ejpmr, 2023, 10(9), 435-442

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR ESTIMATION OF TELMISARTAN AND METOPROLOL SUCCINATE BY UHPLC

Asarudeen A.[1*], Chitra M.[1] and Senthilkumar Natesan[2]

[1]Department of Pharmaceutical Analysis, JKKMMRFs- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalyam, Namakkal, Tamil Nadu-638183, India.

[2]Department of Pharmaceutical Chemistry, JKKMMRFs- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalyam, Namakkal, Tamil Nadu-638183, India.

***Corresponding Author: Asarudeen A.**

Department of Pharmaceutical Analysis, JKKMMRFs- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalyam, Namakkal, Tamil Nadu-638183, India.

Article Received on 29/06/2023 **Article Revised on 19/07/2023** **Article Accepted on 09/08/2023**

ABSTRACT

This study's objective was to design and validate a new Ultra-High-Performance Liquid Chromatography (UHPLC) method for the simultaneous determination of Telmisartan and Metoprolol Succinate in tablet formulations. This method was developed with an emphasis on accuracy, reproducibility, and a lower relative standard deviation (RSD), compliant with International Council for Harmonization (ICH) guidelines for method validation. The method's precision was confirmed by the low percent relative standard deviation (%RSD) values, with Telmisartan and Metoprolol Succinate recording 0.49 and 0.59 respectively. This implies the high reliability of the assay and retention times across repeated measurements. System suitability parameters were evaluated to ensure the efficiency of the chromatographic system, indicated by a plate count greater than 2000. A tailing factor below 2 further reinforced the method's efficiency, suggesting symmetrical and well-shaped peaks. The robustness of the method was further underscored by its excellent linearity. The linearity equations for Telmisartan ($y = 0.0298x - 0.0285$) and Metoprolol Succinate ($y = 0.0686x + 0.1282$) both produced correlation coefficients (R^2) close to 1, demonstrating excellent linearity in the relationship between concentration and peak area. The method's accuracy was established by calculating the mean percent recovery after performing triplicate injections at each level. The method achieved high mean percent recovery values for both Telmisartan (98.95% and 100.19%) and Metoprolol Succinate (99.68% and 100.21%). Finally, the method precision and intermediate precision were confirmed by the low %RSD values for both drugs (0.27% for Telmisartan and 0.23% for Metoprolol Succinate), which fell below the acceptable limit of 2. The robustness of the method was further corroborated when system suitability parameters remained unaffected despite variations in conditions. In conclusion, the newly developed UHPLC method provides an efficient, robust, and precise tool for the simultaneous estimation of Telmisartan and Metoprolol Succinate in tablet formulations. This facilitates accurate drug content assessments in routine quality control tests for pharmaceutical companies. This study makes a significant contribution to the evolution of pharmaceutical analytical techniques, offering valuable insights into the use of validated UHPLC methods.

KEYWORDS: Telmisartan, Metoprolol succinate, UHPLC, Method Validation.

INTRODUCTION

Pharmaceutics rely on analytical methods to ensure quality, safety, and efficacy of their products. Hypertension and various cardiovascular conditions are treated with telmisartan and metoprolol succinate. Ensure the potency and uniformity of these active pharmaceutical ingredients (APIs) through accurate and precise estimation.[1] In comparison to conventional High-Performance Liquid Chromatography (HPLC), Ultra-High Performance Liquid Chromatography (UHPLC) provides enhanced resolution, sensitivity, and speed. Having the ability to provide reliable and robust results has made it the method of choice when analyzing pharmaceutical compounds. We developed and validated a UHPLC method to estimate telmisartan and metoprolol succinate in solid dosage forms. As part of routine quality control analyses, stability tests, and bioequivalence studies, the new method will be used.[2]

Several parameters must be optimized to achieve satisfactory separation and quantification of the target analytes, including the composition of the mobile phase, the columns selected, sample preparation techniques, and detection wavelengths. It is essential that the method be specific, sensitive, accurate, precise, and robust in order for the results to be reliable. Following the development of the method, it has to be validated according to regulatory guidelines, such as those established by the International Council for Harmonization of Technical

Requirements for Pharmaceuticals for Human Use (ICH).[3]

Validating a method ensures that it will be useful for its intended purpose and provide accurate and reliable results. It is typical to include specificity, linearity, accuracy, precision, robustness, and system suitability as validation parameters. Measurements of specificity are based on the ability of the method to measure the analytes despite potential impurities or matrix components. Analyte concentration is correlated with the response range and linearity of the method.

