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AN ANALYTICAL VALIDATED METHOD DEVELOPMENT FOR THE QUANTIFICATION AND DETERMINATION OF DOMPERIDONE IN THE ORAL SUSPENSION

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Abstract

A sensitive and validated high-performance liquid chromatography (HPLC) method was developed for the quantification of domperidone in oral suspension formulations. Domperidone, a dopamine D2 receptor antagonist used for treating gastrointestinal disorders, was analyzed using a C18 column. Domperidone is a widely used medication renowned for its effectiveness in alleviating gastrointestinal disorders, including nausea, vomiting, and gastroparesis. As a dopamine D2 receptor antagonist, it works by restoring balance in the digestive system, providing much-needed relief for those suffering from these uncomfortable conditions. A reverse-phase C18 column (150 mm × 4.6 mm, 5 µm) was used for the chromatographic analysis. The mobile phase was composed of methanol in a 40:60 (v/v) ratio and 0.5% ammonium acetate buffer (pH 6.8) and delivered isocratically at a flow rate of 2.0 mL/min. With an average retention time of 7.6 minutes, detection was done at 280nm. The developed method was validated against ICH and EMA guidelines for key analytical parameters, including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method exhibited excellent linearity over the concentration range of 80–120 µg/mL, with a correlation coefficient (R^2) of 0.9975. The accuracy was confirmed through recovery studies at three concentration levels (50%, 100%, 150%), showing recoveries between 95.08% and 99.22%. The method demonstrated good precision, with relative standard deviation (RSD) values of less than

1% for both intra-day and inter-day analyses. The LOD and LOQ were calculated to be 4.25 µg/mL and 12.88 µg/mL, respectively. Robustness was evaluated by introducing slight variations in chromatographic parameters, which did not significantly affect assay performance. Overall, the developed HPLC method is simple, time-efficient, cost-effective, and robust, making it suitable for routine quality control and quantitative analysis of Domperidone oral suspension formulations without interference from excipients or degradation products.

Introduction

Domperidone is a commonly used medication for nausea and vomiting, and has been the subject of extensive research. This study explores its synthesis, detection, and measurement, with particular emphasis on its pharmaceutical uses. The chemical formula of domperidone is C22H24ClN5O2. Chemically, it is known as five-chloro-1-[1-[3(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]piperidin-4-yl]-1, 3-dihydro-2H-bbenzimidazol-2-one (1) . According to the BCS classification, it is a class II drug with pH-dependent solubility. The experimentally determined aqueous solubility of domperidone and its pKa value is 7.9 (2).

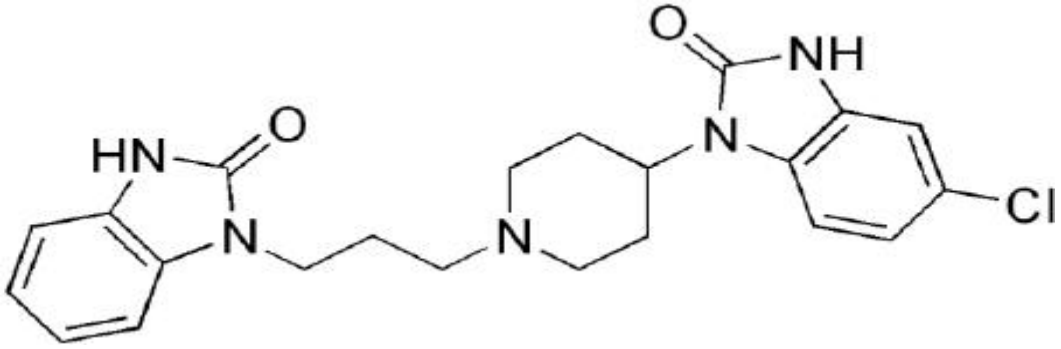

Figure 1: Chemical structure of Domperidone

The synthesis of domperidone involves multiple steps, ending with the coupling of two key intermediates. This reaction, aided by a catalyst such as potassium iodide, results in the formation of the final domperidone molecule (3). Various analytical techniques, such as HPLC, have been used to detect and measure domperidone dosage forms. High-performance liquid chromatography (HPLC) offers a reliable and sensitive method for accurately quantifying domperidone in analytical preparations (4). Many HPLC methods have been developed to analyze domperidone in formulations. However, most of these methods use complex mobile phases, often with buffered aqueous solutions, restricting their use to certain dosage forms (5).

