Research Article

A Novel RP-HPLC Method Development, Optimization and Validation for Estimation of Domperidone as Drug Substances

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ABSTRACT

A Simple, specific, accurate and reliable RP-HPLC method was developed for Domperidone (DOM) in solid dosage formulations. By waters C18, 5µm, 150 x 4.6 mm I.D. column eluted with mixture of Buffer (potassium dihydrogen phosphate + dipotassium hydrogen phosphate & pH adjusted to 7.5 with 0.1 N NaOH) and Acetronitrile in a ratio of 60:40, isocratic elution pattern. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. And at a flow rate of 1.0 ml/minute and a detection wavelength of 280 nm with injection volume of 20 µl at Ambient (30°C) temperature afforded the best separation of these analytes. The retention time of Domperidone was found to be 2.81 min. with runtime of 10 min. The system precision of this method was evaluated by calculating the %RSD of the peak areas of six replicate injections of the standard solution, which were found to be 0.752. The mean recovery was found to be 99.882% for Domperidone. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate. Linearity was established for Domperidone in the range of 5-30 µg/ml with correlation coefficient of 0.998. Furthermore, Specificity studies were demonstrated indicating that the excipients of the formulation did not interfere with the active ingredients of the drug product. The analytical procedure is validated as per ICH Q2B guidelines and shown to be accurate, precise and specific. This method is amenable to the routine analysis and can be successfully employed for Domperidone in different formulations.

Keywords: Domperidone (DOM), RP-HPLC, Accuracy, Precision, Validation.

INTRODUCTION

Domperidone, 1-(3-(4-(5-chloro-2-oxo-2, 3 Dihydrobenzo [D] imidazol-1-yl) piperidin-1 yl) propyl)-1H-benzo [D]imidazol-2(3H)-one (fig.no.1) (Mol.wt.-425.9) acting as a specific blocker of dopamine receptors. It speeds gastrointestinal peristalsis, causes prolactin release, and is used as antiemetic and tool in the study of dopaminergic mechanisms[1]. It is a gastrointestinal emptying (delayed) adjunct and peristaltic stimulant. The gastroprokinetic properties of domperidone are related 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. The antiemetic properties of domperidone are related to its dopamine receptor blocking activity at both the chemoreceptor trigger zone and at the gastric level[2]. It has strong affinities for the D2 and D3 dopamine receptors, which are found in the chemoreceptor trigger zone, located just outside the blood brain barrier, which - among others - regulates nausea and vomiting.

According to the literature survey it was found that few analytical methods such as (HPLC, UV-Visible analysis and LC-MS) were reported for the estimation of Domperidone[3-6]. Many of them suffer from one disadvantage or other such as low sensitivity lack of sensitivity and simplicity etc., the objective of the proposed method is to develop simple and accurate methods for the determination of Domperidone by RP-HPLC method in pharmaceutical dosage forms & it's stability indicative studies[7-8].

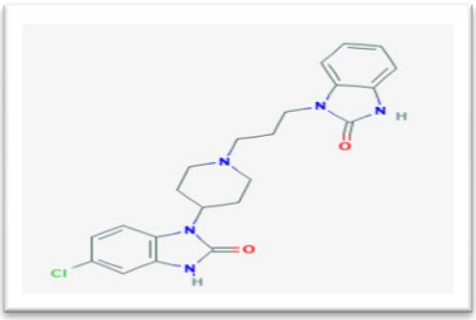

Figure 1: Chemical structure of Domperidone

The present study describes the development of a new rapid, efficient and reproducible RP-HPLC method using isocratic mobile phase for the analysis of Domperidone offers certain advantages in its simplicity and time saving and applicable in routine analysis[9-10]. It also describes the development of validation work as per ICH Q2B guidelines recommended by the Food and Drug Administration (FDA) of the United States[12-14].

MATERIALS AND METHODS

Instruments Used

S.No.	Name Of Instrument	Instrument Model	Name Of Manufacturer
1	UV-Spectrophotometer	UV/1800	Shimadzu
2	HPLC	2695	Waters (Empower Software)
3	Ultra Sonicator	LN	Equitron
4	Digital Ph Meter	S220	Mettler Toledo
5	Analytical Digital Balance (0.01mg Sensitive)	TX/TXB	Shimadzu

Chemicals / Reagents Used

Table 1: List of Chemicals Reagents Used

S. No.	Name	Specifications		Manufacturer/Supplier
		Purity	HPLC Grade	
1.	Doubled distilled water	----	HPLC grade	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9	L.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9	L.R.	Sd fine-Chem ltd; Mumbai

Details of marketed formulation used

Brand name	Manufacturer	Batch No.	Mfg. Date	Exp. Date
Vomistop	Cipla	11U1179	June -2016	Dec-2018

