis.

Preparation of Sample Solutions: Ten tablets were weighed and finely powdered, tablet powder equivalent to 10 mg of Domperidone and 168 mg of Ranitidine hydrochloride accurately weighed and transferred to 100 ml volumetric flask. This mixture was subjected in magnetic stirrer for 60 minutes for complete extraction of drugs using magnetic beads and the solution was made up to the mark with mobile phase.

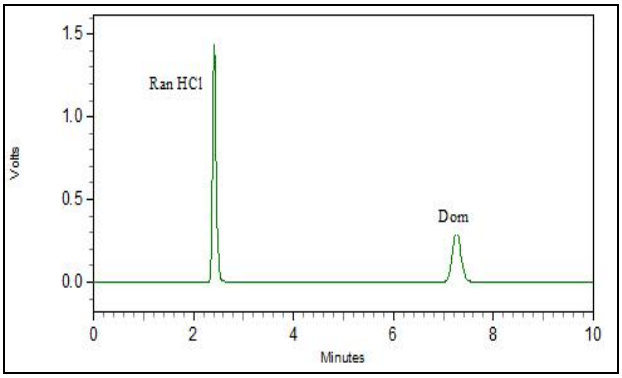
Appropriate volume from this solution was further diluted to get desired concentration level according to the requirement. Final solution filtered through a 0.2 µm membrane filter and 20 µl of this solution was injected for HPLC analysis.

Assay Method: Optimized chromatographic condition, a steady baseline was recorded, the standard solution was injected and the chromatogram was recorded. The retention time of Ranitidine hydrochloride and Domperidone was found to be 2.4 and 7.3 minute respectively. This procedure was repeated for the sample solution. The results obtained from standard and sample is shown in table 1.

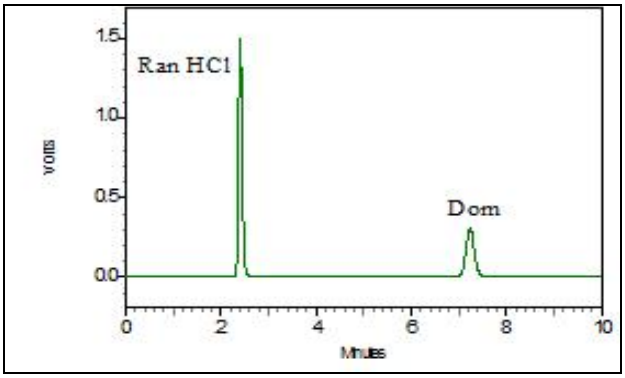
Concentrations of Ranitidine hydrochloride and Domperidone in the formulation were calculated by comparing AUC of the sample with AUC of the standard. Chromatograms of assay are shown in fig. 2. System suitability parameters are shown in table 2.

TABLE 1: ANALYSIS OF FORMULATION

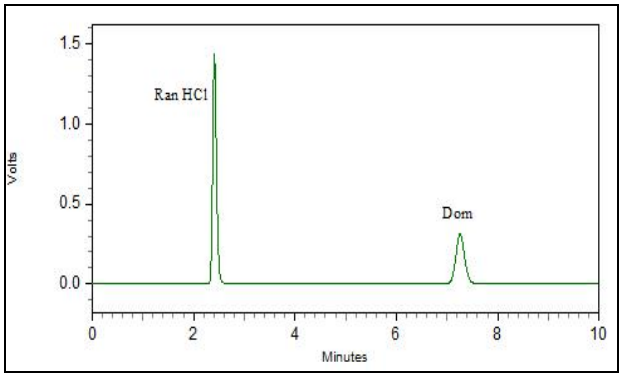
DESCRIPTION	STANDARD		SAMPLE 1st		SAMPLE 2nd	
	Ran HCl	Dom	Ran HCl	Dom	Ran HCl	Dom
Initial amount (mg)	168	10	168	10	168	10
Run 1 (area)	8024502	3913662	8100661	3851832	8176658	3977638
Run 2 (area)	8035554	3900504	8160538	3832100	8190954	3980502
Mean area	8030028	3900504	8130600	3841966	8183806	3979070
Labelled amount recovered (mg)			166.702	9.6529	167.7929	9.9974
% labelled claim			99.23	96.53	99.88	99.97