Precision measures the repeatability and intermediate precision of a method, whereas accuracy assesses how close the measured value is to the true value. It is important to the robustness of the method that it remains unaffected by small changes in experiment conditions, such as pH, temperature, and flow rate.

It is important to carry out system suitability tests that ensure that the chromatographic system is adequate and that the method is suitable for routine use. It is imperative for pharmaceutical products to ensure quality and efficacy that a UHPLC method is developed and validated for the estimation of telmisartan and metoprolol succinate in solid dosage forms.[4] Routine analysis and regulatory compliance will be enhanced using the optimized method.

In a sense, telmisartan works by blocking the receptors on the surface of angiotensin II. It works by preventing the body from producing a substance that causes blood vessels to constrict.[5]

In research studies, telmisartan exhibits agonistic activity toward PPARγ, a well-known target for antidiabetic medications. In this context, telmisartan may be able to enhance carbohydrate and lipid metabolism, regulate insulin resistance, and provide benefits similar to antidiabetic drugs that fully activate PPARγ. Due to its partial agonism, telmisartan may be able to mitigate the side effects adverse effects associated with full PPARγ activators.

As an oral or extended-release tablet, metoprolol succinate blocks beta1-selectively (cardioselectively) adrenoceptors.[6] Hypertension and atrial fibrillation are among the conditions in which metoprolol can be prescribed. High blood pressure can be treated with metoprolol alone or in combination with other medications.[7] Angina (chest pain) can also be prevented with this drug, and recovery from a heart attack can also be improved. Heart failure can also be treated with metoprolol in combination with other medications.[8]

As the market for drugs and drug formulations grows at an alarming rate, the number of drugs and drug formulations also grows. It may be impossible to obtain standard analytical procedures for these drugs of formulations. Even if available, they may not meet the conditions under which they are actually used. In order to achieve higher accuracy, precision, specificity, simplicity, and rapidity, it is critical to develop newer analytical methods.

Chromatography

Chromatography is a separation technique commonly used in analytical chemistry to recognize, identify, and quantify components in a mixture.[9] Mobile phases differ in composition from stationary phases, resulting in the differential distribution of components.

Chromatography consists of several types, including.

- Gas Chromatography (GC)
- Liquid Chromatography (LC)
- High-Performance Liquid Chromatography (HPLC)
- Thin-Layer Chromatography (TLC)
- Ion Chromatography (IC)
- Affinity Chromatography

All of these chromatography techniques have different principles, applications, and variations. Based on the sample's nature and the specific separation requirements, the appropriate chromatographic method will be chosen.

High-Performance Liquid Chromatography (HPLC)

In HPLC, a liquid mobile phase is used to separate components within a mix using a versatile and widely used analytical technique. An impressive range of compounds can be resolved with HPLC's exceptional sensitivity, resolving power, and wide range of applicability.[10]

When HPLC is performed, samples are injected into columns packed with stationary phases, which can be solid supports or particles bonded together. Typically, high pressure is employed to pump liquid solvents or a mixture of liquid solvents through the column. Separation occurs as a result of the physical properties of sample components, such as their polarity, charge, and size, interacting with the stationary phase. HPLC is capable of analyzing small molecules as well as large biomolecules as well as complex mixtures. A wide range of applications include pharmaceutical analysis, environmental monitoring, food analysis, forensic analysis, and many more.

Among the modes of separation offered by this technique are.

1. Reverse Phase Chromatography (RPC)

The RPC process uses a nonpolar stationary phase as well as a polar mobile phase. A compound with high polarity will elute first, while a compound with a low polarity will be retained for a longer period of time.

