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Method Development and Validation of Domperidone Malate in Tablet Dosage Form by Using RP-HPLC Method

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KEYWORDS
HPLC, Domperidone, Validation, Mobile phase, ICH guideline.

ABSTRACT:

A simple, precise, dependable, and replicable HPLC technique was developed to analyze Domperidone in solid dosage forms. The method utilized a C18 column with a mobile phase consisting of KH2PO4 Buffer and Methanol in a ratio of 60:40 (v/v), with detection performed at 253 nm. Domperidone exhibited a retention time of 6.7 minutes. Validation of the method was conducted following ICH guidelines, demonstrating satisfactory linearity, accuracy, and precision. The limit of detection and limit of quantification were determined to be 0.36 µg/mL and 1.56 µg/mL, respectively. This method proved suitable for the routine analysis of Domperidone, both individually and in combination with other dosage forms.

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1. Introduction

Over the last few decades much attention is paid towards the quality of pharmaceuticals that enter the market. The major challenge for both bulk drug industries and pharmaceutical industries is to produce quality products. It is necessary to conduct vigorous quality control checks in order to maintain the quality and purity of output from each industry. Purity of active pharmaceutical ingredient depends on several factors such as raw materials, their method of manufacture and the type of crystallization and purification process. Concept about purity changes with time and it is inseparable from the developments in analytical chemistry. The pharmacopoeias specify not only purity but also puts limits which can be very stringent on levels of various impurities. Modern separation methods clearly play a dominant role in scientific research today because these methods simultaneously separate and quantify the components hence making the separation and characterization of impurities easier[1]. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Different pharmacopoeias such as British pharmacopoeia (BP) and the United States pharmacopoeia (USP) are slowly incorporating limits to allowable levels of impurities present in the APIs or formulation. The International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances, products and residual solvent. Impurity profile is description of the identified and unidentified impurities present in a typical batch of API produced by a specific controlled production process. It is one of the most important fields of activity in contemporary industrial pharmaceutical analysis. Domperidone malate is a dopamine receptor antagonist that exerts its pharmacological effects primarily on the gastrointestinal tract[2]. It is widely prescribed for its prokinetic properties, aiding in the management of gastrointestinal motility disorders such as gastroparesis and gastroesophageal reflux disease (GERD). Additionally, Domperidone malate is utilized to alleviate symptoms of nausea and vomiting. The accurate quantification of Domperidone malate is essential for ensuring proper dosage administration and therapeutic efficacy[3]. High-Performance Liquid Chromatography (HPLC) has emerged as a robust

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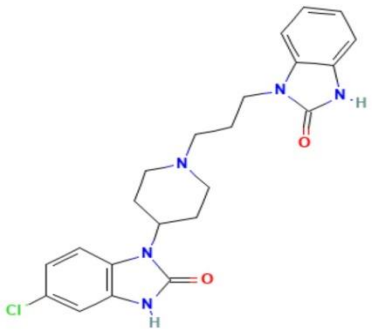
analytical technique for the quantification of Domperidone malate due to its sensitivity, specificity, and reproducibility. In this study, we aim to develop and validate an HPLC method for the determination of Domperidone malate in pharmaceutical formulations. The method optimization involves careful selection of chromatographic conditions, including the choice of stationary phase, mobile phase composition, and detection wavelength[4]. Validation parameters such as linearity, precision, accuracy, and robustness will be assessed to ensure the reliability and suitability of the method for routine analysis. The development of a validated HPLC method for Domperidone malate analysis holds significant importance in pharmaceutical quality control and research. It provides a means for precise quantification, thereby facilitating the evaluation of drug formulations' potency and stability. Moreover, this method can be employed in pharmacokinetic studies to investigate Domperidone malate's absorption, distribution, metabolism, and excretion profiles[5]. Overall, the establishment of an HPLC method for Domperidone malate analysis contributes to ensuring the quality, safety, and efficacy of pharmaceutical products containing this important therapeutic agent



Structure of Domperidone malate

Materials and Method

Instrumentation:

HPLC waters auto sampler were used for the method development of Domperidone malate, Column with 250x4.5mm, 5µm, Hibar C18 column was used.

Mobile phase preparation:

Buffer was prepared by adding 5.82 g of KH_2PO_4 in 1000 ml of milliQ water . Mobile phase was prepared in the ratio of 60 volume buffer and 40 volumes of Methanol.

Standard preparation:

Weigh accurately about 100 mg of Domperidone malate working standard and transfer into a 100 ml volumetric flask. Dissolve in 50 ml of mobile phase and sonicate for about 10 minutes to dissolve the material. Dissolve, dilute to volume with mobile phase and mix well. Pipette 5 ml of this solution into a 100ml volumetric flask, dilute to volume with mobile phase and mix.

Sample preparation:

Weigh 20 tablets powder it and Transfer 150mg of this powder into 500ml volumetric flask and add 250ml of mobile phase sonicate for 30 minutes with intermediate shaking for 30 minutes mechanically and bring to volume with mobile phase. Filter the solution through 0.45µm syringe filter. Transfer 10ml of this solution into 250ml volumetric flask, dilute to volume with mobile phase and mix.

