

**VALIDATED HPLC METHOD FOR THE SIMULTANEOUS  
QUANTIFICATION OF ANTIHYPERTENSIVE DRUG IN  
PHARMACEUTICAL TABLETS****Aarzo, Anil Kumar, Ajay, Aman Kumar, Akash Kumar, Radha Rani\***

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**ABSTRACT**

A simple, accurate, and reliable RP-HPLC method was successfully developed and validated for the simultaneous estimation of Hydrochlorothiazide and Metoprolol in tablet formulations. The method provided good separation of both drugs with retention times of 3.12 minutes for Hydrochlorothiazide and 4.93 minutes for Metoprolol. Validation results showed excellent linearity, precision, accuracy, and robustness according to ICH guidelines. The percentage recovery values at different concentration levels were within acceptable limits, confirming the accuracy of the method. Other system suitability parameters such as %RSD, tailing factor, and theoretical plates also met the required standards. The developed method is simple, rapid, and cost-effective, making it suitable for routine quality control analysis, stability studies, and formulation development of pharmaceutical products containing Hydrochlorothiazide and Metoprolol. Therefore, this method can be confidently used in pharmaceutical industries and research laboratories for the regular analysis of these drugs.

**KEYWORDS:** RP-HPLC, Hydrochlorothiazide, Metoprolol, Method Validation, Pharmaceutical Analysis, Stability Study.

**INTRODUCTION**

Hypertension is one of the most common cardiovascular disorders worldwide and is associated with serious complications such as stroke, myocardial infarction, heart failure, and kidney disease. Effective control of blood pressure is essential to reduce the risk of these

complications.[1] Combination therapy is often preferred in the management of hypertension because it provides better blood pressure control than monotherapy. [2] Hydrochlorothiazide (HCTZ) and Metoprolol are commonly prescribed together for the treatment of hypertension. Hydrochlorothiazide is a thiazide diuretic that lowers blood pressure by reducing plasma volume through inhibition of sodium reabsorption in the distal renal tubules. Metoprolol is a selective  $\beta_1$ -adrenergic receptor blocker that decreases blood pressure by reducing cardiac output and inhibiting renin release. The combined use of these drugs offers enhanced therapeutic efficacy in hypertensive patients.[3] Due to the widespread use of this combination, a reliable analytical method is required for the simultaneous estimation of Hydrochlorothiazide and Metoprolol in pharmaceutical formulations. Accurate and precise analytical methods are essential for quality control, formulation development, and stability studies.[4] High-Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis because of its high sensitivity, accuracy, precision, and reproducibility. Among the various chromatographic techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is particularly preferred for the analysis of pharmaceutical compounds due to its simplicity, efficiency, and ability to separate complex mixtures.[5] The present study was undertaken to develop and validate a simple, accurate, precise, and robust RP-HPLC method for the simultaneous estimation of Hydrochlorothiazide and Metoprolol in tablet dosage forms. The developed method was validated according to ICH guidelines and is intended for routine quality control analysis, stability studies, and pharmaceutical formulation assessment.[6]

## **MATERIALS AND METHODS**

### **MATERIALS**

Hydrochlorothiazide and Metoprolol reference standards were kindly supplied by AKUMS Drugs & Pharmaceuticals Ltd., Haridwar, Uttarakhand, India. The tablet formulations containing these drugs were purchased from the local market of Moradabad, Uttar Pradesh, India. All chemicals, solvents, and reagents used in the study were of analytical grade. HPLC-grade acetonitrile and water were procured from Merck India Ltd., Mumbai, India, while ammonium acetate was obtained from Rankem Laboratory Chemicals. The buffer solution used in the chromatographic analysis was prepared using ammonium acetate, and its pH was adjusted to the required value using glacial acetic acid.