Chromatogram of Standard Runs 1

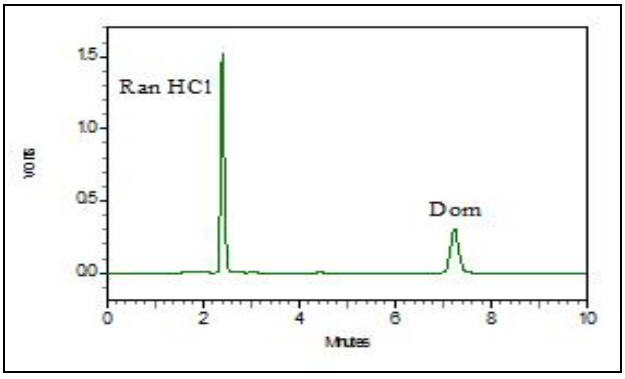


Test Sample Runs 1



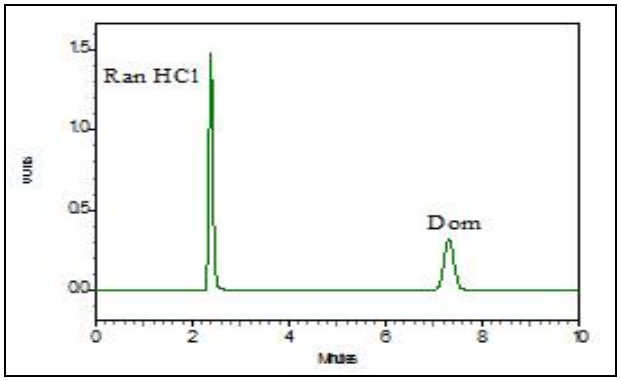
Chromatogram of standard runs 2

Chromatogram of Standard

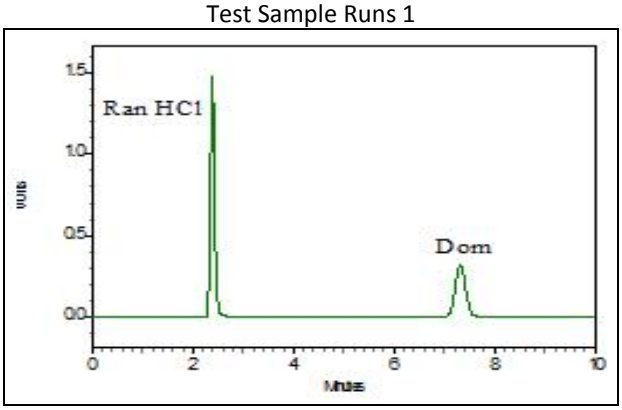


Test Sample Runs **2**

Chromatograms of Ranitalia RD Tablet Sample



Test Sample Runs 1



Test Sample Runs 2

Chromatograms of Rantac-D Tablet Sample

FIG. 2 CHROMATOGRAMS OF ASSAY

TABLE 2: SYSTEM SUITABILITY PARAMETERS

PARAMETERS	STANDARD RUNS 1		STANDARD RUN 2	
	Ran HCl	Dom	Ran HCl	Dom
Theoretical plates	2553	9474	2570	9498
Resolution	16.53		16.8	
Tailing factor (U.S.P)	1.1538	1.071	1.1012	1.092

Validation of Proposed Method: Validation (evaluation of suitability) of an analytical technique is a procedure aimed at obtaining experimentally justified evidence of the ability of this technique to give results characterized by the required accuracy and precision. Typical analytical parameters used in assay validation include: -

Precision: The precision (repeatability, reproducibility) of an analytical procedure is evaluated in terms of the standard deviation (SD) and relative standard deviation (% RSD) determined in a series of measurements. The precision was studied by preparing the standard solution of 25 ppm and 420 ppm of Domperidone and ranitidine hydrochloride respectively and injected repeatedly for six times. The obtained results are shown in table 3.