Chromatographic condition:

Chromatographic condition for Domperidone malate was carried with C-18 column with 250 x 4.6 mm i.d. and 5 µm particle size. The mobile phase consisted of Buffer: Methanol (60: 40v/v) and that was set at a flow rate of 1.0 ml/min. The mobile phase was degassed and filtered through 0.45µm membrane filter before pumping into HPLC system. The eluent was monitored by UV detection at 253 nm.

Result and discussion:

Specificity

The blank, sample and standard solutions were prepared as per described in the methodology and injected in the HPLC system. The retention time of all corresponding peak observed in the resulting chromatograms were recorded[8]. The obtained results are presented as follows. No peak should be observed due to blank solution at the same retention time of main peak as observed in the standard solution and sample solution. The main peak should be spectrally pure.

Journal of Chemical Health Risks
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Table No. 01 Specificity data

Name of Solution	Retention Time (minute)	Peak Purity
	Domperidone malate	Domperidone malate
Blank	No interference	NA
Standard Solution	6.789	1.0
Sample Solution	6.778	1.0

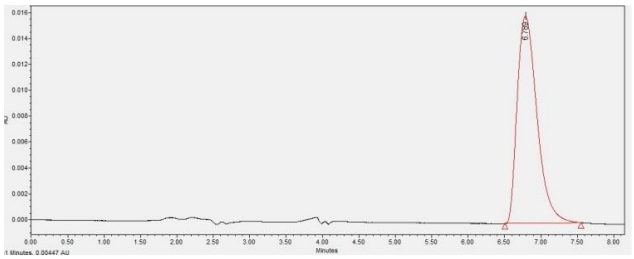

Fig: 1. A typical HPLC Chromatogram for Domperidone malate Standard

Precision

Six sample preparations were prepared from the same samples as described in methodology and % assay was calculated. The mean relative standard deviation and 95% confidence interval of the results was calculated. The results obtained for assay are presented in the following table.

Table No. 02: Precision of Domperidone malate

Sample No.	Area	% Assay
	Domperidone malate	
1	967732	101.50
2	961515	101.30
3	959850	101.60
4	958366	101.50
5	959919	101.40
6	952787	100.90
Mean Percentage		101.40
%RSD		0.23

Intermediate Precision

The analysis was carried out as described in the repeatability exercise. A different analyst carried out the analysis on a different day, using a different HPLC and different column[6]. The obtained results for % assay and overall comparative data presented in the following tables.

Table No. 03 Intermediate precision

Sample No.	Area	% Assay
	Domperidone malate	
1.	944458	101.20
2.	946087	101.10
3.	944633	100.90
4.	945514	101.00
5.	946597	101.10
6.	946701	101.40

The relative standard deviation of the assay results for six individual sample preparations is within the acceptance criteria of not more than 2.0%. The relative standard deviation of assay obtained from 12 samples preparations (Repeatability and Intermediate precision) is within the acceptance criteria of not more than 2.0%[7]. The absolute difference between the mean assay result obtained in repeatability and intermediate precision is within the acceptance criteria of not more than 2.

Linearity and Range

A series of solutions were prepared by quantitative dilutions of the stock solution of main drug standard to obtain concentration at 50% to 150% of the working concentration sample. Each solution was injected and the peak area was recorded slope, Y-intercept and

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correlation of the regression were calculated. The value of concentration, peak area and concentration are presented in the results table[8]. A graph of peak area v/s concentration was plotted. For range injected six replicates of lower and higher concentration levels and calculated the mean and relative standard deviation and also recorded the concentration levels over which the results are linear.

Table 04: Linearity area of Domperidone malate

Level %	Theoretical Concentration (ppb)	Area
		Domperidone malate
50%	80	464239
80%	128	754331
100%	160	948052
120%	192	1137649
150%	240	1416652

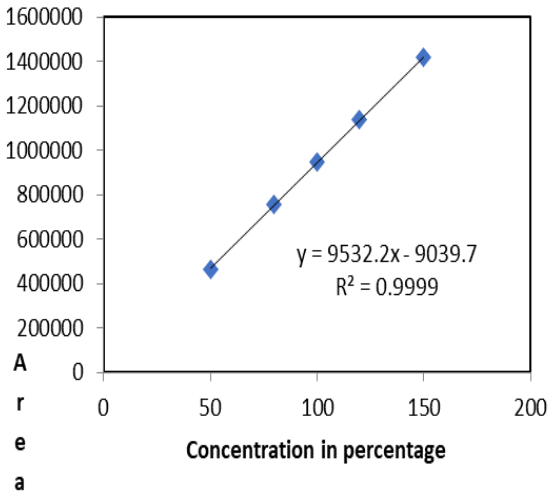

Fig : 2 Linearity curve of Domperidone malate

Table No. 05: Range area of Domperidone malate

Injection No.	AREA	
	50%	150%
1.	463797	1415336
2.	461819	1412383
3.	461934	1413209
4.	461244	1412005
5.	461654	1411497
6.	462377	1411098
Mean	462138	1412588
% RSD	**0.18**	**0.10**

Accuracy

Recovery solutions were prepared by spiking the drug substances diluent in the concentration level 50%, 100% and 150% of the working concentration of sample in triplicate[9]. The % recovery was calculated for each of the recovery solution and the mean recovery was determined. The results are presented in the following tables.The % recovery at 50%, 100% and 150% levels is within the acceptance criteria 97.0% to 103%. Based on the above obtained recovery results, it is concluded that method for assay by HPLC is accurate.