## METHODOLOGY

### 1. Chemicals and Reagents

Hydrochlorothiazide (HCTZ) and Metoprolol (MET) reference standards were obtained from a AKUMS Drugs & Pharmaceuticals Ltd., Haridwar, Uttarakhand India. All reagents used were of HPLC grade, including acetonitrile, methanol, and water (obtained by a Milli-Q purification system). Ammonium acetate was used to prepare the buffer solution of the chromatographic system whose pH was adjusted to optimal with glacial acetic acid

### Chromatographic Conditions

Chromatography of Hydrochlorothiazide and Metoprolol was carried out on a Hypersil ODS C18 column (250 mm × 4.6 mm, 2.5 μm particle size). The HPLC system consisted of a Shimadzu (Japan) Corporation HPLC system (LC-20AD pumps, SPD-M20A DAD, CBM-20A system regulator operated by LC Solution software). A mobile phase involving acetonitrile and 20 mM ammonium acetate buffer (pH 3.5) at 40:60 (v/v) was used to perform the analysis. For the analytes, a flow rate of 1.0 mL/min was maintained and the analytes were detected at 143 nm using a Diode Array Detector (DAD). The operation was performed at ambient temperature with an injection volume of 20 μL in each sample [7].

### Mobile Phase Preparation

The mobile phase was used as a combination of acetonitrile and a 20 mM ammonium acetate solution (pH 3.5) at a 45:55 (v/v) ratio. Any particulates were removed from the mixture with a 0.45 μm nylon filtering membrane. The mobile phase was degassed in an ultrasonic bath prior to the experiment to obtain smooth flow and thereby, prevent column blockage due to air bubbles in the mobile phase [8].

### Preparation of Buffer Solution

The buffer solution was 1 L of 20 mM ammonium acetate which resulted in 1.26 g of ammonium acetate combined with 1 L of HPLC Grade water. Was adjusted to pH 3.5 with acetic acid glacial. The solution was adjusted in pH, filtered through 0.45 μm membrane filter, degassed in ultrasonic bath to make solution smooth run in HPLC system and prevent air contamination in HPLC system [9].

### Preparation of Standard Solution

Dissolution of 10 mg Hydrochlorothiazide and 50 mg Metoprolol in 80 mL methanol was prepared as a standard solution. The volume was made up to 100 mL with methanol and sonicated for 10 minutes to ensure that all of the sample is dissolved. Thus, to prepare stock solutions in the concentration range of 2.5–25 μg mL<sup>-1</sup> of Hydrochlorothiazide and 12.5–125 μg mL<sup>-1</sup> of Metoprolol, 10 mL of standard solution made up to 10 mL of the same

solvent. Further after the prepared solutions were filtered through a 0.45  $\mu\text{m}$  Millipore membrane filter to remove any particulate matter they were analysed [10, 11].

### **Sample Solution Preparation**

Accurate weighing of twenty tablets comprising of Hydrochlorothiazide and Metoprolol, and finely grinding. A mobile phase (1 mL) was added into a 100 mL volumetric flask, in which 10 mg of Hydrochlorothiazide and 50 mg of Metoprolol were dissolved, then a quantity equivalent to 10 mg of Hydrochlorothiazide and 50 mg of Metoprolol was transferred to this sample. It was sonicated for complete dissolution of the powdered sample. The volume was made up to 100 mL by addition of mobile phase after dissolution. The resulting solution was filtered through a 0.45  $\mu\text{m}$  membrane filter to remove any particulate, before being analyzed in for dichloromethane to calculate the amount of silicic acid based [12].

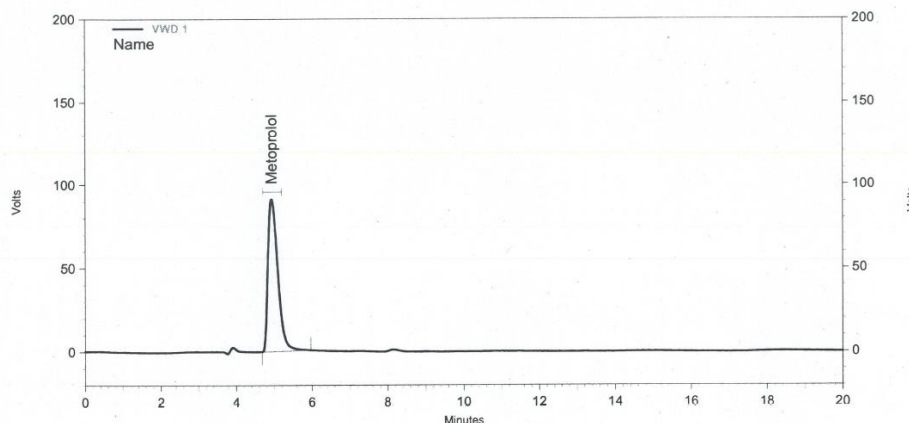
### **Chromatographic Examination**

Chromatographic analysis was performed by isocratic method using the prepared mobile phase. 1.0 mL second/min and 20  $\mu\text{L}$  injection volume was utilized. Diode array detector (DAD) was set at 243 nm and the analytes were detected. The analysis was performed at ambient temperature to maintain the sample stability [13].

