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RP-HPLC Method Devlopment and Validation of Domperidone in Solid Dosage Form

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A simple, accurate, reliable and reproducible HPLC method was developed for the determination of Domperidone (DOM) in solid dosage forms. The method employed C_{18} column, water: methanol (55:45) as mobile phase and detection was made at 291nm. The retention times was found to be 4.5 min for DOM. The method was validated as per ICH guidelines. The method shows good linearity, accuracy, and precision, limit of detection and limit of quantification. The method was suitable for routine analysis of DOM individually and in combined dosage forms.

Keyword: HPLC, Domperidone

INTRODUCTION: 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 [1]. It is a official compound of B. P.[2]. A survey of literature reveals that HPLC method is not available for estimation of the drug Domperidome in tablet dosage form.[3-12]

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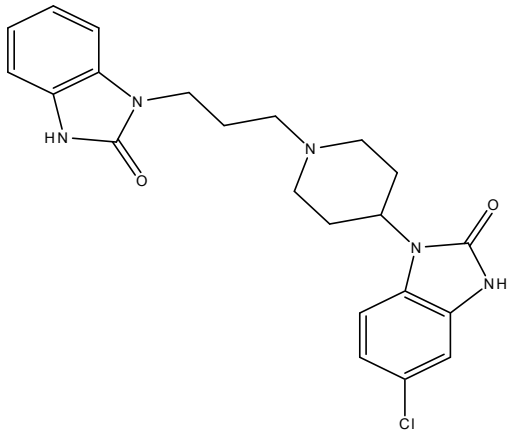
DOMPERIDONE FIG. 1: CHEMICAL STRUCTURE



2. Experimental Work

2.1. Chemicals, Reagents and Apparatus

Domperidone was procured from Cadila pharmaceuticals Ltd., Gujarat, India. HPLC grade

methanol and water were purchased from RANCHEM, RFCL Ltd., New Delhi, India. Water from Milli Q (Millipore Bedford, MA) was employed in all experiments. The HPLC instrumentation comprises of A Agilent Technologies's HPLC (1220 infinity LC, Agilent Technologies, U.S.A) equipped with UV-Visible Detector, Qualisil, C18, BDS column (250 mm X 4.6 mm; 5µ.), Hamilton 20 µL syringe, A Citizen analytical balance (Sartorius), An ultra-sonic sonicator (Equitron), Borosil volumetric flask of 10, 25, 50,100 ml capacity, pipettes – 1ml, 5ml, 10ml, beakers, measuring cylinders etc.

2.2. Preparation of the mobile phase

Water and methano (55:45) were mixed to achieve a mobile phase of a mixture. The mobile phase was filtered through a nylon 0.45 µm membrane filter and was degassed for 3 min before use.

2.3. Chromatographic conditions

The analytical wavelength was set at 291 nm and 20 µL of samples were manually injected with Hamilton syringe. The chromatographic separations were accomplished using mobile phase, consisting of buffer (water: methanol (55:45). Mobile phase was pumped in isocratic system at a flow rate of 1.0 mL/min.

2.4. Preparation of standard stock solutionsA.

a. Standard Domperidone Stock Solution (100 µg/mL)

Standard DOM powder (5 mg) was weighed accurately and transferred in to 50 mL volumetric flask and dissolved in and diluted to 50 mL with methanol to prepare working standard solution having concentration of 100 µg/mL. Standard HPLC gradewater -water:methanol (55:45)100µg/mL

2.5. Sample Solution

Contents of 20 tablate having Domperidone (DOM) were weighed accurately. A quantity of the powder equivalent to about 20 mg of pure drug of DOM was taken in to 100 mL volumetric flask, completely dissolved and filtered through whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings and diluted to the mark with methanol. One mL of extract was transferred into 10 mL volumetric flask and diluted to the mark with methanol to get an approximate concentration of 20 µg/mL of DOM.

3. Method validation

3.1. Solution stability

Sample solutions were kept at 25°C and 2-10°C for 24 h and 3 days, respectively. Assay of initial time period was compared with these two time points. The falls in the assay values were evaluated. The difference between assays should not be more than 2 % for formulation, and 0.5% for API.

3.2. Linearity (Calibration Curve):

A calibration curve was plotted over a concentration range of 2 - 20 µg/mL for DOM. Accurately measured standard stock solution of DOM (0.2,0.4, 0.6, 0.8,1.0, 1.2, 1.4, 1.6,1.8 &2.0 mL) were transferred to a separate series of 10 mL of volumetric flasks and diluted to the mark with methanol. Twenty microlitre of each solution in the concentration was injected under operating chromatographic conditions described above. Calibration curves was constructed for DOM by plotting area versus concentrations. Each reading was average of five determinations.

3.3 Precision

A. Repeatability (Precision on replication)

Method precision of experiment was performed by preparing the standard solution of DOM (5 µg/mL) for six times and analyzed as per the proposed method.

B. Intermediate precision (Reproducibility)

The Intra-day precision (C.V) was determined for standard solution of DOM (2 - 21 µg/mL) for five times on the same day. The Inter-day precision (C.V) was determined for standard solution of DOM (2-21 µg/mL) for five days.

3.4. Accuracy (% Recovery)

The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablet (DOM 3 µg/mL) with three different concentrations of standards DOM 1,2,3 µg/mL).