RESULTS AND DISCUSSION

Method Development

Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of domperidone, so that the same wave number can be utilized in HPLC UV detector for estimating the domperidone. While scanning the domperidone solution we observed maxima at 280 nm. The UV spectrum has been recorded on UV-SPECTROPHOTOMETER of model no-UV/1800 by SHIMADZU. The scanned UV spectrum is attached in

Mobile Phase Preparation

The mobile phase used in this analysis consists of a mixture of Buffer (potassium dihydrogen phosphate + dipotassium hydrogen phosphate & pH adjusted to 7.5 with 0.1 N NaOH) and Acetronitrile in a ratio of 60:40. 600 ml of this buffer solution was added and properly mixed with 400 ml of acetronitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 5 minutes before using in the experiment

Preparation of standard solution of Domperidone

25 mg of Domperidone was weighed accurately and transferred into 25 ml volumetric flask. About 10 ml of HPLC dimethyl sulfoxide was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 1000 µg/ml of Domperidone. From the stock solution again 1 ml was taken in a 10 ml volumetric flask & volume was make up to the mark by mobile phase. This solution contains 100 µg/ml of Domperidone which has been injected to HPLC.

Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

Different chromatographic conditions used and their Optimizations

The different HPLC chromatographic conditions were used to find out the optimum chromatographic condition for best elution of drugs.

Trail 1

Mobile phase-		Water:ACN(80:20)
Wavelength	-	280nm
Flow rate	-	0.8 ml/ min.
Run time	-	10 min.
Column	-	Hiq Sil, C-18, V size(250mm*4.6mmØ)

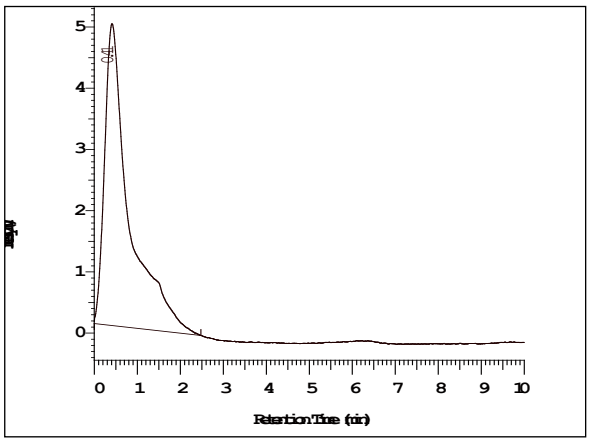

Figure 2: Trail 1 Chromatogram

Result

No peaks were separated and a negative peak was also found. Hence chromatogram was not acceptable.

Limit: % rsd <1 , Resolution factor >2

Trail 2

Mobile phase	-	Water: Methanol (20:80)
Wavelength	-	280nm
Flow rate	-	0.8 ml/ min.
Run time	-	10 min.
Column	-	Hiq Sil, C-18, V size (250mm*4.6mmØ)

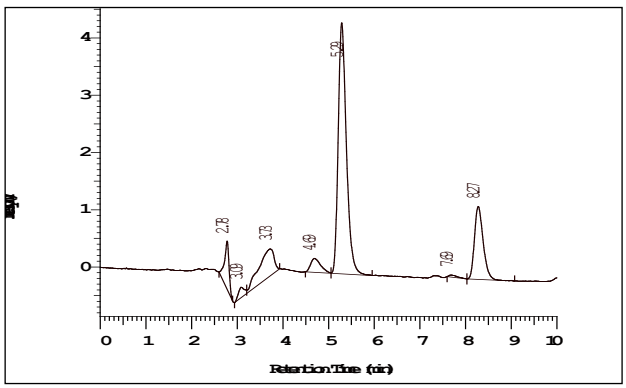

Figure 3: Trail 2 Chromatogram

Result

Peak shape was not proper. Peak tailing and a negative peak were found. Hence chromatogram was not acceptable.

Limit: % rsd <1, Resolution factor >2

Final method

By waters C_{18}, 5µm, 150 x 4.6 mm I.D. column eluted with mixture of Buffer (potassium dihydrogen phosphate + dipotassium hydrogen phosphate & pH adjusted to 7.5 with 0.1 N NaOH) and Acetronitrile in a ratio of 60:40, isocratic elution pattern. And at a flow rate of 1.0 ml/minute and a detection wavelength of 280 nm with injection volume of 20 µl at Ambient(30°C) temperature afforded the best separation of these analytes. The retention time of Domperidone was found to be 2.81 min. with runtime of 10 min.