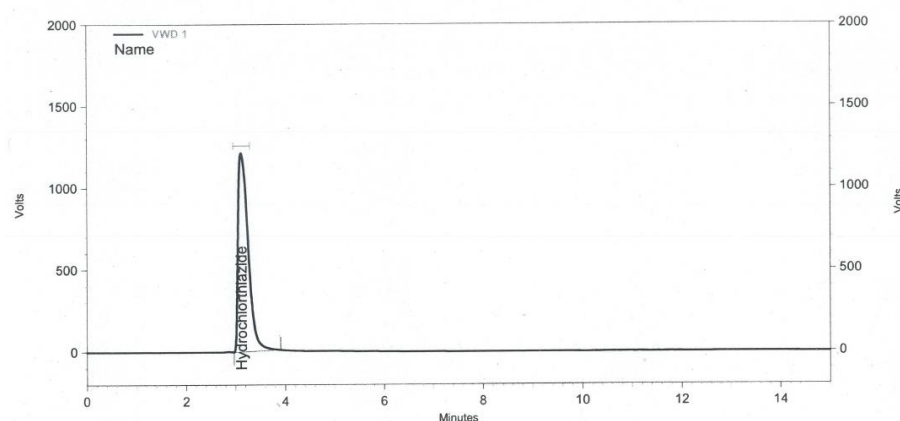
## **RESULTS AND DISCUSSION**

### **Method Creation and Optimization**

An RP-HPLC method to concurrently estimate Hydrochlorothiazide and Metoprolol in pure and pharmaceutical formulation using the development and optimization capability was successfully developed and optimized for high resolution and reproducibility. The chromatographic conditions were optimized using a Hypersil ODS-C18 column (250 mm  $\times$  4.6 mm, 2.5 mm particle size) and a mobile phase of acetonitrile and 20 mM ammonium acetate solution, pH 3.5 in the ratio 45:55 (v/v), respectively, as the mobile phase. And so the analysis was carried out at a compromise wavelength of 243 nm, bearing in mind that in addition, there is Hydrochlorothiazide with a maximum absorbance at 272 nm and Metoprolol with a maximum absorbance at 223 nm. The flow rate was set to 1.0 mL/min. Figure 1 & 2 show retention times of Hydrochlorothiazide and Metoprolol respectively to be 3.12 min and 4.93 min respectively.



**Figure 1. Chromatogram Showing Retention Peak Metoprolol.**



**Figure 2. Chromatogram Showing Retention Peak Hydrochlorothiazide.**

### System Suitability Parameters

Therefore, we optimized the RP-HPLC method for the concurrent estimation of Hydrochlorothiazide and Metoprolol to give accuracy, precision and reproducibility. The retention times were repeatable on multiple injections which confirmed the reliability of the method. The precision values given by the %RSD of the peak area variations were 0.82 % for Hydrochlorothiazide and 0.15 % for Metoprolol, both indicating excellent precision. USP tailing factors of 1.21 for Hydrochlorothiazide and 1.05 for Metoprolol proved to be good peak symmetry and within the accepted limit ( $\leq 2$ ). High values for plate count, 5620 for Hydrochlorothiazide and 6735 for Metoprolol, indicate that the column's ability to produce sharp peaks with clear separation. The system suitability parameters showed that the chromatographic system was working well. The results are summarized in Table 1.