The study aims to create a sensitive, fast, and simple high-performance liquid chromatography (HPLC) method to simultaneously measure domperidone in various

dosage forms. This approach eliminates the need for derivatization or complex sample preparation. The method is more cost-effective due to lower reagent use and easier sample extraction. It also requires less sample and has a shorter chromatographic run time compared to earlier methods. Rigorous validation is crucial to ensure the method's accuracy and reliability, with key parameters such as specificity, linearity, accuracy, precision, ruggedness, and system suitability being evaluated. Statistical tools like the mean, standard deviation, and correlation analysis are vital for analyzing and interpreting data, enabling researchers to draw meaningful conclusions from their experiments data.

Chemicals and Reagents

Domperidone (100% purity) was obtained from Hetero Labs Limited (Unit-III India) and supplied by Fedro Pharmaceuticals Labs Pvt. Ltd., Peshawar, Pakistan. HPLC-grade chemicals such as Dimethyl formamide (≥ 99.5%), Methanol (≥ 99.9%), Ammonium acetate (≥ 97.0%), and 37% HCl were purchased from Daejung Chemicals (South Korea). These reagents were used to prepare the solvent and mobile phase. Additionally, a pharmaceutical suspension containing 5 mg of domperidone per 5 ml was formulated and provided by Fedro Pharmaceutical Labs Pvt. Ltd, Peshawar, Pakistan. Distilled water was prepared with a distillation apparatus and used to make 0.1 M HCl, 0.5% w/v Ammonium Acetate buffer, and the placebo.

Instrumentation

The quantification of domperidone was carried out using a Shimadzu 10AT series HPLC system (Japan), which includes a pump (LC-10AT VP), a column oven (CTO-10AS VP), and a UV/VIS detector (SPD-10A VP). The system featured a Nelson network chromatographic interface and was managed by Shimadzu LC Classic software (version 10). Chromatographic analysis was conducted on a reverse-phase C18 column (150 mm x 4.6 mm, 5 µm) from Welchrome. The column oven was set to 25°C. The mobile phase was a mixture of 0.5% ammonium acetate buffer (pH 6.8) and methanol in a 4:1 ratio, delivered isocratically. Samples were pumped at 2.0 mL/min, ensuring a good balance between peak resolution and analysis time. Filtration prior to analysis used a nylon syringe filter with a 0.45µm pore size and a 25mm diameter from membrane solution. Additional instruments involved in sample preparation included a digital analytical balance (Model ATX124), pH meter (3505, Esses-UK), magnetic stirrer, centrifuge (K001, Pakistan), and filtration setup analysis.

Experimental Parameters and Chromatographic Condition Optimization

Method optimization involved testing different reverse-phase columns to find the best one for domperidone analysis. The mobile phase was a 40:60 (v/v) mix of 0.5%

ammonium acetate buffer and methanol, filtered through a 0.45µm membrane, sonicated for degassing, and adjusted to pH 7.0. Isocratic elution at 2.0 mL/min provided the best peak resolution and analysis efficiency. The column oven temperature was maintained between 25 °C and 50°C, with sample injection volumes from 50µL to 100µL. Detection was carried out at 280 nm. Domperidone, sourced from Hetero Labs Limited, India, and tested by Fedro Pharmaceutical Pvt. Ltd., Peshawar, Pakistan, served as the internal standard. The developed HPLC method showed excellent accuracy, sensitivity, and robustness for measuring domperidone.

Table 1: *Chromatographic Conditions*

Parameters	Conditions
Column	C18 (150mm x 4.6mm, 5µm)
Mobile phase	0.5% Ammonium Acetate buffer pH 6.8: Methanol (40:60 V/V)
Flow rate	2.0 mL/min
Temperature	25-50°C
Detection wavelength	280 nm
Injection volume	50-100 µL
Retention time	8.2 min

Solution and Buffer

The study employed various solutions and buffers. An ammonium acetate buffer (0.5% w/v, pH 6.8) was prepared by dissolving 5 grams of ammonium acetate in 800 mL of water, stirring until fully dissolved, and then bringing the volume to 1000 mL with distilled water. A 1M hydrochloric acid stock solution was made by diluting 8.2 mL of 37% hydrochloric acid to 1000 mL with water, with further dilutions to 0.1M using distilled water. The diluent used in the study comprised 50 mL of dimethylformamide, an appropriate volume of methanol, and 1N HCl (6).