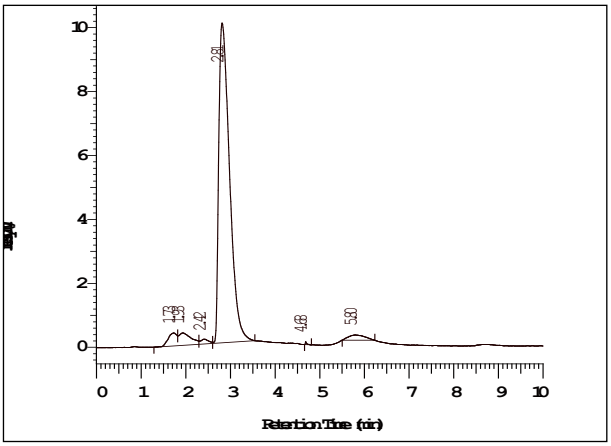

Figure 4: Optimized Chromatogram of Domperidone

Result

The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Domperidone for 10 min. The retention time were found to be 2.81 min.

Method Validation

Specificity

Preparation and running of Domperidone

As per the label claim, each tablet contains 10mg of Domperidone. To estimate this 500 mg of the powdered tablet has been dissolved in 25 ml of dimethyl sulfoxide. Further dilution was done by taking 1ml of this solution in 10ml volumetric flask, dissolve and make up with mobile phase. To extract the drug in the solution, it has been sonicated for 5 minutes followed by cyclo-mixing for 5 minutes. Resulting solution was filtered by using Millipore syringe filter (0.42 micron). Resulting clear solution was injected in HPLC in duplicate as per the above mentioned HPLC method. Area obtained in both the injections is as mentioned below.

Result

No peaks were found at the retention of Domperidone. Specificity studies indicating that the excipients did not interfere with the analysis. No interference is observed with the drug.

Accuracy

In nine different 10 ml volumetric flasks, 1 ml of the pre-analyzed capsule solution (100 µg/ml) was taken and added 1, 2, 3 ml of standard solution of bulk (API) mixture (100µg/ml) and the volume was made up to 10 ml with mobile phase.

The solutions were then injected into the HPLC system and the peak areas were recorded. The data are shown in the below table.

Linearity and Range

Result

Linearity range was found to be 5-30 µg/ml for Domperidone. The correlation coefficient was found to be 0.998

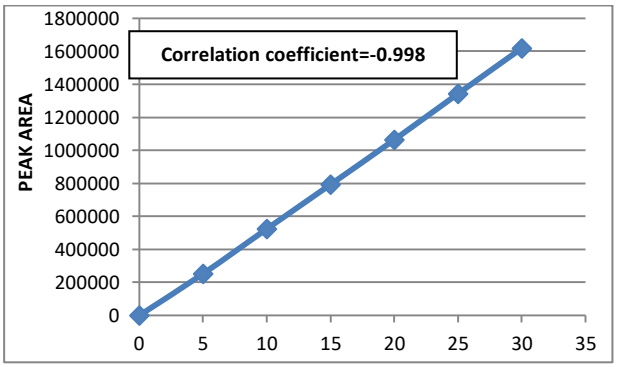

Figure 5: Standard curve for Domperidone

Table 2: Data of recovery studies

Accuracy levels	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	99.63	Mean= 99.67667%
S_2 : 80 %	8	10	99.92	S.D. = 0.223681
S_3 : 80 %	8	10	99.48	% R.S.D.= 0.224407
S_4 : 100 %	10	10	99.19	Mean= 99.19%
S_5 : 100 %	10	10	99.25	S.D. = 0.06
S_6 : 100 %	10	10	99.13	% R.S.D.= 0.06049
S_7 : 120 %	12	10	99.25	Mean= 99.49%
S_8 : 120 %	12	10	99.54	S.D. = 0.219317
S_9 : 120 %	12	10	99.68	% R.S.D. = 0.220441

Result

The mean recovery was found to be 99.882% for Domperidone. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Domperidone. The percent relative standard deviations were calculated for Domperidone are presented in the table-26.

Table 3: Data showing repeatability analysis

HPLC Injection Standard of Domperidone	Area	Retention Time
Replicate – 1	161765	2.81
Replicate – 2	161811	2.81
Replicate – 3	163199	2.81
Replicate – 4	161143	2.82
Replicate – 5	161913	2.82
Replicate – 6	161905	2.81
Average	161966.2	2.814
Standard Deviation	752.4714	0.005477
% RSD	0.752	

Result

The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml of Domperidone showed a RSD of 0.752%. It was concluded that the analytical technique showed good repeatability.

Limit: % rsd <1, Resolution factor >2

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established.

S. No.	Parameter	Limit	Result
1	Resolution factor	Rs > 2	9.15
2	Tailing factor	T ≤ 2	Domperidone=0.12
3	Theoretical plate	N > 3000	Domperidone=3246

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Domperidone API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The sample preparation is simple and the analysis time is short. The analytical procedure is validated as per ICH Q2B guidelines and shown to be accurate, precise and specific. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Domperidone in different formulations.

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