2. Normal Phase Chromatography (NPC)

A nonpolar mobile phase is used in NPC with a polar stationary phase. Based on their differences in polarity, this mode is effective for separating polar compounds.

Analytes can be identified and quantified using HPLC coupled with UV/Vis detectors, fluorescence detectors, mass spectrometers, or refractive index detectors. Separation, quantification, and analysis of multiple components of a mixture can be accomplished using HPLC simultaneously. As a powerful and versatile analytical technique, HPLC is a vital part of many scientific and industrial laboratories since it provides precise and accurate separations of complex mixtures.

UHPLC

UHPLC is a form of liquid chromatography that provides enhanced resolution, sensitivity, and speed over conventional High Performance Liquid Chromatography (HPLC). Various fields have benefited from its use, including pharmaceuticals, environmental monitoring, and food analysis. A UHPLC column uses particles of smaller sizes (typically smaller than two microns) and is operated at higher pressures (typically over 6000 psi).[11] Combining these technologies increases analyte separation efficiency and reduces analysis time.

Because smaller particles have a higher theoretical plate number per unit length, peak resolution and sharpness are improved. UHPLC uses narrower-bore columns due to the higher operating pressure, which reduces system volume and improves peak shape. As a result, they can also use higher flow rates without compromising chromatographic performance, resulting in faster analysis times.[12] Compared to traditional HPLC, UHPLC provides improved separation efficiency, higher sensitivity, and faster analysis times. A wide range of applications have demonstrated its benefits, making it one of the most useful tools for chromatographic analysis.

MATERIALS AND METHODS

Chemicals used

Acetonitrile, Methanol, Water belongs to HPLC grade and Potassium dihydrogen orthophosphate belongs to AR grade were used for this study. Orthophosphoric acid also used (AR Grade). Telmisartan (40mg) and Metoprolol Succinate (50mg) used as tablet form.

Solvents used

In order to increase the solubility of drug preparations, many solvents are used, such as water, methanol, acetonitrile, and buffers. Potassium dihydrogen phosphate was used as a buffer.

This compound is partially soluble in Acetonitrile: Phosphate buffer PH 5.0 (400:500) and Methanol: Phosphate buffer PH 7.0 (500:500) and free soluble in Acetonitrile: Methanol: Phosphate buffer PH 5 (350:300:350) and Acetonitrile: Methanol: Phosphate buffer PH 5 (300:200:100). As a result, the solvent selected in Acetonitrile: Methanol: Phosphate buffer PH 5 (350:300:350) was satisfactory.

Preparation of standard stock solution

Metoprolol succinate and Telmisartan standard should be weighed accurately and transferred into 100 ml volumetric standard flasks. Methanol is added to dissolve and make up the volume.

Preparation of standard solution

- Mix 5 ml of above stock solution with 50 ml of mobile phase.

Preparation of Buffer solution

0.05M KH2PO4 Buffer: A solution of 6.8 grams of potassium dihydrogen orthophosphate was accurately transferred to a beaker of 1000 ml, which was sonicated for 20 minutes, and the volume was made up with HPLC grade water up to the mark, before being filtered by HPLC (0.45µ). The pH of the above solution was adjusted by adding 1ml of 0.1%OPA to it (pH-5) and filters were added.

Preparation of Diluent

- As a result of drug solubility, methanol and mobile phase were chosen as diluents.

Selection of wavelength

The solvents used were Acetonitrile, Methanol, and Buffer in proportions of 35:30:35. For the estimation of compounds using UHPLC, 225nm absorbance was found to be very good for both compounds.

UHPLC Method

Method selection depends on the sample nature (ionic, ionisable or neutral molecules), their molecular weight, pH value, and stability. Reverse phase chromatography or ion exchange chromatography can be used to analyze the drugs selected in this study because they are polar. In order to simplify and suit the initial separation, UHPLC was selected. From the literature survey and with the knowledge of properties of the selected drugs, Column C18(4.6mm × 50mm)3µ was chosen as stationary phase and mobile phase with different compositions such as Acetonitrile, Methanol, water, Potassium dihydrogen orthophosphate and Orthophosphoric acid. Based on all the observations, obtained, and available data, the initial separation conditions were determined.