Preparation of Standard Solution, Sample, and Placebo

The study used a 0.1 mg/mL stock solution of domperidone, prepared by dissolving 100 mg in a mixture of dimethylformamide, methanol, and 1M HCl (v/v/v). For sample preparation, 10 mL of a solution containing 10 mg of domperidone was transferred to a volumetric flask, then combined with the same solvent mixture, shaken, sonicated, and diluted to volume with methanol. The solution was then filtered through a 0.45 µm membrane before analysis. A placebo was prepared separately by blending Tween 80, sorbitol, sodium saccharin, citric acid, glycerin, soaked gum, and essence in a specific sequence, adjusting the final volume to 250 mL. Calibration curves for standards of

domperidone were created using solutions with concentrations ranging from 80 µg/mL to 120 µg/mL, in 10 µg/mL steps.

Validation of the Developed Method

The proposed method was validated according to established standard guidelines (7). Emphasis was placed on linearity, precision, specificity/selectivity, accuracy, sensitivity (including the lower limit of detection (LOD) and lower limit of quantification (LOQ)), sample stability, and robustness (8).

Linearity

The method's linearity was evaluated by creating calibration curves with five different concentration points of the standard solutions in the mobile phase, ranging from 80 to 120µg/mL. The ratio of the analyte's peak area to that of the internal standard was plotted against their respective concentrations. (9) The slope (a), correlation coefficient (r), and (b) percent recovery were obtained through regression analysis using the least squares method.

Precision

The method's precision was evaluated by examining the consistency of repeated injections and analyses (10). Injection repeatability was assessed by performing five replicate injections of samples containing 250 ng/ml of Domperidone. For each injection, the retention time of the drug and the domperidone dose were recorded, and the results were expressed as the mean and coefficient of variation (CV, %RSD). Analysis repeatability was evaluated by analyzing five replicates (n=5) of a sample with a known domperidone concentration, and the percent recovery was calculated. Results were reported as the mean with %RSD (n=5). To determine intermediate precision (both intra-day and inter-day reproducibility), samples with different domperidone concentrations were analyzed three times a day at 8-hour intervals on day 1 (intraday). Inter-day precision was assessed by analyzing the same sample across three consecutive days. Results were expressed as mean ± standard deviation, covariance (n=5).

Selectivity/Specificity

Specificity describes an analytical method's capacity to accurately identify the analyte even when other components are present (11).

Linearity & Range

Linearity of a method indicates its ability to produce test results that are directly proportional to the analyte concentration in samples. To create the calibration curve, a mixed standard solution of domperidone in methanol and water at a concentration of 500µg/mL was prepared. A series of dilutions containing domperidone was then made,

establishing a linear relationship by plotting concentrations against peak areas. According to ICH Guideline Q2 (R1), the concentration range for domperidone was set at 80µg/mL, 90µg/mL, 100µg/mL, 110µg/mL, and 120µg/mL, evenly spaced within the expected working range.

Accuracy

Percent recovery was utilized to assess the accuracy of the proposed method. Accuracy in an analytical procedure indicates how closely the measured or found value aligns with the accepted, actual, or reference value. This measure is sometimes referred to as trueness (11). In the accuracy and recovery study, known sample quantities were used to represent three concentration levels: 50%, 100%, and 150%. Results were obtained with three replicates at each level, covering the lowest (50%) and highest (150%) concentrations within the expected working range. The accuracy assessment measured analyte recovery through percentage recovery analysis across the assay's range.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The detection and quantification limits were established using standard solutions from 80µg/mL to 120µg/mL, representing 80% to 120% of the standard solution. A linear regression analysis was performed on this data.

System Suitability

System suitability testing is crucial to confirm the chromatographic system's quality and performance (12). The required extent of testing depends on the purpose of the test method. For dissolution or release profile tests using an external standard method, the minimum recommended system suitability parameters are k', T, and RSD. For acceptance, release, stability, or impurity/degradation tests that use either external or internal standards, it is recommended to include k', T, R, and RSD as the baseline parameters for system suitability. System suitability was assessed through chromatograms from standard solutions, focusing on key parameters such as theoretical plates, peak asymmetry, capacity factor, and resolution of domperidone peaks.