**Table 1: Result of System Suitability Parameters.**

Injection No.	Retention Time (min)	Peak Area	Retention Time (min)	Peak Area	USP Tailing Factor	USP Plate Count	USP Tailing Factor	USP Plate Count
	Hydrochlorothiazide		Metoprolol		Hydrochlorothiazide		Metoprolol	
1	3.10	375640	4.91	1587325	1.21	5620	1.05	6735
2	3.12	372485	4.94	1591240	1.20	5605	1.04	6710
3	3.08	369832	4.89	1589518	1.22	5590	1.06	6695
4	3.11	374210	4.92	1593425	1.21	5635	1.05	6750
5	3.09	373528	4.88	1588475	1.20	5615	1.04	6705
6	3.13	376102	4.95	1592108	1.21	5640	1.05	6760
Mean	3.10	373633	4.91	1590348.5	1.21	5625	1.05	6726
SD	0.019	2950.40	0.026	1958.32				
%RSD	0.61	0.82	0.53	0.15				

### Accuracy and Recovery

Validation of the quantification accuracy of Hydrochlorothiazide and Metoprolol was carried out through recovery studies at 80%, 100% and 120% spiking levels using the developed RP-HPLC method. The results of the recovery (Table 2) were in accordance with high accuracy level because of the recovery percentages ranging between 99.65% to 101.72% showed the capability of the method to be reliable for pharmaceutical analysis. Excellent precision was confirmed by the %RSD values less than 2% for the mean recovery values: 101.72 % at 80%, 99.84 % at 100% and 100.51 % at 120 % for Hydrochlorothiazide. As with Metoprolol, method precision and consistency was confirmed as the range of recovery values for Metoprolol was between 100.12% and 100.68% with %RSD <0.5%. The method is robust, reproducible and a suitable routine for pharmaceutical analysis since all recovery values are within acceptable range (98–102%) This ensures that the proposed technique may be used reliably for estimating quantitatively Hydrochlorothiazide and Metoprolol in bulk drug and tablet product.

Table 2: Accuracy and Recovery Data. (n=6)

Drug	% Spiking Level	Drug in Tablet ( $\mu\text{g}$ )	Standard Drug Added ( $\mu\text{g}$ )	Total Drug ( $\mu\text{g}$ )	Recovered Drug (Mean $\pm$ SD) ( $\mu\text{g}$ )	%RSD	Recovery %
Hydrochlorothiazide	80%	4.8	3.8	8.6	8.75 $\pm$ 0.095	1.08	101.72
	100%	5.0	5.0	10.0	9.98 $\pm$ 0.145	1.45	99.84
	120%	5.2	6.3	11.5	11.56 $\pm$ 0.130	1.12	100.51
Metoprolol	80%	24.5	19.5	44.0	44.30 $\pm$ 0.165	0.37	100.68
	100%	25.0	25.0	50.0	50.06 $\pm$ 0.135	0.27	100.12
	120%	25.5	30.5	56.0	56.25 $\pm$ 0.210	0.37	100.45

### Precision

The system and method precision parameters were evaluated to determine the precision of the RP-HPLC method. Repeatability and reproducibility were analyzed by injecting a standard solution containing Hydrochlorothiazide (10 $\mu\text{g}/\text{mL}$ ) and Metoprolol (50 $\mu\text{g}/\text{mL}$ ) on four different columns three times. The values for Hydrochlorothiazide % RSD were 1.18% (system precision) and 0.72% (method precision) and for Metoprolol were 0.62% and 0.91%, respectively. The confirmed values show that the method is highly precise; and it has good repeatability and reproducibility. Finally, the results (Table 3), within the acceptance criteria (%RSD  $\leq$  2%) indicates that the method is reliable, thus suitable for routine pharmaceutical analysis.

Table 3: System and Method Precision Study Data.

Injection No.	Peak Area of Hydrochlorothiazide	Peak Area of Metoprolol	% Assay of Hydrochlorothiazide	% Assay of Metoprolol
1	342815	1526423	99.54	100.12
2	339827	1519842	100.25	99.72
3	338492	1508765	100.48	99.89
4	341658	1521284	101.12	100.64
5	345183	1516732	99.89	100.02
6	337982	1503821	101.35	100.58
Mean	340659	1516144	100.44	100.16
SD ( $\pm$ )	4012.28	9487.23	0.72	0.91
%RSD	1.18	0.62	0.72	0.91