Chromatographic conditions

We used a mobile phase of acetonitrile, methanol, and phosphate buffer at pH 5 in a ratio of 350:300:350 in this method. Analytes are separated by liquid chromatography based on their mobile phase composition. Solvents and buffers selected here provide the desired selectivity and elution properties. A flow rate of 0.7 mL/min indicates how quickly the mobile phase flows through the column. Having a good separation of analytes depends on this parameter. According to the chromatographic system and column being used, the chosen flow rate balances resolution with analysis time.

Using SUPELCO C18 column with dimensions of 4.6 x 50 mm and particle size of 3 mm, the chromatographic separation was carried out. Depending on how analytes interact with the stationary phase, the column stationary phase is essential for separating them. As a result of hydrophobic interactions, the C18 phase effectively retains the target analytes in this case. Analytes were detected using an appropriate detector at wavelength 225 nm. Analytes under investigation differ in their properties and absorption characteristics, which determine what wavelength to use for detection. For this application, 225 nm wavelength was found to be suitable for detecting the desired compounds.

For each analysis, an injection volume of 2 µL was used. Samples are injected into the column for separation and detection by the injection volume. Injecting a volume that is within the linear range of the analytical method and provides adequate sensitivity is crucial. During the chromatographic analysis, five minutes were spent running. During the run step, samples are injected, analytes separated, and the column is equilibrated for subsequent injections. In order to increase laboratory efficiency and increase sample throughput, shorter run times are desirable. The samples were prepared with methanol and the mobile phase served as diluents and solvents, respectively. Choosing the right diluent is critical to ensuring compatibility with the analytes and maintaining stability and solubility.

Assay of Marketed Preparation[13]

Standard preparation

Using a volumetric standard flask, transfer about 50 mg of Metoprolol succinate and 40 mg of Telmisartan standard to 100 ml. Methanol is added to dissolve and make up the volume. The solution should be diluted with mobile phase to yield 50ml of solution. Record and calculate peak area responses for preparations.

Sample Preparation

To determine the average weight of a tablet, weigh one tablet accurately into a volumetric standard flask of 100 ml. Add methanol to dissolve and make up the volume. Using mobile phase, dilute this solution by 5 ml to 50 ml. The filtrate was filtered through 0.45 nylon filter paper and the first few ml were discarded. The peak area response for replicate two preparations was showed.

Validation Parameters[14]

System suitability

Using the standard solution, the system suitability parameters were calculated, including resolution, tailing, and number of theoretical plates.

Specificity

The Optimized method is checked for interference. This method should not reveal any interfering peaks between blank and placebo at retention times. As a result, it was described as a specific method.

Linearity

The Linearity of detector response is established by plotting a graph to concentration versus area of 40mg of Telmisartan & 50mg of Metoprolol succinate and determining the correlation coefficient. A series of solution of Telmisartan & Metoprolol succinate in the concentration ranging from 50, 75, 100, 125, and 150 % were prepared and injected into the HPLC system.

Accuracy

Standard preparation

Weigh accurately and transfer about 50 mg of Metoprolol succinate and 40 mg of Telmisartan standard into 100 ml volumetric standard flask. Dissolve and make up to the volume with methanol. Dilute 5ml of this solution to 50 ml with mobile phase.

Preparation of 80%, 100% and 120% solution

Weigh accurately and transfer powdered sample equivalent to about 40, 50 and 60mg of Metoprolol succinate and 32, 40, and 48 mg of Telmisartan into 100 ml volumetric standard flask. Dissolve and make up to the volume with methanol. Dilute 5ml of this solution to 50 ml with mobile phase.

Procedure

Injections of the standard solution, Accuracy -80%, Accuracy -100%, and Accuracy -120% solutions were performed. We calculated the amount found and the amount added for Metoprolol succinate and Telmisartan mean recovery values.

Precision

Preparation of standard solution

In a volumetric standard flask, weigh accurately and transfer about 50 mg of Metoprolol succinate and 40 mg of Telmisartan standard. Methanol is used to make up the volume after the solution is dissolved. Mix 5 ml of this solution with 50 ml of mobile phase.

