World Journal of Pharmaceutical and Life Sciences

Impact Factor: 8.21 www.wjpls.org **Coden USA: WJPLA7**

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF ITOPRIDE HYDROCHLORIDE IN SOLID ORAL FORMULATIONS

Dhanush Ram Turkane[*1]

Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Junwani, Bhilai - 490020, Chhattisgarh, India.

***Corresponding Author: Dhanush Ram Turkane**

Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Junwani, Bhilai - 490020, Chhattisgarh, India. **DOi:** https://doi.org/10.5281/zenodo.21020756

How to cite this Article: Dhanush Ram Turkane[*1] (2026). Rp-Hplc Analytical Method Development And Validation For Quantitative Estimation Of Itopride Hydrochloride In Solid Oral Formulations. World Journal of Pharmaceutical and Life Sciences, 12(7), 110–116.

This work is licensed under Creative Commons Attribution 4.0 International license.

Article Received on 25/05/2026 **Article Revised on 15/06/2026** **Article Published on 01/07/2026**

ABSTRACT

The present study was aimed at the development and validation of a simple, rapid, precise, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative estimation of Itopride Hydrochloride in tablet dosage forms. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of potassium dihydrogen phosphate buffer and methanol (60:40, v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 220 nm using a UV detector. The retention time of Itopride Hydrochloride was found to be approximately 5.84 minutes, enabling rapid analysis. The method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, sensitivity, and system suitability. The method exhibited excellent linearity over the concentration range of 50–150%, with a correlation coefficient (R^2) of 0.9998. Accuracy studies showed mean recovery of 100.24%, while precision studies demonstrated low %RSD values below 0.5%, indicating excellent repeatability and reproducibility. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.32 µg/mL and 1.08 µg/mL, respectively, confirming the sensitivity of the method. Robustness studies revealed that minor deliberate changes in chromatographic conditions did not significantly affect analytical performance. Forced degradation studies under acidic, alkaline, oxidative, and thermal stress conditions demonstrated that the method could effectively separate degradation products from the main drug peak, confirming its stability-indicating nature. The developed RP-HPLC method was found to be accurate, precise, robust, sensitive, and suitable for routine quality control analysis and stability studies of Itopride Hydrochloride in pharmaceutical dosage forms.

KEYWORDS: Itopride Hydrochloride, RP-HPLC, Method Development, Method Validation, Stability-Indicating Method, Forced Degradation Study.

INTRODUCTION

Quality, safety, and efficacy are essential requirements for pharmaceutical products in the pharmaceutical industry. Analytical method development plays a crucial role in monitoring active pharmaceutical ingredients (APIs) during manufacturing and quality control processes.[1] Accurate and reliable analytical methods enable the determination of drug purity, potency, and the presence of impurities that may affect the therapeutic performance and safety profile of pharmaceutical products. Itopride Hydrochloride is a gastroprokinetic agent widely used for the treatment of functional dyspepsia, gastroesophageal reflux disease (GERD), and other gastrointestinal motility disorders. It acts through dopamine D2 receptor antagonism and acetylcholinesterase inhibition, thereby enhancing gastrointestinal motility and gastric emptying.[2] Figure 1 illustrates the chemical structure of Itopride Hydrochloride. Due to its extensive clinical use and therapeutic importance, a validated and reliable analytical method is required for its accurate quantification in pharmaceutical dosage forms. Among the various analytical techniques available, Reverse-Phase High-

Performance Liquid Chromatography (RP-HPLC) is one of the most widely employed methods because of its high sensitivity, specificity, reproducibility, and suitability for routine quality control analysis.[3] The present study aims to develop and validate a simple, rapid, precise, and economical RP-HPLC method for the quantitative estimation of Itopride Hydrochloride in solid oral dosage forms.[4] During method optimization, critical chromatographic parameters such as mobile phase composition, detection wavelength, flow rate, and column selection were systematically evaluated to achieve optimum separation and peak characteristics. The developed method was validated according to the recommendations of the International Council for Harmonisation with respect to specificity, linearity, accuracy, precision, robustness, and system suitability parameters. The validated RP-HPLC method is expected to provide a reliable analytical tool for the routine quality control and quantitative determination of Itopride Hydrochloride in pharmaceutical formulations.[5]