Robustness

Method robustness was tested by examining how different experimental conditions affected the peak area, peak height, and retention time of both analytes (13). Small, intentional adjustments were applied to various chromatographic conditions, including column oven temperature (±5°C), mobile phase composition, flow rate (±0.2 ml/min), detector wavelength (±2 nm), and detector voltage (±50 mV).

Statistical Interpretation and Correlation of Data

Various statistical tools, such as the mean (X), standard deviation (SD), and relative standard deviation (%RSD), were computed using MS Excel and used for quantifying both analytes.

Results and Discussion

Validation of the Developed Method

The optimal methanol-to-ethanol ratio was set at 60:40 with a flow rate of 2.0 mL/min. Chromatographic separation was conducted on a C18 column kept at 25 ºC. Both the sample and standard solutions were injected with 20 µL. The average retention time for domperidone was 7.6 minutes. Figure 2 shows a typical chromatogram of the domperidone standard.

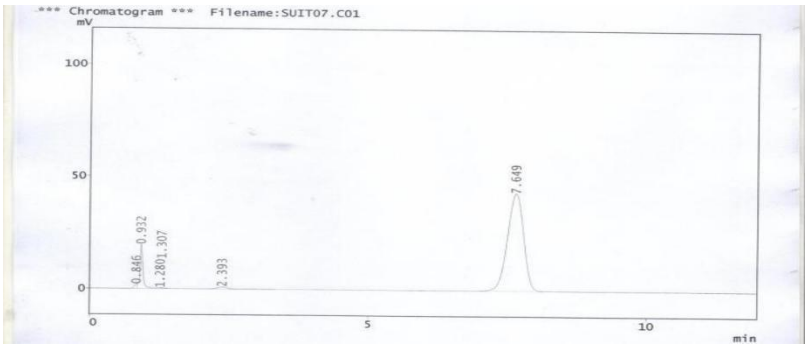

Figure 2: Chromatogram of Domperidone

Specificity

The HPLC method's specificity was evaluated by analyzing a standard domperidone solution (100 µg/mL), alongside the sample, placebo, and diluent. The standard solution produced a clear peak at 7.649 minutes with a peak area of 870349 at 280 nm. The sample containing domperidone showed a peak at 7.158 minutes with a peak area of 845035 at 280 nm. No peaks were detected at 280 nm for the placebo and diluent, indicating no interference. This confirms the method's specificity for accurately detecting and measuring domperidone in the pharmaceutical formulation.

Linearity & Range

The Linearity and Range study was conducted at various concentrations between 80 µg and 120 µg for domperidone. The method demonstrated linearity within this range, as evidenced by the correlation coefficient and percent recovery, which are detailed in Table 2.

Table 2: *Linear regression data of the HPLC method for the determination of Domperidone*

Domperidone (µg/ml)	Recovered sample	Percent Recovery
80 µg/ml	79.68 µg/ml	99.6 %
90 µg/ml	89.75 µg/ml	99.72 %
100 µg/ml	100.92 µg/ml	100.92 %
110 µg/ml	109.65 µg/ml	99.68 %
120 µg/ml	117.34 µg/ml	97.78 %
Correlation Coefficient	0.9975	
Slope	8231.93	
Intercept	36761	
Standard Error	10606.25	

Accuracy and Recovery

Samples at 50% of the target concentration had an average assay result of 47.54%, with a percent recovery of 95.08%. This suggests the assay slightly underestimates concentration in this range, though the recovery remains acceptable. For samples at 100%, the mean result was 98.51%, with a percent recovery exactly at 98.51%, indicating accurate measurement at the target level. At 150%, the average result was 148.84%, with a percent recovery of 99.22%. As shown in Table 3, these results reflect a slight overestimation at this level, but the recovery still falls within acceptable limits range.

Table 3: *Accuracy of the HPLC method for the determination of Domperidone*

Domperidone on %	50%	100%	150%
Replicate 1	407399	843547	1271532
Replicate 2	407628	848086	1275209
Replicate 3	404787	835939	1272052
Mean	406605	842524	1272931
St. Dev.	1578.3	6137.8	1989.87
RSD%	0.388%	0.7%	0.15%
Assay result	47.54%	98.51%	148.84%
Percent Recovery	95.08%	98.51%	99.22%

Precision

Perform five replicate scans of a 100% standard solution. Then, measure the response peak for each under intermediate conditions. For repeatability, inject six replicates of the Standard and record the response peak area for each. The Relative Standard Deviation of these six measurements is shown in Table 4.