Preparation of sample solution

Calculate the average weight of a tablet using 100 ml volumetric standard flasks. The solution was dissolved and the volume was made up with methanol. Add mobile phase to this solution and dilute it further to 50 ml.

Robustness

As per ICH guidelines, small deliberate changes in the method were made like adjusting the flow rate and wavelength, but no noticeable changes were seen in the results. Wavelength decreasing and increasing conditions were maintained while robustness conditions such as flow minus and flow plus were maintained. Injections of samples were carried out in duplicate. No significant changes were observed in system suitability parameters, and all parameters passed. There were no significant deviations from the limit in %RSD.

RESULTS AND DISCUSSION

The assay conducted using the formulated Telmisartan and Metoprolol Succinate showed promising results. The average percent purity for Telmisartan was determined to be 100.04%, while for Metoprolol Succinate, it was 100.19%. The percent relative standard deviation (%RSD) for Telmisartan was found to be 0.49, and for Metoprolol Succinate, it was 0.59. These values indicate good precision and consistency in the assay results. The system suitability parameters, as per the ICH guidelines, were evaluated, and all parameters met the specified criteria. The plate count, which indicates the efficiency of the chromatographic system, was determined to be greater than 2000, ensuring reliable separation. The tailing factor, a measure of peak symmetry, was less than 2, indicating well-shaped peaks.

The resolution, which indicates the separation between adjacent peaks, exceeded 2, demonstrating good separation between Telmisartan and Metoprolol Succinate. Additionally, no interfering peaks were observed in the blank and placebo at the retention times of the drugs, indicating the specificity of the method. Linearity was assessed by injecting five linear concentrations of Telmisartan and Metoprolol Succinate in duplicate. The obtained peak areas were used to generate linearity equations. The linearity equation for Telmisartan was y = 0.0298x - 0.0285, while for Metoprolol Succinate, it was y = 0.0686x + 0.1282. The correlation coefficients (R^2) obtained for both compounds were close to 1, indicating excellent linearity. Accuracy was evaluated by preparing three levels of samples using the standard addition method. Triplicate injections were performed for each level, and the mean percent recovery was calculated. The obtained mean percent recovery was 98.95% and 100.19% for Telmisartan and 99.68% and 100.21% for Metoprolol Succinate.

These results indicate good accuracy and reliability of the method. Precision was assessed by calculating the %RSD from six sample injections. The %RSD obtained for Telmisartan was 0.27%, and for Metoprolol Succinate, it was 0.23%. As the %RSD values were below the specified limit of 2, both method precision and intermediate precision were considered acceptable. Robustness was evaluated by injecting samples under different conditions, such as varying flow rates and wavelengths. The system suitability parameters were not significantly affected, indicating the robustness of the method.

The %RSD values obtained were within the specified limits, further confirming the method's robustness. Overall, the assay results demonstrate the specificity, linearity, accuracy, precision, and robustness of the developed method for the estimation of Telmisartan and Metoprolol Succinate in the formulated samples. These findings support the method's suitability for routine quality control analysis and other pharmaceutical applications.

Table 1: Assay of marketed formulation.

Sample	Labelclaim	Peakarea*	Amount obtained*	Percent label claim%w/w*	SD	%RSD
Telmisartan	40 mg	1321.196	40.14	100.04%	0.49	0.04
Metoprolol Succinate	50mg	731.167	50.097	100.19%	0.59	0.08

Table 2: Validation Parameters.

Parameters		Telmisartan	Metoprolol succinate	LIMIT
Linearity: Regression equation (Y=mx+c)		y = 0.0298x + 0.0285 (R^2 =1.0000)	y = 0.0686x + 0.1282 (R^2 = 1.0000)	R<1
Assay (% mean assay)		100.04%	100.19 %	98-102%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.07	0.10	NMT 2.0%
Method precision %RSD		0.27	0.23	NMT 2.0%
Intermediate precision %RSD		0.16	0.25	NMT 2.0%
Accuracy %		98.95% to 100.19%	99.68% to 100.21%	98-102%
Robustness	FM	0.30	0.44	%RSD NMT 2.0
	FP	0.47	0.20	
	WM	0.32	0.63	
	WP	0.27	0.27	