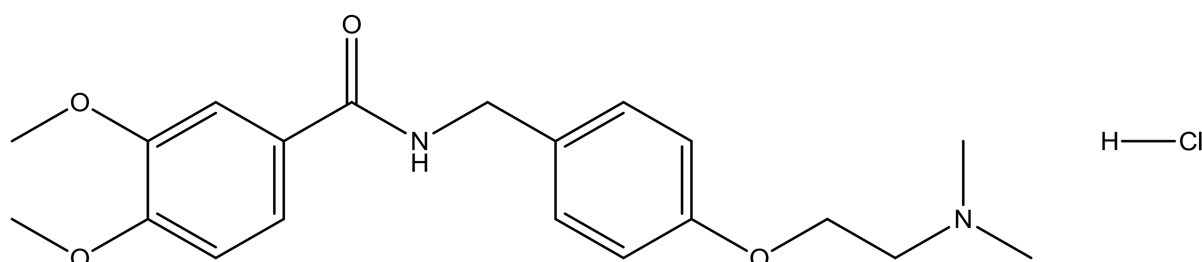

Figure 1: Chemical Structure of Itopride Hydrochloride.[6]

Materials and Methods

Instrumentation

RP-HPLC analysis was performed using a Waters HPLC system equipped with an autosampler and UV-Visible detector. The optimal chromatographic separation of Itopride Hydrochloride was achieved using a C18 column (250 mm × 4.6 mm, 5 µm particle size), which provided satisfactory peak symmetry, resolution, and retention characteristics suitable for quantitative analysis.

Chemicals and Solvents

Itopride Hydrochloride, serving as the analytical reference standard, was procured in pure form. Analytical-grade potassium dihydrogen phosphate (KH_2PO_4) and methanol of chromatographic purity (HPLC-grade) were used for method development and analysis.[7] All solutions, mobile phases, and dilutions employed throughout the study were prepared using ultrapure water obtained from a Milli-Q purification system.[8]

Formulation of Buffer and Mobile System

Precisely 5.82 g of potassium dihydrogen phosphate (KH_2PO_4) was accurately weighed and dissolved in ultrapure water to make up a final volume of 1000 mL, thereby preparing the phosphate buffer solution. The buffer was filtered through a 0.45 µm membrane filter and subsequently degassed under vacuum to remove dissolved gases.[9] The mobile phase was prepared by mixing the phosphate buffer and methanol in the ratio of 60:40 (v/v). The resulting mobile phase was filtered and degassed prior to use and served as the chromatographic eluent for the separation and quantitative estimation of Itopride Hydrochloride.[10]

Standard Solution Development

An accurately weighed quantity of 100 mg of Itopride Hydrochloride reference standard was transferred into a 100 mL volumetric flask. Approximately 50 mL of the mobile phase was added, and the solution was sonicated for 10 minutes to ensure complete dissolution of the drug. The volume was then made up to the mark with the same mobile phase to obtain the stock standard solution containing 1000 µg/mL of Itopride Hydrochloride.[11] Subsequently, 5 mL of this stock solution was transferred into a 100 mL volumetric flask and diluted to volume with the mobile phase to obtain the working standard solution containing 50 µg/mL of Itopride Hydrochloride.[12]

Preparation of Sample

Twenty tablets containing Itopride Hydrochloride were accurately weighed and finely powdered. A quantity of powder equivalent to 150 mg of Itopride Hydrochloride was transferred into a 500 mL volumetric flask. Approximately 50 mL of water was added, followed by 250 mL of the mobile phase. The mixture was sonicated for 30 minutes with intermittent shaking to ensure complete extraction of the drug from the tablet matrix.[13,14] After cooling to room temperature, the volume was made up to 500 mL with the mobile phase. The resulting solution was filtered through a 0.45 µm membrane filter to remove insoluble excipients. Subsequently, 1 mL of the filtrate was transferred into a 250 mL volumetric flask and diluted to volume with the mobile phase to obtain the final sample solution for RP-HPLC analysis.[15]

Chromatographic Method Parameters

Chromatographic analysis was carried out using a reverse-phase HPLC system equipped with a C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) as the stationary phase.[16] The mobile phase was delivered at a flow rate of 1.0 mL/min under isocratic conditions. Detection of Itopride Hydrochloride was performed using a UV detector set at 220 nm, corresponding to the drug's optimum absorption wavelength.[17] Under the optimized chromatographic conditions, the total run time for each analysis was approximately 8 minutes, and a fixed injec-

tion volume of 20 µL was used for all standard and sample solutions.[18]

RESULTS AND DISCUSSION

Specificity

The specificity of the developed RP-HPLC method was evaluated by comparing the chromatograms of the blank, standard, and sample solutions. Analysis of Itopride Hydrochloride demonstrated the absence of interfering peaks at the retention time of the analyte, indicating that the method was free from interference arising from excipients, solvents, or other matrix components. The selectivity of the method was further confirmed by the spectral purity of the Itopride Hydrochloride peak. The chromatograms of both standard and sample solutions exhibited identical retention times and acceptable peak purity values, confirming the specificity of the method. The specificity data are presented in Table 1, while the representative chromatograms are shown in Figure 2.