Table 4: *Intermediate Precision Data for Domperidone*

Day	No. of Sample	Peak Area	Assay	Average Assay	RSD
	01	835272	97.66%		
	02	834263	97.55%		
	03	835577	97.70%		
Inter Day	04	835653	97.71%		
	05	829639	97.0%	97.52%	0.31%
	01	837921	97.97%		
Intra Day	02	837656	97.94%		
	03	837582	97.93%	98.04%	0.19%
	04	839060	98.11%		
	05	841420	98.26%		

Robustness

Robustness studies performed independently by two analysts confirmed the reliability and accuracy of the developed assay. Analyst 1 and Analyst 2 reported average assay recoveries of 98.94% (RSD 0.3870%) and 98.68% (RSD 0.2316%), respectively, demonstrating strong reproducibility between analysts. Additional robustness tests, which varied sonication time and oven temperature, showed consistent results with low RSD values. The mean assay percentage under the 'Change in Sonication Time' condition was 98.96% (RSD 0.5459%), and for the 'Change in Oven Temperature' condition, it was 98.69% (RSD 0.7600%). Collectively, these results confirm the assay's robustness against small adjustments in key parameters, as detailed in Tables 5 and 6.

Table 5: *Robustness Analysis Data of Change in Sonication Time and Change in Column Temperature*

Variable	Sample	Area of Sample	Area of Standard	Assay	Average Assay	SD	RSD
Change in Sonication Time	1	847339	855958	99.07%	98.96%	4620.53	0.54%
	2	841396	854436	98.38%			
	3	850496	855384	99.44%			
Change in Column Temperature	1	851136	855958	99.52%	98.69%	6398.52	0.76%
	2	842488	854436	98.51%			

Table 6: *Robustness analysis data of Analyst One and Two*

Variable	Sample	Area of Sample	Area of Standard	Assay %	Average Assay %	SD	RSD
Analyst 01	1	849863	855958	99.37 %	98.94%	3269.79759	0.38%
	2	845270	854436	98.83 %			
	3	843535	855384	98.63 %			
Analyst 02	1	843123	855958	98.58 %	98.68%	1954.71796	0.23%
	2	842557	854436	98.51 %			
	3	846190	855384	98.94 %			

System Suitability

The average absorbance for the sample at 100% concentration was derived from the replicates, yielding a mean of 862066.5 with a standard deviation of 8640.44 and a relative standard deviation of 0.86881%. This demonstrates excellent precision. The chromatographic parameters include a theoretical plate count of 4578, a peak asymmetry factor of 0.982 (indicating a symmetrical peak), a capacity factor of 8.3, and a resolution of 9.5. These results indicate effective separation and retention of the analyte in the HPLC system, as detailed in Table 7.

Table 7: *System Suitability Parameters*

Sr. No.	Sample 100%	Absorbance	Mean	Standard Deviation	Relative Standard Deviation
01	Replicate 1	856803			
02	Replicate 2	856739	864567	7511.44	0.86881%
03	Replicate 3	871530			
04	Replicate 4	862067			
05	Replicate 5	865448			
06	Replicate 6	874816			
Theoretical plates			4578		
Peak asymmetry			0.982		
Capacity factor			8.3		
Resolution			9.5		

Limit of Quantification (LOQ) and Limit of Detection (LOD)

A linear regression analysis was conducted, resulting in a slope of 8231.93, an intercept of 36761.2, and a correlation coefficient of 0.995, demonstrating strong linearity between concentration and area response. The LOD for Domperidone is 4.25 µg/mL, while the LOQ is 12.88 µg/mL, calculated using the linear regression equation based on

the response's standard deviation and slope. The relevant data are shown in Table 8 below.

Table 8: *Analytical Data for Determination of LOD and LOQ for Domperidone*

Limit of Detection & Limit of Quantitation		
Concentration of Domperidone (µg/ml)	Range %	Area Values Domperidone
80 µg/ml Domperidone	80 %	688881
90 µg/ml Domperidone	90 %	775941
100 µg/ml Domperidone	100 %	872497
110 µg/ml Domperidone	110 %	948008
120 µg/ml Domperidone	120 %	1014444
Slope	8231.93	
Intercept	36761.200	
Correlation Coefficient	0.995	
Regression Standard Deviation	10606.25	
LOD µg per mL	4.25 µg	
LOQ µg per mL	12.88 µg	