TABLE 3: PRECISION STUDIES

SR. NO.	AREA	
	RANITIDINE HCL	DOMPERIDONE
1	8173693	3947902
2	8154886	3949403
3	8121214	3958580
4	8088886	3947902
5	8068677	3934338
6	8106854	3968414
Mean	8119035	3951089.833
Standard deviation	39675.7	11489.33
% RSD	0.488675	0.290789

Accuracy: The % recovery has been studied and established within the range of 80 % to 120 %. The spiking study for each concentration along with amount recovered and calculation of % recovery is shown in table 4.

TABLE 4: RECOVERY STUDY

DESCRIPTION	CONCENTRATION (%)					
	80		100		120	
	Ran HCl	Dom	Ran HCl	Dom	Ran HCl	Dom
Weight of sample taken	13.44 mg	8 mg	16.8 mg	10 mg	20.16 mg	12 mg
Run 1 (area)	6414706	3193768	8049991	3938665	9647632	4714753
Run 2 (area)	6487340	3224454	8053693	3910864	9605488	4714533
Run 3 (area)	6484704	3243998	7988194	3934534	9636529	4763579
Mean area	6462250	3220740	8030626	3928021	9629883	4730955
Std. Deviation	41195.4	25320.12	36793.78	15001.28	21843.9	28253.43
% RSD	0.647	0.786159	0.458168	0.381904	0.226835	0.5972035
Amount recovered	13.259	8.06	16.477	9.84	19.757	11.85
% Recovery	98.65	100.75	98.07	98.4	98	98.75

% Recovery= amount recovered/ amount introduced X 100

The response obtained for the various concentrations is plotted and observed to be linear (correlation coefficient – 0.9999 for ranitidine hydrochloride and 0.9986 for Domperidone). The graphical representation of accuracy studies is depicted in figure 3 and accuracy data is shown in table 5.

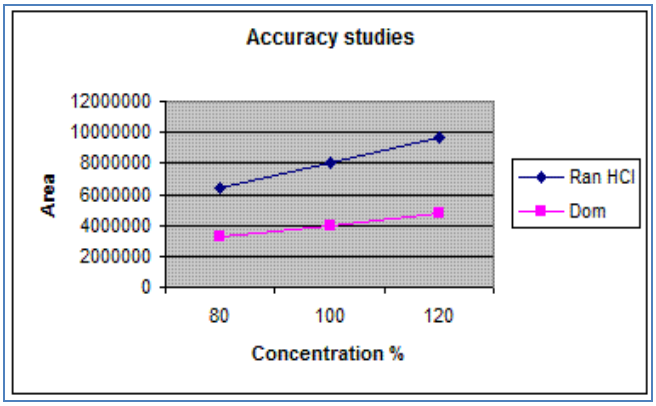


FIG. 3: LINEARITY PLOT FOR ACCURACY STUDY

TABLE 5: ACCURACY STUDY

CONCENTRATION (%)	AREA (Mean)	
	Ran HCl	Dom
80	6462250	3220740
100	8030626	3928021
120	9629883	4730955
Correlation Coefficient	0.9999	0.9986

Linearity: The linearity of the method was studied at six concentrations (%) of 150, 125, 100, 75, 50, and 25. The obtained response was linear which apparently indicates the capability of method to reproduce/repeat the results within the linear range, consistently meeting the standard norms for method validation (ICH

guidelines on validation analytical methods, Q2B). The results obtained are shown in table 6. The response obtained for each of the concentration is plotted by taking % concentration on *x*-axis and area on *y*-axis. The graphical representation for linearity studies is depicted in figure 4.