Table 1: Specificity Data for Itopride Hydrochloride.

Solution Type	Retention Time (min)	Peak Purity
Blank	No interference	—
Standard Solution	5.842	1.000
Sample Solution	5.831	1.000

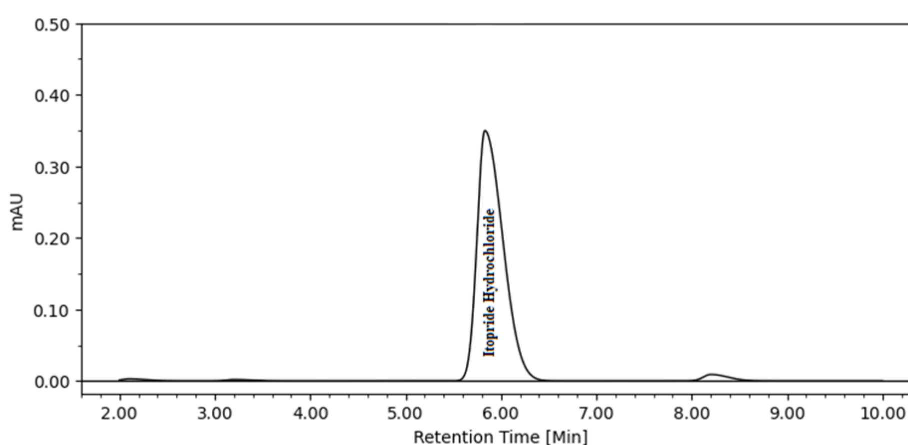

Figure 2: Chromatogram Showing Retention Peak.

Precision

The precision of the developed RP-HPLC method was evaluated by analyzing six independently prepared sample solutions from the same batch of Itopride Hydrochloride tablets. The low percentage relative standard deviation (%RSD) of the assay values demonstrated excellent repeatability of the method. Intermediate precision was assessed by a second analyst on a different day using a separate chromatographic column. Comparable results were obtained, confirming the ruggedness and reproducibility of the method. The results of repeatability and intermediate precision are presented in Table 2, Figure 3, and Table 3.

Table 2: Precision of Itopride Hydrochloride.

Sample No.	Peak Area	% Assay
1	945862	99.4
2	952174	100.1
3	949387	99.8
4	956428	100.5
5	951216	100.0
6	947735	99.7
Mean	—	**99.92**
%RSD	—	**0.39**

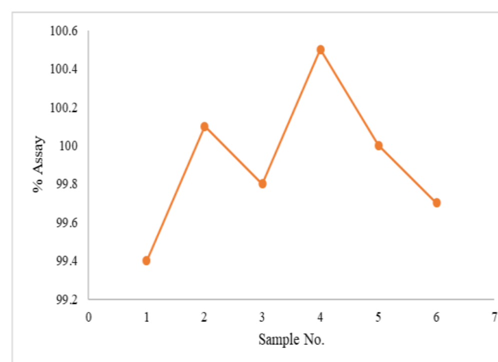

Figure 3: Precision % Assay of Itopride Hydrochloride.

Table 3: Intermediate Precision of Itopride Hydrochloride.

Sample No.	Peak Area	% Assay
1	938742	99.6
2	944318	100.2
3	941576	99.9
4	947865	100.5
5	943227	100.1
6	949104	100.7
Mean	—	**100.17**
%RSD	—	**0.40**

Linearity and Range

The linearity of the developed RP-HPLC method for Itopride Hydrochloride was evaluated over a concentration range corresponding to 50%–150% of the target assay concentration. A series of standard solutions were prepared and analyzed under optimized chromatographic conditions. The calibration curve demonstrated an excellent linear relationship between concentration and peak area, with a correlation coefficient (R^2 = 0.9998), indicating outstanding proportionality within the studied range. The linearity data are presented in Table 4, and the corresponding calibration curve is shown in Figure 4.

Table 4: Linearity of Itopride Hydrochloride.