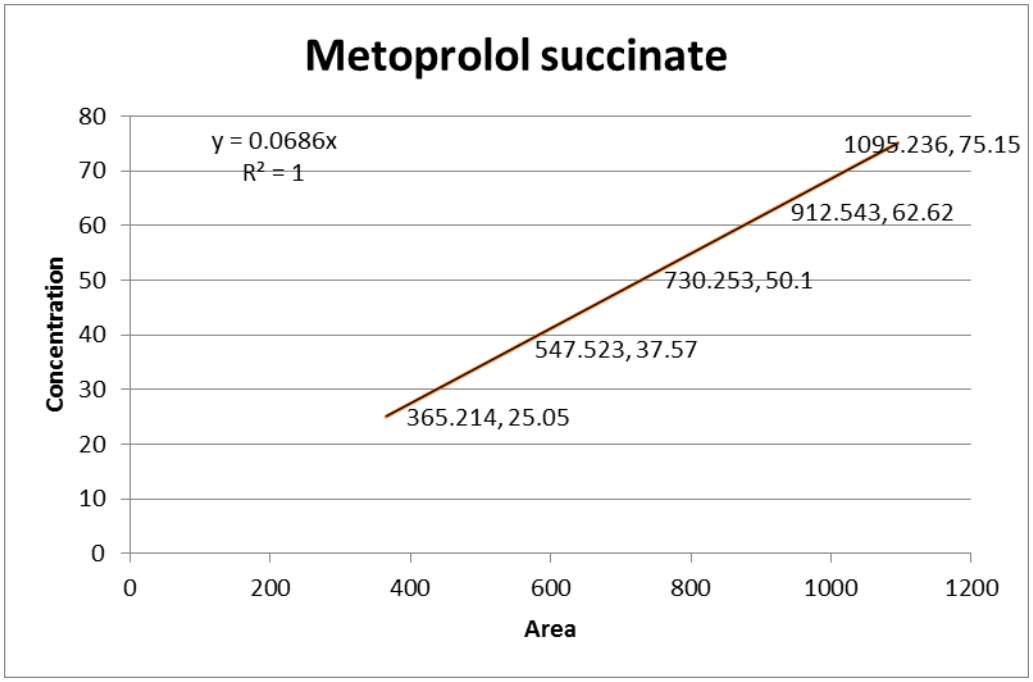

Figure 1: Linearity study on metoprolol succinate.