Conclusion

This study successfully developed and validated a high-performance liquid chromatography (HPLC) method, providing a precise, specific, and reproducible way to estimate domperidone in suspension forms. Motivated by the need for reliable quality control tools that guarantee safety, efficacy, and consistency in pharmaceuticals, the method uses a straightforward mix of methanol and ammonium acetate buffer as the mobile phase, optimized on a C18 reverse-phase column. Detection at 280 nm was chosen based on domperidone's absorbance for accurate measurement. Validation followed ICH Q2(R1) guidelines, confirming the method's specificity, linearity, accuracy, precision, and robustness. Linearity tests showed a strong, proportional response between concentration and signal (R^2 = 0.9975). Recovery studies verified the method's accuracy, and low %RSD values in precision tests confirmed its reliability and repeatability. Robustness assessments demonstrated the method's stability against minor operational variations, proving its suitability for routine use in laboratories. Valid across the working concentration range with high reproducibility, it adheres to ICH Q2(R1) standards, making it ideal for regulatory quality control and practical for daily pharmaceutical production. Overall, it offers an effective tool for ensuring compliance

and safeguarding patient health by maintaining the consistent therapeutic quality of domperidone oral suspensions.

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Authorship Contribution

Conceptualization, Methodology, Software, Resources, Writing- original draft (____) Conceptualization, Project administration, Supervision, Writing - review & editing (_____). Review & editing, formal analysis (_____). Writing - review & editing, funding

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Data Availability Statement

The data generated will be available upon reasonable request.

References

Ali MS, Ghori M, Khatri AR. Stability indicating simultaneous determination of domperidone (DP), methylparaben (MP) and propylparaben by high performance liquid chromatography (HPLC). J Pharm Biomed Anal. 2006;41(2):358–65.

Basu A, Chakraborty J. Development and Validation of a Sensitive and Specific High Performance Liquid Chromatographic Method for Assay of a Binary Mixture of Pantoprazole and Domperidone in Oral Solid Dosage Formulations. Int J Pharm Sci Res. 2019;10(10):4881.

Haridhar K, Gowtham A, Tn D, Chandan C. Method Development and Validation of Domperidone Malate in Tablet Dosage Form by Using RP-HPLC Method Journal of Chemical Health Risks. 2024;14:1135–42.

HSE. Guidelines for the validation of analytical methods for testing agricultural chemical residues in food. Shoku-An No.0124001. 2002;(3):1–10.

Methods A. Lod/loq. 2.

Moertel CG, Reitemeier RJ, Gage RP. A Controlled Clinical Evaluation of Antiemetic Drugs. JAMA: The Journal of the American Medical Association. 1963;186(2):116–8.

Mondal S, Haque A, Islam MS, Islam A. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Domperidone and Naproxen in Tablet Dosage Form [Internet]. Article in Journal of Applied Pharmaceutical Science. 2011. Available from: https://www.researchgate.net/publication/216643627

Pandey VP, Vanjinathan S, Singh S. Determination of domperidone and omeprazole in capsule dosage form by UV/visible spectrophotometer. Asian Journal of Chemistry. 2010;22(3):2305–8.

Prasad YE, Rajasekhar KK, Rajendra Prasad Y, Shankarananth V, Yaminikrishna H V, Saikumar S, et al. Spectrophotometric method for the estimation of domperidone in bulk and pharmaceutical formulations Available online through www.jpronline.info. J Pharm Res. 2009;2(10):1593–4.

Ravichandran S, Keerthibala K. Development and validation of RP-HPLC method for the simultaneous estimation of paracetamol, domperidone and esomeprazole magnesium in tablet dosage form. Int J Pharmtech Res. 2016;9(7):200–6.

Shabir GA. Development and Validation of a Reversed-phase HPLC Method for the Determination of Hydroxybenzene in a Cream Formulation Shabir: RP-HPLC Method for Determination of Hydroxybenzene [Internet]. Available from: www.ijpsonline.com

Soboleva E, Ambrus Á. Application of a system suitability test for quality assurance and performance optimisation of a gas chromatographic system for pesticide residue analysis. In: Journal of Chromatography A. Elsevier; 2004. p. 55–65.

Yu L, Shao R, Guo Q, Hong H, Zhu N. The methodology for preparing domperidone: strategies, routes and reaction processes. Vol. 12, RSC Advances. Royal Society of Chemistry; 2022. p. 22869–80.