Concentration (%)	Peak Area
50	471250
80	754180
100	941520
120	1132580
150	1416720

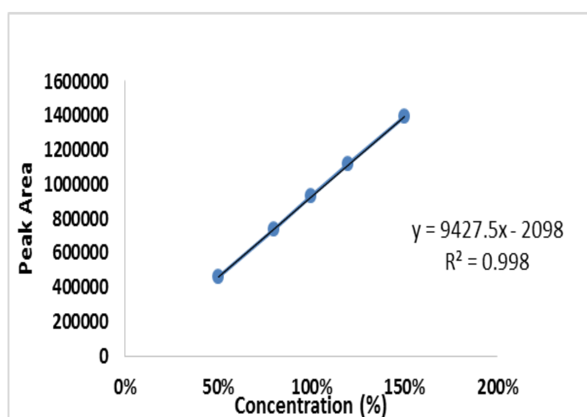

Figure 4: Linearity Curve of Itopride Hydrochloride.

Accuracy

The accuracy of the developed RP-HPLC method for Itopride Hydrochloride was evaluated by recovery studies performed at three concentration levels, namely 50%, 100%, and 150% of the target concentration. Known amounts of Itopride Hydrochloride standard were added to pre-analyzed sample solutions, and the percentage recovery was determined. The mean recovery values obtained were within the acceptable range of 98–102%, demonstrating the accuracy of the method. The recovery data are presented in Table 5.

Table 5: Accuracy (Recovery) of Itopride Hydrochloride.

Level (%)	% Recovery
50%	99.1, 99.4, 99.7
100%	100.2, 100.6, 100.9
150%	100.4, 100.8, 101.1
Mean	**100.24**

System Suitability

System suitability testing was performed before method validation and sample analysis to ensure that the chromatographic system was functioning properly and capable of producing reliable results. The evaluated parameters included retention time, peak area, tailing factor, theoretical plates, and percentage relative standard deviation (%RSD) of peak areas obtained from six replicate injections of the standard solution. The results demonstrated good peak symmetry, high column efficiency, and excellent repeatability of injections. All system suitability parameters complied with the acceptance criteria recommended by regulatory guidelines, including a tailing factor of less than 2.0, theoretical plates greater than 2000, and %RSD of peak areas below 2.0. These findings confirm that the chromatographic system was suitable for the routine quantitative analysis of Itopride Hydrochloride. The results are presented in Table 6.

Table 6: System Suitability Parameters for Itopride Hydrochloride.

Injection No.	Retention Time (min)	Peak Area	Tailing Factor	Theoretical Plates
1	5.84	941520	1.02	4285
2	5.83	938742	1.01	4312
3	5.84	944318	1.03	4268
4	5.85	941576	1.02	4295
5	5.83	947865	1.01	4338
6	5.84	943227	1.02	4305
Mean	**5.84**	—	**1.02**	**4301**
%RSD (Area)	—	**0.34**	—	—

Robustness

The robustness of the developed RP-HPLC method for Itopride Hydrochloride was evaluated by deliberately introducing small variations in chromatographic conditions, including column temperature, mobile phase composition, flow rate, and detection wavelength. The influence of these variations on retention time, tailing factor, theoretical plates, %RSD, and assay values was assessed. The results demonstrated that minor changes in analytical parameters did not significantly affect chromatographic performance. All system suitability parameters remained within acceptable limits, confirming the reliability and robustness of the method. The robustness data are presented in Table 7.

Table 7: Robustness Parameters for Itopride Hydrochloride.

Condition Modified	Retention Time (min)	Tailing Factor	Theoretical Plates	%RSD	Assay (%)
Standard Condition	5.84	1.02	4301	0.34	99.92
Column Temperature: 20°C	6.25	1.11	4125	0.37	100.10
Column Temperature: 30°C	5.42	1.08	4058	0.32	99.85
Mobile Phase Ratio (65:35 Buffer:Methanol)	6.82	1.06	4456	0.35	100.24
Mobile Phase Ratio (55:45 Buffer:Methanol)	4.96	1.15	3982	0.38	99.76
Detection Wavelength: 218 nm	5.83	1.03	4218	0.31	100.05
Detection Wavelength: 222 nm	5.84	1.04	4265	0.33	100.18
Flow Rate: 0.8 mL/min	6.71	1.09	4176	0.36	99.88
Flow Rate: 1.2 mL/min	4.91	1.12	4015	0.35	99.94

Detection threshold (LOD) and quantification threshold (LOQ)

The developed RP-HPLC method demonstrated high analytical sensitivity with an LOD of 0.32 µg/mL and an LOQ of 1.08 µg/mL. These values indicate that the method is capable of reliably detecting and quantifying trace levels of Itopride Hydrochloride, making it suitable for routine quality control and assay applications (Table 8).