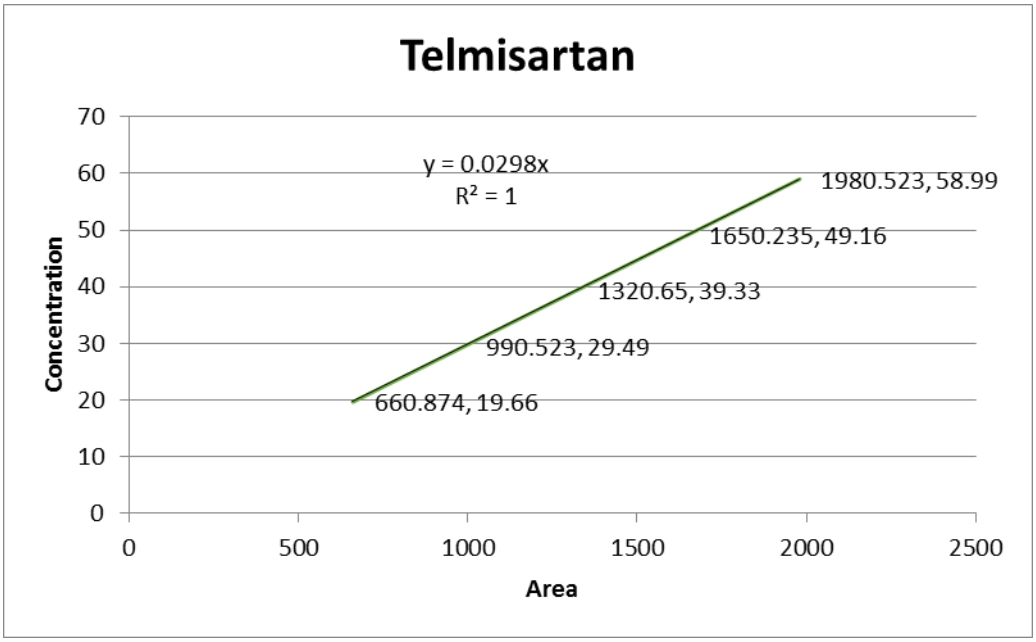

Figure 2: Linearity study on telmisartan.

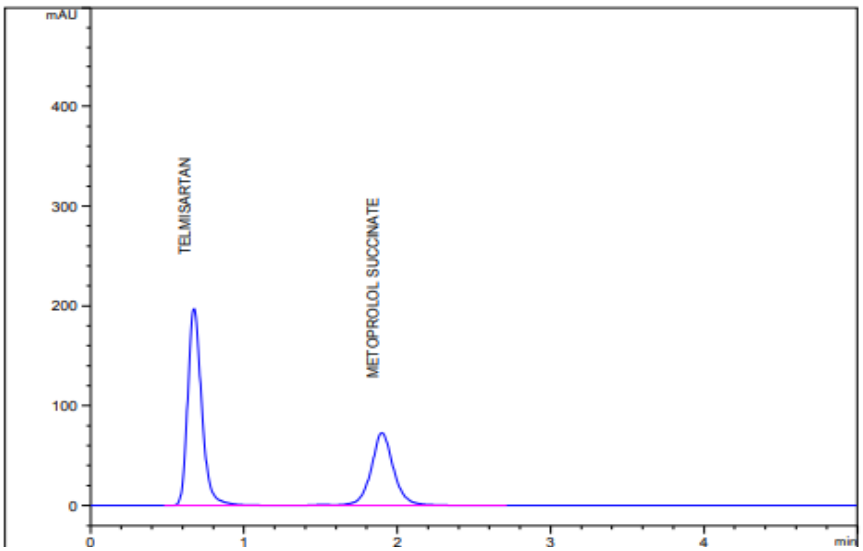

Figure 3: Wavelength plus standard Chromatogram of Telmisartan and Metoprolol Succinate.

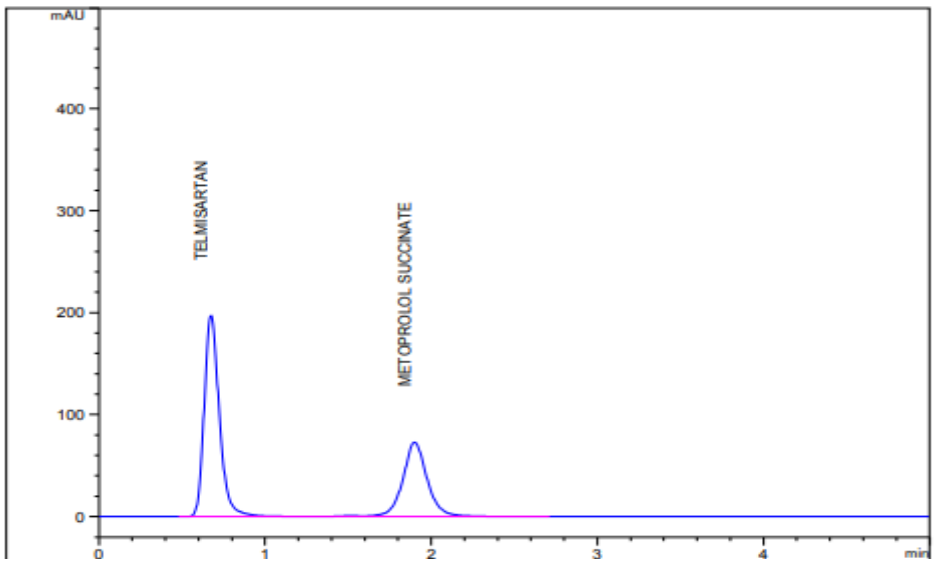

Figure 4: Wavelength plus sample Chromatogram of Telmisartan and Metoprolol Succinate.