Table-1

Formulation	Amount labeled (mg)	Amount found (mg) %Amount FoundS.D. (n=3)	Amount labeled (mg)
Dom(PURE)	20	101.86 ± 1.09	20.22
Formulation (MARKETED)	20	101.45 ± 1.60	20.0

3.5. Limit of Detection

Limit of detection was calculated using following equation as per ICH guidelines. LOD = 3.3 × N/S Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve. It is expressed as signal to noise ratio of 2:1.

3.6. Limit of Quantification

Limit of quantification was calculated using following equation as per ICH guidelines. LOQ = 10 × N/S Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

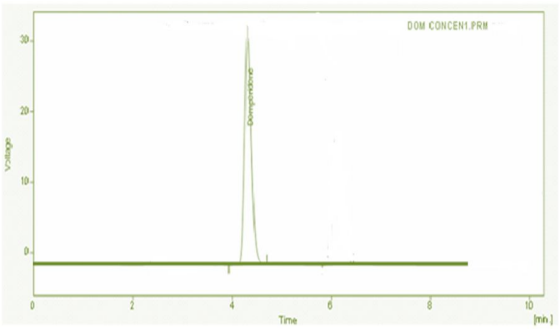
System suitability: Number of theoretical plates was determined by employing the formula n=16(t/w) 2 where t=retention time and w = width of the peak. Tailing factor was derived from the formula t = w/2t where w = half of the width, t = retention time**.**

4. Results and discussion

4.1. Selection of mobile phase:

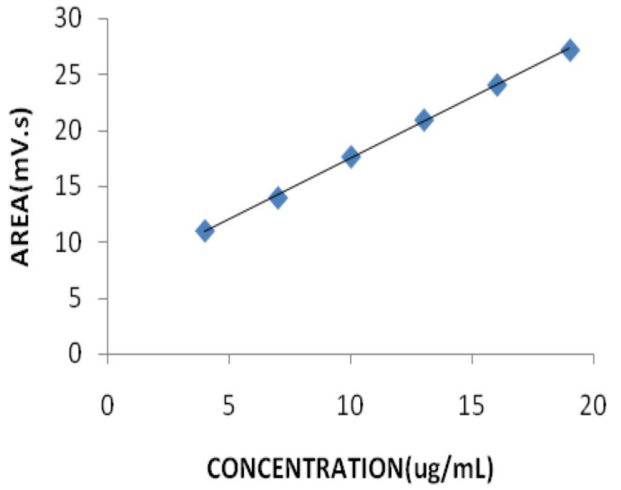
Of the various combinations of the mobile phases tried, the one consisting a water: methanol (55:45) at 1 mL/min flow rate, was found to be most suitable. DOM resolved well and could be detected at 291 nm. With retention times of 4.5 minutes. **(Figure 2)** Good linearity could be achieved for DOM the concentration ranges of 2-10 µg/mL **(Figure 3)** . Results obtained by applying the RP-HPLC method showed that the concentrations of DOM can be determined in prepared mixtures. The proposed method has been applied to the assay DOM in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The market formulation was found to contain 101.45 ± 1.60 of the labeled amount of DOM **(Table 1).**

Fig-2 Chromatogram of Domperidone



Retention time 4.5min for domperidone

Fig-3 Calibration curve of Domperidone



4.2. Validation:

Linearity and range-The six-point calibration curves that was constructed were linear over the selected concentration range for DOM ranging between 2 -21 µg/mL. The linearity of the calibration graphs and adherence of the system to Beer's law was validated by the high value of the correlation coefficient 0.993 and the intercept values 8.05 for DOM.

(Table -2).

Table 2: Summary of Validation Parameters of RP-HPLC

Recovery%	99
Repeatability (RSD, n=5)	0.05-1.11
Precision(CV) Intra-day (n=3) Inter-day (n=3) Specificity Specific	0.01 - 0.45 0.05 - 0.24 0.05 - 0.28 Specific
Solvent suitability	99.96 – 100.11
Limit of Detection (g/ml)	0.02 -0.24
Limit of Quantitation (g/ml)	0.09-0.72

Accuracy-Good recoveries was obtained in the method for the compounds which was found to be 100.43 ± 1.15 for DOM. **(Table 3).**

Table 3: Determination of Accuracy

DOM (µg/ml)	Amt of sample	Amt. of drug added	Amt. recovered	recovered % Recovery
10	0	10.09	104.3	104
10	4	12.17	99.97	99
10	8	16.20	99.92	99
10	12	20.06	103.2	103
10	16	24.04	99.94	99

Precision-The % R.S.D was found to be 0.05 - 0.24 for intra-day and 0.05 - 0.28 for inter-day variations **(Table-2).**

Limit of Detection and Limit of Quantitation- The value for LOD was found to be 0.02- 0.24 µg/mL and LOQ was found to be 0.09- 0.72 µg/mL for DOM. **(Table- 2).**

System suitability testing for HPLC- The number of theoretical plates, tailing factor and retention times was well within the accepted values in the method. The values are presented in **(Table 4).**

Table-4 System Suitability Parameters

S.No	System Suitability Parameters	DOM
1.	Retention Time	4.5
2.	Tailing Factor	1.2
3.	Theoretical Plate	2532

5. Conclusion

Thus it can be concluded that domperidone can be quantified simultaneously by the proposed HPLC method using an isocratic mobile phase consisting water: methanol: (55: 45) using a UV detector at 291 nm. The proposed method is simple, sensitive, rapid, accurate and precise. It can be applied successfully for the estimation of domperidone in bulk and its pharmaceutical formulation.

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