Table 8: Detection and Quantification Limits of Itopride Hydrochloride.

Parameter	Value (µg/mL)
Limit of Detection (LOD)	0.32
Limit of Quantification (LOQ)	1.08

Forced Degradation Investigations

The forced degradation study revealed that Itopride Hydrochloride underwent slight to moderate degradation under the applied stress conditions. The highest degradation was observed under oxidative stress, where approximately 78.85% of the drug remained, while acidic, alkaline, and thermal conditions resulted in drug recovery values ranging from 89.85% to 92.05% (Table 9). In all cases, degradation products were well resolved from the main drug peak, confirming the specificity and stability-indicating nature of the developed RP-HPLC method. This demonstrates that the method is suitable for routine stability testing and quality control analysis of Itopride Hydrochloride pharmaceutical formulations.

Table 9: Results of Forced Degradation Study for Itopride Hydrochloride.

Stress Condition	Average Peak Area	% Assay Remaining
Acidic Hydrolysis	858420	91.20
Alkaline Hydrolysis	845630	89.85
Oxidative Degradation	742180	78.85
Thermal Degradation	866540	92.05

CONCLUSION

The present study successfully developed and validated a reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative estimation of Itopride Hydrochloride in tablet dosage forms. The developed method was found to be simple, accurate, precise, robust, sensitive, and reliable for routine pharmaceutical analysis. Validation studies demonstrated satisfactory performance with respect to specificity, linearity, accuracy, precision, robustness, and system suitability parameters in accordance with ICH guidelines. The method exhibited excellent linearity over the concentration range of 50–150% with a correlation coefficient (R^2) of 0.9998. Precision studies yielded low %RSD values, indicating good repeatability and reproducibility, while recovery studies confirmed the accuracy of the method. The retention time of Itopride Hydrochloride was established at approximately 5.84 minutes, allowing rapid and efficient analysis. The low LOD (0.32 µg/mL) and LOQ (1.08 µg/mL) values demonstrated the high sensitivity of the method. Furthermore, forced degradation studies under acidic, alkaline, oxidative, and thermal stress conditions confirmed the stability-indicating capability of the method, as degradation products were well separated from the principal drug peak without interference. Therefore, the developed RP-HPLC method can be effectively employed for routine quality control, assay determination, and stability studies of Itopride Hydrochloride in pharmaceutical dosage forms due to its simplicity, accuracy, reproducibility, and reliability.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the support of Shri Shankaracharya Institute of Pharmaceutical Sciences and Research in the successful completion of this research work.

Conflict of Interest
The authors declare that there is no conflict of interest associated with this research work.

Funding
Nil.