TABLE 6: LINEARITY DATA

Concentration (%)	Area (mean)	
	Ran HCl	Dom
25	1950249	1000736
50	3997528	2006946
75	5906906	2912497
100	8023651	3933307
125	9764192	5134203
150	11815201	5970638
Correlation coefficient	0.999	0.998
Slope	785612	40288
y-intercept	35471	32155

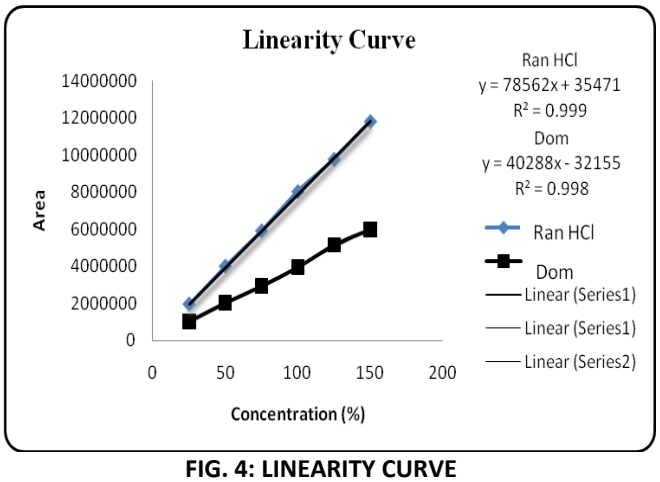
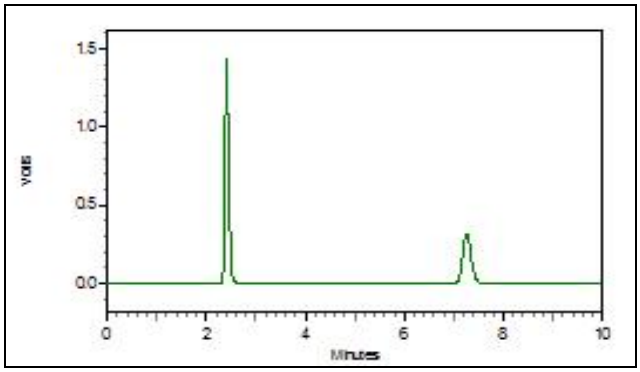
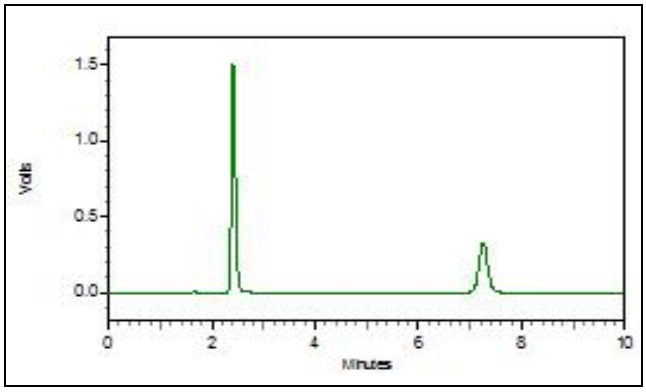


FIG. 4: LINEARITY CURVE

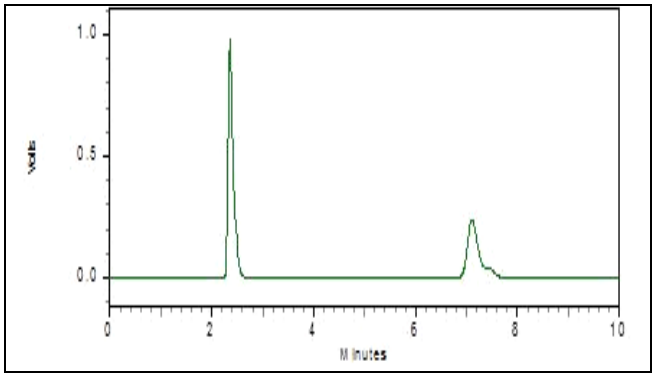
Drug stability studies: The stability of analytes solution was determined at regular interval of 1st, 3rd, 7th day. Chromatograms of drug stability studies are shown in fig. 5. The results of drug stability studies are shown in table 7.