Journal of Chemical Health Risks
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JCHR (2024) 14(3), 1135-1142 | ISSN:2251-6727

Table No. 06 Accuracy area

Level	Area	%Recovery
	Domperidone malate	
50%	451911	99.30
	448985	98.60
	448046	98.40
100%	925966	101.70
	923744	101.50
	929363	102.10
150%	1381883	101.20
	1379866	101.00
	1388678	101.70
Mean Recovery		100.60

System Suitability

Standard solution was prepared as per the methodology and injected into HPLC system before starting every validation parameter[10]. The percentage relative standard deviation for 6 replicate injections, tailing factor and heoretical plates of standard solution has been carried out.

Robustness

Robustness study was carried out on standard solution and system suitability solution using the method mentioned in the methodology section by making the following alterations in the chromatographic conditions, like Changing the column oven temperature (20°C and 30°C)[11], Changing the ratio of organic phase (45 Volume and 35 Volume of acetonitrile), Changing the wavelength of UV detector (252 nm and 254 nm), Changing the flow rate of mobile phase (0.8 mL and 1.2 mL)[12].

Condition	Retention Time	Tailing	Theoretical	%RSD
	Domperidone malate	Factor	Plates	Domperidone malate
Specificity	6.36	0.887	2948	0.23
Solution stability	6.67	1.219	3209	0.1
Linearity	6.27	0.959	2641	0.15

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and Range				
Repeatability	6.63	0.913	2921	0.41
Intermediate Precision	6.94	1.193	2901	0.15
Accuracy	6.82	1.055	3376	0.26

Table No. 07 System suitability data

Condition	**Retention Time**	**Tailing Factor of Domperidone malate**	**Theoretical Plates of Domperidone malate**	**%RSD (Area)**	**Assay %**
	Domperidone malate			**Domperidone malate**	
Normal (Unaltered) (Repeatability)	6.66	0.895	2905	0.41	101.4
Column oven temperature (20°C)	7.39	1.37	2532	0.26	101.79
Column oven temperature (30°C)	5.63	1.32	2266	0.15	101.62
Mobile Phase Composition {Mixture of buffer and Methanol (65 : 35).	8.55	1.17	3405	0.37	99
Mobile Phase Composition {Mixture of buffer and Methanol (55 : 45).	4.02	1.34	2321	0.09	99
Wavelength for detection (252 nm)	6.35	1.16	2601	0.26	99.2
Wavelength for detection (254 nm)	6.36	1.37	2734	0.36	99.5

Table No. 08 Robustness data

Journal of Chemical Health Risks

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JCHR (2024) 14(3), 1135-1142 | ISSN:2251-6727

The % relative standard dev ation for 6 replicate injection of standard solution, tailing factor and theoretical plate were not significantly changed with altered condition[13]. Hence the method is robust to the specified changes i.e. mobile phase composition, detector wavelength (±2 nm), column oven temperature and flow rate of mobile phase[14].

Forced Degradation Studies

The forced degradation studies is perform to establish the stability indicating nature of the related substance and to observe any degraded compounds[15]. Domperidone malate for injection samples are subjected to stress with 0.1 N HCL, 0.1 N NaoH, 3% H2O2, thermal degradation at 105˚C[16].

Table No. 09 Forced degradation area

STUDY	AVG AREA	%
Acid stress	860170	90.94
Alkali stress	864560	92.92
Oxidation stress	739797	79.58
Thermal stress	863668	93.14

LOD LOQ:

The LOQ and LOD solution to be prepared in diluent to get a concentration close to the theoretically determined limit of quantification. LOD and LOQ limits could be determined simply by using the relative standard deviation of area counts and signal to noise ratio of each analyte to be calculated. LOD was found to be 0.36 µg/mL whereas LOQ was 1.56 µg/mL.

Conclusion:

A simple, precise, cost-effective, robust, and accurate HPLC method has developed for the determination of Domperidone malate from dry injection. The method was validated as per ICH guidelines Q2 (R1) for analytical method validation. The actives as Domperidone malate were determined in the dosage formulation of dry powder injection. The method's demonstrated linearity, precision, and reproducibility underscore its suitability for routine analysis in quality control settings. By providing a precise tool for the quantification of Domperidone malate, this method contributes significantly to ensuring the potency and safety of pharmaceutical formulations. Hence, the developed method can be used in the routine analysis of Domperidone malate in dosage forms.

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Declaration of interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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