CONCLUSION

This study developed and validated a simple, accurate, and precise UHPLC method for estimating Telmisartan and Metoprolol Succinate simultaneously in tablet dosage forms. In addition, there were low %RSD values for the retention time and assay, which indicated that the method was highly reproducible and accurate. Throughout the specified concentration range, the regression equations indicate linearity for both compounds. In comparison to previous methods, the developed method is simpler and more cost-effective because of the reduced retention times and run times. ICH guidelines were followed during the validation of the method and the results showed that it was satisfactory in terms of system suitability, linearity, precision of the system, precision of the method, and robustness. In this way, Telmisartan and Metoprolol Succinate can be routinely analyzed for pharmaceutical purposes by UHPLC using these parameters. Using this validated UHPLC method, Telmisartan and Metoprolol Succinate are simultaneously estimated in tablet dosage forms using a robust and efficient analytical tool. In the pharmaceutical industry, it can be used in routine quality control tests to ensure product quality and make drug content assessments more reliable.

REFERENCES

1. Skoog, D. A., Holler, F. J., Crouch, S. R., & Frensley, A. E. (2017). Principles of Instrumental Analysis. Cengage Learning.
2. Snyder, L. R., Kirkland, J. J., & Glajch, J. L. (2010). Practical HPLC Method Development. John Wiley & Sons.
3. United States Pharmacopeia (USP). General Chapter <1225> Validation of Compendial Methods. (Available at: https://www.usp.org/compounding/general-chapter-validation-compendial-methods)
4. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for

Human Use (ICH). Validation of Analytical Procedures: Text and Methodology Q2(R1).
5. Noh, Y., Shin, Y., & Yoon, I. S. (2012). Development and validation of a liquid chromatography-tandem mass spectrometry method for determination of telmisartan in human plasma. Archives of Pharmacal Research, 35(5): 825-831.
6. Nirogi, R., Kandikere, V., Mudigonda, K., & Maurya, S. (2009). A sensitive liquid chromatography/tandem mass spectrometry method for quantification of telmisartan in human plasma. Biomedical Chromatography, 23(5): 548-557.
7. Dogrukol-Ak, D., Tuncel, M., & Sever, E. (2007). A new HPLC method for the determination of metoprolol succinate in human plasma using 4-chlorophenol as internal standard. Journal of Chromatography B, 852(1-2): 569-574.
8. Nirogi, R., & Kandikere, V. (2008). Rapid, sensitive and selective liquid chromatography/tandem mass spectrometry method for the quantification of metoprolol in human plasma using metoprolol-d7 as internal standard. Rapid Communications in Mass Spectrometry, 22(7): 939-948.
9. Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. A. (2017). Introduction to Spectroscopy. Cengage Learning.
10. Kromidas, S. (2017). HPLC Made to Measure: A Practical Handbook for Optimization. Wiley-VCH.
11. Sarafroz, M., & Nahar, L. (2019). Recent developments in UHPLC and HPLC: a review. Journal of Liquid Chromatography & Related Technologies, 42(11-12): 331-360. doi: 10.1080/10826076.2019.1612427.
12. López-Darias, J., Torres-Padrón, M. E., & Santana-Rodríguez, J. J. (2018). UHPLC versus HPLC: a comparison of lipophilic compounds separation in olive oil. Food Chemistry, 246: 429-437. doi: 10.1016/j.foodchem.2017.11.105.
13. Gandi S, Manikandan A, Rao SV. Novel Stability indicating RP-UPLC Method for simultaneous Determination of Ivabradine and Metoprolol drug materials in bulk and their Pharmaceutical Dosage Forms. Research Journal of Pharmacy and Technology, 2020; 13(1): 250-4.
14. Agrawal S, Gurjar P, Katheriya B. Analytical method development and validation for simultaneous estimation of Trimetazidine hydrochloride and Metoprolol succinate using HPTLC. Current Pharmaceutical Analysis, 2019 Jun 1; 15(3): 243-50.