First Day



Third Day



Seventh Day

FIG. 5: CHROMATOGRAMS OF STABILITY

TABLE 7: STABILITY STUDY

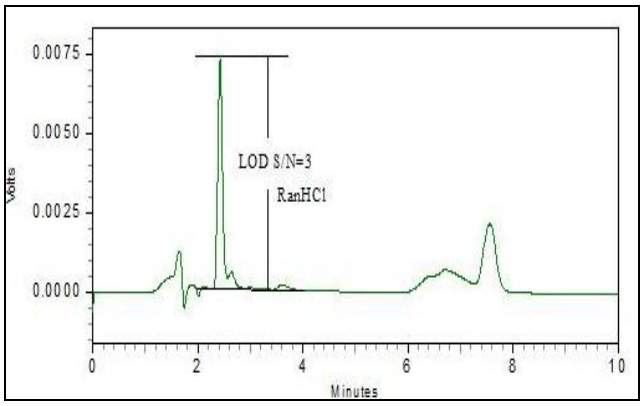
DESCRIPTION	FIRST DAY		THIRD DAY		SEVENTH DAY	
	Ran HCl (area)	Dom (area)	Ran HCl (area)	Dom (area)	Ran HCl (area)	Dom (area)
Run 1	8049991	3938665	7902193	3951199	7768677	3828580
Run 2	8053693	3910864	7944816	3868522	7760854	3879403
Run 3	7988194	3934534	7981524	3871330	7795042	3831292
Mean area	8030626	3928021	7942844	3897017	7774858	3846425
Std. Deviation	36793.78	15001.28	39702.24	46943.99	17912.43	28591.96
%RSD	0.458168	0.381904	0.499849	1.204613	0.230389	0.743339

Observing the stability studies, we were concluded that drugs should be stable for three day and on seventh day both drugs show change on peak shape and some in peak area.

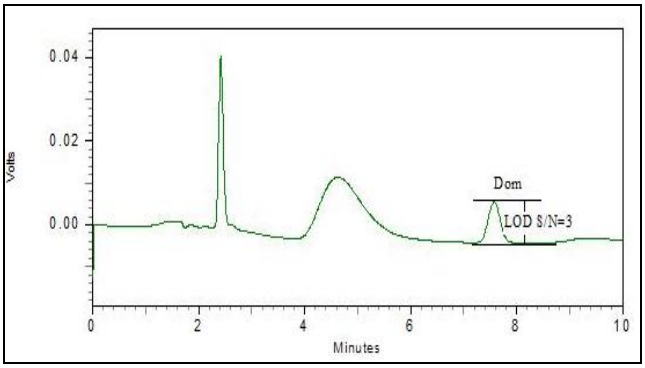
LOD (Limit of Detection): Adjust the instrument sensitivity; record the instrument noise level in the elution time range of drugs. When the instrument is in the most sensitive condition, calculate the average noise. (Test for twice to get mean value). Accurately weighed and transferred 10 mg Domperidone standard into a 10 ml volumetric flask (A) the volume was made up to the mark with mobile phase. Take 1 ml from (A) into a 10 ml volumetric flask (B) and added ranitidine hydrochloride standard 16.8 mg the volume was made up to the mark with the mobile phase. Appropriate volume from this solution was further diluted to get appropriate concentration level according to the requirement. Inject 20 µl and record the peak height of drugs, calculate the ratio of peak height to noise (S/N) the sample concentration with signal to noise ratio (S/N) near is the LOD concentration. LOD result is shown in table 8 and LOD chromatograms are shown in fig. 6.

TABLE 8: LOD RESULT

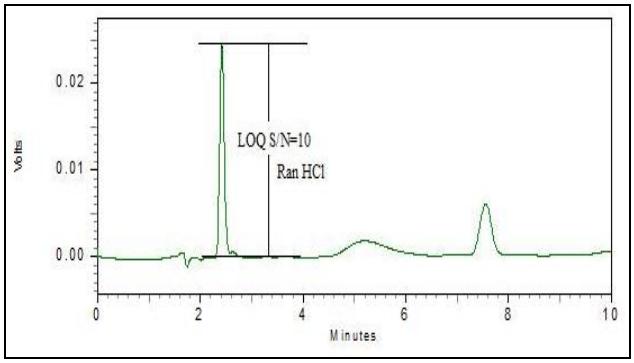
DESCRIPTION	CONC. (ppm)	PEAK HEIGHT (volts)	AVERAGE NOISE (volts)	S/N
Ranitidine HCl	0.8203125	0.00792	0.0026275	3
Domperidone	0.78125	0.00788	0.0026275	3