REFERENCE

1. ICH. *Validation of Analytical Procedures: Text and Methodology Q2(R1)*. International Conference on Harmonization, 2015. https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf
2. Basha, M., Chandu, B. R., Reddy, P. S., Ahmed, S., & Venkatesh, B. (2014). Development and validation of RP-HPLC method for the estimation of Metoclopramide in bulk and tablet dosage form. *Journal of Chemical and Pharmaceutical Sciences, 7*(2): Article 140–143.
3. RaviKKumar VR, Rathi S, Singh S, Patel B, Singh S, Chaturvedi K, Sharma B. A Comprehensive Review on Ulcer and Their Treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2023 Dec 21; 39: e20230006. doi: 10.62958/j.cjap.2023.006. PMID:38755116.
4. Singh, V., Arora, S., Akram, W., Alam, S., Kumari, L., Kumar, N., Kumar, B., Kumar, S., Agrawal, M., Singhal, M., Kumar, S., Singh, S., Singh, K., Saha, S., & Dwivedi, V. (2024). Involvement of molecular mechanism and biological activities of Pemirolast: A therapeutic review. *New Emirates Medical Journal*, *5*, Article e02506882308410. https://doi.org/10.2174/010250688230841024060705 3814
5. Rajput DS, Gupta N, Singh S, Sharma B. A Comprehensive Review: Personalized Medicine for Rare Disease Cancer Treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2023 Dec 23; 39: e20230008. doi: 10.62958/j.cjap.2023.008. PMID: 38830754.
6. Patel, M., Shah, D., Parmar, R., Mehta, T., & Patel, G. A review on pharmacological and analytical aspects of Metoclopramide Hydrochloride. *International Journal of Pharmaceutical Sciences Review and Research,* 2011; 9(1): 33–37.
7. Singh S, Chaurasia A, Rajput DS, Gupta N. Mucoadhesive Drug Delivery System and There Future Prospective: Are a Promising Approach for Effective Treatment? Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2023 Dec 20; 39: e20230005. doi: 10.62958/j.cjap.2023.005. PMID: 38751344.
8. Kumar, S., Saha, S., Sharma, B., Singh, S., Shukla, P., Mukherjee, S., Agrawal, M., Singh, K., & Singh, T. (2023). The role of resveratrol in Alzheimer's disease: A comprehensive review of current research. *Current Functional Foods*, *2*(2): Article e121223224364, 13 pages. https://doi.org/10.2174/012666862926924423112707 1411
9. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, Bumrela S, Jain PC, Goswami P, Singh S. Recent Innovations and Future Perspectives in Transferosomes for Transdermal Drug Delivery in Therapeutic and Pharmacological Applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2024 Oct 24; 40: e20240031. doi: 10.62958/j.cjap.2024.031. PMID: 39442957.
10. Vaghela MC, Rathi S, Shirole RL, Verma J, Shaheen, Panigrahi S, Singh S. Leveraging AI and Machine Learning in Six-Sigma Documentation for Pharmaceutical Quality Assurance. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2024 Jul 18; 40: e20240005. doi: 10.62958/j.cjap.2024.005. PMID: 39019923.
11. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, Bumrela S, Jain PC, Goswami P, Singh S. Recent Innovations and Future Perspectives in Transferosomes for Transdermal Drug Delivery in Therapeutic and Pharmacological Applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2024 Oct 24; 40: e20240031. doi: 10.62958/j.cjap.2024.031. PMID: 39442957.
12. Kumar, S., Saha, S., Pathak, D., Singh, T., Kumar, A., Singh, K., Mishra, A. K., Singh, S., & Singh, S. (2024). Cholesterol absorption inhibition by some nutraceuticals. *Recent Advances in Food, Nutrition & Agriculture*, *16*(1): 2–11. https://doi.org/10.2174/012772574X285280240220065812
13. Singh, S., Chaurasia, A., Rajput, D. S., & Gupta, N. (2024). An overview on mucoadhesive buccal drug delivery systems & approaches: A comprehensive review. *African Journal of Biological Sciences (South Africa)*, *6*(5): 522–541, DOI: 10.33472/AFJBS.6.5.2024.522-541
14. Kumar, S., Singh, S., Rajput, D., Sharma, B., Chaturvedi, K., Singh, N., Saha, S., Singh, K., & Mukherjee, S. (2024). Pharmacological approaches and herbal interventions for Alzheimer's disease. *The Natural Products Journal*, *14*(8): Article e220124225945. https://doi.org/10.2174/012210315527526623112309 0138
15. Ravikkumar VR, Patel BD, Rathi S, Parthiban S, Upadhye MC, Shah AM, Rehan SSA, Samanta S, Singh S. Formulation and Evaluation of Drumstick Leaves Tablet as An Immunomodulator. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2024 Jun 21; 40: e20240004. doi:10.62958/j.cjap.2024.004. PMID: 38902996.
16. Sharma, A., Bara, G., Keshamma, E., Sharma, B., Singh, S., Singh, S. P., Parashar, T., Rathore, H. S., Sarma, S. K., & Rawat, S. (2023). Cancer biology and therapeutics: A contemporary review. *Journal of Cardiovascular Disease Research*, *14*(10): 1229-1247.
17. Singh S, Chaurasia A, Gupta N, Rajput DS. Effect of Formulation Parameters on Enalapril Maleate Mucoadhesive Buccal Tablet Using Quality by Design (QbD) Approach. Zhongguo Ying Yong Sheng

Li Xue Za Zhi, 2024 Jun 27; 40: e20240003. doi: 10.62958/j.cjap.2024.003. PMID: 38925868.

18. Patel SK, Prathyusha S, Kasturi M, Godse KC, Singh R, Rathi S, Bumrela S, Singh S, Goswami P. Optimizing Irbesartan Fast Dissolving Tablets Using Natural Polysaccharides for Enhanced Drug Delivery and Patient Compliance. *Int Res J Multidiscip Scope (IRJMS)*, 2025; 6(1): 1181-1190. https://doi.org/10.47857/irjms.2025.v06i01.02542