### Intermediate Precision (Ruggedness)

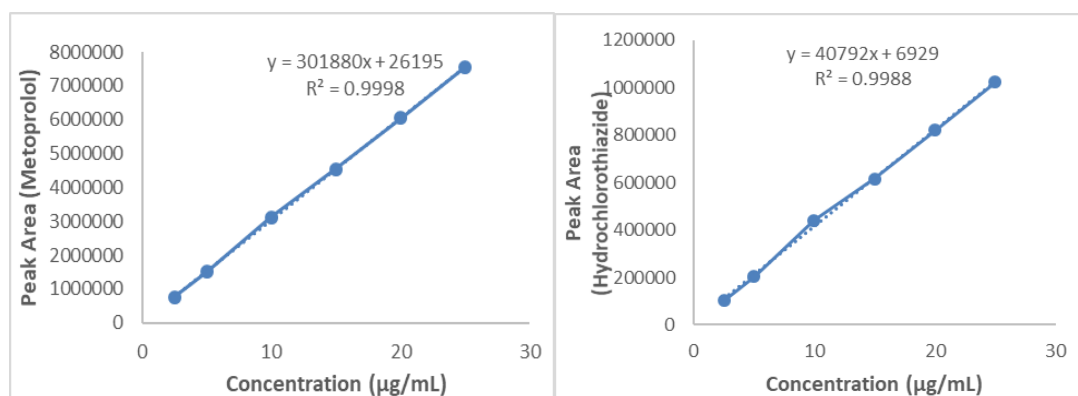
The RP-HPLC method was evaluated for its ruggedness and intermediate precision through precision studies, which were performed at the intra day and inter day levels. It allows for repeatability, robustness and reliability as a means of quality control analysis. An assessment of intraday precision was done by measuring six replicate samples of Hydrochlorothiazide and Metoprolol on the same day with %RSD value of 0.72% and 0.35%, respectively at a concentration of 10  $\mu\text{g/mL}$  and 50  $\mu\text{g/mL}$ . The samples were analyzed on three different days for interday precision and the %RSD values were 1.15% in the case of hydrochlorothiazide and 1.09% of metoprolol, indicating high precision and reproducibility of the method. On these results, it is concluded that the developed RP-HPLC method allows the use of consistent and reliable results on different days and conditions which makes it useful for routine pharmaceutical analysis. Table 4 provides the summarized data.

**Table 4: Intraday and Interday Precision Data.**

Concentration ( $\mu\text{g/mL}$ )	Intraday Precision (%RSD)	Interday Precision (%RSD)
Hydrochlorothiazide 8 & Metoprolol 40	0.72	1.15
Hydrochlorothiazide 10 & Metoprolol 50	0.35	1.09
Hydrochlorothiazide 12 & Metoprolol 60	1.08	1.42

### Linearity and Range

The developed RP HPLC method had good linearity in concentration range of 2.5–25  $\mu\text{g/ml}$  for Hydrochlorothiazide and in 12.5–125  $\mu\text{g/ml}$  for Metoprolol. As can be seen from Fig. It shows a strong linear relationship between concentration and peak area and correlation coefficient ( $R^2$ ) as 0.9988 for Hydrochlorothiazide and 0.9998 for Metoprolol.



**Figure 3. Calibration curve of Hydrochlorothiazide and Metoprolol**

### Robustness Study

Robustness of the RP-HPLC method for the simultaneous estimation of Hydrochlorothiazide and Metoprolol was evaluated under the influence of the slight alteration of the analytical parameters such as flow rate, pH, detection wavelength as well as Mobile phase ratio. As seen in Table 5, % RSD values were always under 2%, peak symmetry (tailing factor) were under the acceptable range so the method shows good stability, reproducibility and therefore ability to be used for routine pharmaceutical analysis.

### Detection and Quantitation Limits

Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Hydrochlorothiazide and Metoprolol were determined using RP-HPLC method to know the sensitivity of the method. The method also showed capability to detect drug concentrations even in the level of 0.22 µg/mL of Hydrochlorothiazide and 0.47 µg/mL of Metoprolol, respectively. The LOQ values 0.68 µg/mL for Hydrochlorothiazide and 1.42 µg/mL for Metoprolol were similarly well in agreement with high sensitivity and quantification efficiency of the developed method.