LOD Chromatogram for Ran HCl



LOD Chromatogram for Dom



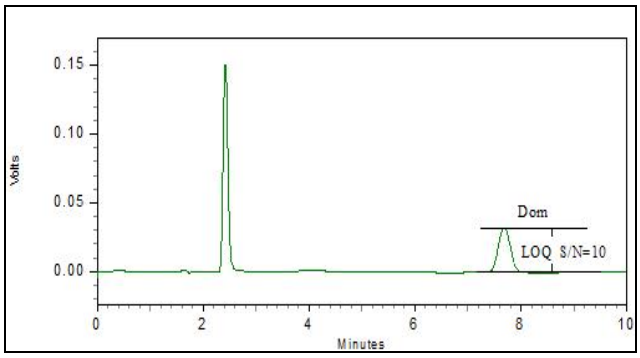
LOQ Chromatogram for Ran HCl

FIG. 6: CHROMATOGRAMS OF LOD

LOQ (Limit of Quantification): Accurately weighed and transferred 10 mg Domperidone standard into a 10 ml volumetric flask (A) the volume was made up to the mark with mobile phase. Take 1 ml from (A) into a 10ml volumetric flask (B) and added ranitidine hydrochloride standard 16.8 mg the volume was made up to the mark with the mobile phase. Appropriate volume from this solution was further diluted to get appropriate concentration level according to the requirement. Inject 20 µl and record the peak height of drugs and calculate the ratio of peak height to noise (S/N) the sample concentration with signal to noise ratio (S/N) near 10 is the LOQ concentration. LOQ result is shown in table 9 and LOQ chromatograms are shown in fig. 7.

TABLE 9: LOQ RESULT

DESCRIPTION	CONC. (ppm)	PEAK HEIGHT (volts)	AVERAGE NOISE (volts)	S/N
Ranitidine HCl	6.5625	0.02634	0.0026275	10
Domperidone	3.125	0.0310	0.0026275	10



LOQ Chromatogram for Dom

FIG. 7: CHROMATOGRAMS OF LOQ

RESULTS AND DISCUSSION: Summary of method validation for assay of Ranitidine HCl and Domperidone is shown in table 10.

CONCLUSION: The developed RP-HPLC method enables a quantitative determination of Ranitidine hydrochloride and Domperidone in formulations. The application of this method in routine analysis can be justified since easy sample preparation steps are involved using simple reagents and solvents. The validation demonstrated that the procedure is suitable for the intended purpose because the method was considered linear, precise, accurate, and specific. The statistical analysis proves that the method is reproducible and selective for the estimation of Ranitidine hydrochloride and Domperidone in pharmaceutical formulations.

TABLE 10: SUMMARY OF METHOD VALIDATION

VALIDATION PARAMETER	PROCEDURE	RESULTS
Precision	Six replicate injection of Assay Concentration	% RSD = 0.488675 (Ran HCL) %RSD = 0.290789 (Dom)
Accuracy	80%, 100% and 120% of Assay Concentration	Accuracy studies under limit and lies between
Stability of analytical solution	Perform the study at 1^{st}, 3^{rd}, 7^{th} day	Both drugs show degradation after 3^{rd} day
Linearity	Linearity studies were performed by introducing 150, 125, 100, 75, 50 and 25% of Assay Concentration	R^2 = 0.999 (Ran HCl) R^2 = 0.998 (Dom)
LOD	S/N ratio =3	0.8203125 ppm for Ranitidine HCl 0.78125 ppm for Domperidone
LOQ	S/N ratio =10	6.5625 ppm for Ranitidine HCl 3.125ppm for Domperidone

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REFERENCES:

1. Indian Pharmacopoeia, 2006.
2. United State Pharmacopoeia 30- NF 25, 2007, p- 3102.
3. British Pharmacopoeia, vol.1 & 2, The British Pharmacopoeia Commission, London, 2009, p- 5068-5073, 2091-2095.
4. Triphati, K.D. Essential of Medical Pharmacology, Jaypee Brother Medical Publisher (P) LTD. *New Delhi reprint*. 2004, p- 679-697.
5. M. S. Charde, S.G. Walode, M.R. Tajne and A.V. Kasture (2006) UV-Spectrophotometric Estimation of Ranitidine and Domperidone in Tablet formulation. *Indian J. Pharm. Sci., 2006, 68 (5): 658-659.*
6. P. Ravi Kumar, P. Bhanu Prakash, M. Murali Krishna, M. Santha Yadav (2006) Simultaneous Estimation of Domperidone and Pantoprazole in Solid Dosage Form by UV Spectrophotometry. *E-Journal of Chemistry, Vol. 3, No.12, pp 142-145, July 2006*.
7. R. Sahu, Preeti Nagar, S. Bhattacharya and Deepti Jain (2006) Simultaneous Spectrophotometric Estimation of Famotidine and Domperidone in combined tablet dosage form. *Indian J. Pharm. Sci., 2006, 68 (4): 503-506.*
8. S. Pillai and I. Singhvi (2007) Spectrophotometric simultaneous estimation of Ranitidine hydrochloride and Ondansetron hydrochloride from tablet formulation. *Indian J. Pharm. Sci., 2007, 69 (4): 601-604.*
9. Tasnuva Haque, Md Mesbah Uddin Talukder, Susmita Laila, Kanij Fatema, Abdul Kalam Lutful Kabir. Simultaneous Estimation of Naproxen and Ranitidine HCl by Using UV Spectrophotometer. *S. J. Pharm. Sci., 2008,* 1(1&2): 18-24.
10. S. Lakshmana, A. Shirwaikar, Annie Shirwaikar, C. Dinesh Kumar. A. Joseph and R. Kumar (2008) Simultaneous Estimation of Esomeprazole and Domperidone by UV-Spectrophotometric method. *Indian J. Pharm. Sci., 2008, 70 (1): 128-131.*
11. Y. Rajendra Prasad, K. K. Rajasekhar, V.Shankarananth, H.V.Yaminikrishna, S.Saikumar, P. Venkata Raghav Reddy (2009) Spectrophotometric method for the estimation of domperidone in bulk and pharmaceutical formulations. *Journal of Pharmacy Research 2009, 2(10), 1593-1594.*
12. Kalra Kapil, Naik S, Jarmal Garima and Mishra N (2009) Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Domperidone in Tablet Formulation. *Asian J. Research Chem. 2(2): April.-June, 2009.*
13. Bhavna Patel, Zarna Dedania, Ronak Dedania, Chetan Ramolia, G Vidya Sagar (2009) Simultaneous Estimation of Lansoprazole and Domperidone in Combined Dosage form by RP-HPLC*. Asian J. Research Chem. 2 (2) April-June, 2009.*

14. Sivakumar Thanikachalam, Manavalan Rajappan, Valliappan Kannappan (2008) Stability-Indicating HPLC Method for Simultaneous Determination of Pantoprazole and Domperidone from their Combination Drug Product. *Chromatographia, 2008, 67, January (1, 2).*
15. R. Kalirajan, K. Anandarajagopal, Seena Mary Mathew, B. Gowramma, S. Jubie and B. Suresh (2008) Simultaneous determination of Rabeprazole and Domperidone in dosage forms by RP-HPLC. *Rasayan J. Chem, Vol.1, No.2, 2008, 232-235.*
16. Laksmi Sivasubramanian and V. Anil Kumar (2007) Simultaneous HPLC estimation of Omeprazole and Domperidone from tablets. *Indian J. Pharm. Sci., 2007, 69 (5): 674-676.*
17. A. Karthik, G. Subramanian, A. Ranjith Kumar and N. Udupa (2007) Simultaneous estimation of Paracetamol and Domperidone in tablets by reverse phase HPLC method. . *Indian J. Pharm. Sci., 2007, 69 (1): 142-144.*
18. J. V. Susheel, M. Lekha and T.K. Ravi (2007) High performance thin layer chromatographic estimation of Lansoprazole and Domperidone in tablet. *Indian J. Pharm. Sci., 2007, 69 (5): 684-686.*