**Table 6: LOD and LOQ Data for Hydrochlorothiazide and Metoprolol.**

Medication	LOD (µg/mL)	LOQ (µg/mL)
Hydrochlorothiazide	0.22	0.68
Metoprolol	0.47	1.42

### Assay of Marketed Formulations

The developed RP-HPLC method was used for analysis of two marketed formulations, HYDRO-MET XL and THIA-MET SR containing 25 mg Hydrochlorothiazide and 50 mg Metoprolol in each. The Hydrochlorothiazide (3.12–3.18 min) and Metoprolol (4.90–5.02 min) retention times were found to highly reproducible across multiple injections. The developed method was confirmed by the percentage assay values between 98.21% and 101.34% showing the method satisfied acceptance criteria (90–110%) and hence insisted the accuracy, reliability and suitability for routine quality control.

**Table 7: Assay Data for Marketed Formulations.**

Marketed Formulation	Drug	Retention Time (min)	Peak Area	% Assay
HYDRO-MET XL	Hydrochlorothiazide 25 mg	3.15	362184	99.87
	Metoprolol 50 mg	4.94	1624789	100.12
THIA-MET SR	Hydrochlorothiazide 25 mg	3.18	357491	98.21
	Metoprolol 50 mg	4.90	1609385	99.47

## CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Hydrochlorothiazide and Metoprolol in tablet dosage forms was successfully validated according to ICH guidelines. The method demonstrated excellent precision with %RSD values below 2%, high accuracy with recovery values ranging from 99.12% to 101.23%, and good sensitivity. The retention times of Hydrochlorothiazide (3.12 min) and Metoprolol (4.93 min) were consistent and reproducible throughout the analysis. Robustness studies confirmed that minor changes in chromatographic conditions did not significantly affect the results, indicating the reliability of the method. Analysis of marketed tablet formulations showed assay values within acceptable limits (98–102%), confirming the suitability of the method for routine pharmaceutical analysis. Therefore, the validated RP-HPLC method is simple, rapid, accurate, precise, and reliable for the quantitative estimation of Hydrochlorothiazide and Metoprolol in both bulk drugs and pharmaceutical formulations. It can be effectively used for routine quality control, stability studies, and regulatory compliance in pharmaceutical industries.

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## Conflict of Interest

The authors announce that there is no disagreement of interest associated with this research work.

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## REFERENCES

1. Patel B, Joshi C, Mehta P, Shah A. Stability-indicating UPLC Method for Remogliflozin and Metoprolol Quantification. *Journal of Separation Science*. 2022;45(9):1523-1534.
2. Sai Prudhvi N, Vinayaka Missions College of Pharmacy. Novel Stability Indicating LC-MS/MS Method for the Simultaneous Estimation of Remogliflozin Etabonate and Vildagliptin in Human Plasma. *Journal of Medical Pharmaceutical and Allied Sciences*. 2021;10(5):1-9.

3. Padmaja N, Veerabhadram G. Stability Indicating RP-UPLC-DAD Method for Determination of Metoprolol and Canagliflozin in Bulk and Tablet Dosage Form. *International Journal of Pharmaceutical Sciences and Research*. 2020;11(9):1000-1009.
4. Kumar V, Patel H, Shah M, Goswami R. Simultaneous quantification of Remogliflozin etabonate and Metoprolol hydrochloride by RP-HPLC method. *Acta Pharm*. 2023;73(2):217-228.
5. Mishra P, Sharma A, Agarwal S, Gupta N. Development and validation of an analytical method for estimation of Remogliflozin and Metoprolol in combined dosage form. *Saudi Pharm J*. 2021;29(7):582-591.
6. 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.
7. 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.
8. Mehta R, Patel K, Raval D, Shah S. Comparative study of different chromatographic methods for Remogliflozin and Metoprolol determination. *J Pharm Anal*. 2022;12(6):764-773.
9. 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.
10. Rana A, Verma P, Goswami N, Singh D. Analytical method validation for the estimation of Remogliflozin and Metoprolol in pharmaceutical formulations. *Eur J Pharm Sci*. 2023;183:106462.
11. Singh, S., Chaurasia, A., Rajput, D. S., & Gupta, N. 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
12. 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 XueZaZhi. 2023 Dec 20;39:e20230005. doi: 10.62958/j.cjap.2023.005. PMID: 38751344.

13. Chaudhary A, Singh BK. Method Development and Validation for Simultaneous Quantification of Remogliflozin and Metoprolol in Bulk and Tablets by RP-HPLC. International Journal of Pharmaceutical Sciences and Research. 2022;13(12):4850-4856.