ejpmr, 2023,10(9), 455-461

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANALYTICAL UHPLC METHOD FOR SOLID DOSAGE FORM ANALYSIS OF TAMSULOSIN AND DEFLAZACORT: DEVELOPMENT AND VALIDATION

Sathish Kumar G.[1]*, Vijayamirtharaj R.[1], Senthil Kumar N.[2]

[1]Department of Pharmaceutical Analysis, JKKMRF's-Annai JKK Sampoorani Ammal College of pharmacy, Komarapalayam, Namakkal, Tamil Nadu-638183, India.

[2]Department of Pharmaceutical Chemistry, JKKMRF's-Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu-638183, India.

***Corresponding Author: Sathish Kumar G.**
Department of Pharmaceutical Analysis, JKKMRF's-Annai JKK Sampoorani Ammal College of pharmacy, Komarapalayam, Namakkal, Tamil Nadu-638183, India.

Article Received on 29/06/2023 **Article Revised on 20/07/2023** **Article Accepted on 10/08/2023**

ABSTRACT

To treat various medical conditions, the pharmaceutical compounds Deflazacort and Tamsulosin are commonly used in solid dosage forms. As an antagonist of the alpha-1 adrenergic receptor, Tamsulosin can effectively treat BPH and lower urinary tract symptoms. Inflammatory and autoimmune conditions are treated with Deflazacort, a synthetic glucocorticoid. An RP-HPLC based method was developed and validated for estimating Tamsulosin and Deflazacort in a combined dosage form simultaneously. With UHPLC, separation efficiency is improved, solvent consumption is reduced, and analysis is rapid as compared with conventional chromatography. Assays revealed that Deflazacort had an average percentage purity of 100.24%, and Tamsulosin had an average percentage purity of 100.50%, with coefficients of variation of 0.71 and 0.27, respectively. The validation parameters all met ICH requirements, with a plate count exceeding 1000 and a tailing factor below 2. Deflazacort and Tamsulosin were injected in duplicate at five linear concentrations. Tamsulosin's linearity equation was y = 0.2195x + 0.06119, while Deflazacort's linearity equation was y = 0.903x - 0.0634, both with high correlation coefficients (R^2 = 0.9999). Three levels of accuracy samples were used to evaluate the accuracy of the standard addition method. Triplicate injections were performed for each level, resulting in mean % recovery ranging from 99.63% to 100.90% for Tamsulosin and 99.80% to 100.90% for Deflazacort. Tamsulosin % RSD was 0.50% and Deflazacort % RSD was 0.21%, respectively. It was confirmed that the method is specific since no interfering peaks were detected in placebo or blank samples during retention times for the target drugs. Flow rate and wavelength variations were introduced to assess robustness conditions, with minimal impact on system suitability parameters, keeping all parameters within acceptable limits. A RP-HPLC method was developed to estimate Tamsulosin and Deflazacort simultaneously in a combined dosage form, and it was shown to be rapid, specific, sensitive, precise, and accurate. A valuable tool for routine analysis and research, this method is suitable for use in various fields like pharmaceutical analysis, quality control, and biopharmaceutics.

KEYWORDS: UHPLC, Tamsulosin, Deflazacort, Validation, Analytical methods.

INTRODUCTION

Pharmaceutics research and development place a great deal of emphasis on ensuring product quality and efficacy in order to guarantee patient safety and therapeutic effectiveness. The analytical methods employed in drug formulations and active pharmaceutical ingredients (APIs) play a crucial role in determining their identity, purity, potency, and stability.[1]

Because of its superior speed, resolution, sensitivity, and efficiency compared with conventional High-Performance Liquid Chromatography (HPLC), Ultra-High-Performance Liquid Chromatography (UHPLC) has become a powerful tool for pharmaceutical analysis.[2]

Deflazacort and Tamsulosin are two pharmaceutical compounds commonly used in solid dosage forms to treat various medical conditions. BPH and lower urinary tract symptoms can be treated effectively with Tamsulosin, an antagonist of the alpha-1 adrenergic receptor.[3] A synthetic glucocorticoid called Deflazacort, however, is used to treat inflammatory and autoimmune conditions.

To quantify both drugs simultaneously in solid dosage forms, an analytical method that is precise, accurate, and robust must be developed and validated since they are commonly administered or combined in fixed-dose formulations.

The determination of Tamsulosin and Deflazacort in different matrices has been achieved through

spectrophotometry, HPLC, and UHPLC.[4] Compared to conventional chromatography, UHPLC offers distinct advantages, including improved separation efficiency, reduced solvent consumption, and rapid analysis. UHPLC may be able to measure Tamsulosin and Deflazacort simultaneously, but there is limited research on the method, so more research is needed. In this study, Tamsulosin and Deflazacort in solid dosage forms will be determined by an analytical UHPLC method. Using the proposed method, accurate quantification of both drugs will be possible within a shorter analysis time due to excellent chromatographic resolution and peak separation. A rigorous validation process will be carried out in accordance with regulatory guidelines so as to determine the method's reliability, sensitivity, and specificity for routine quality control analyses of Tamsulosin and Deflazacort in pharmaceutical formulations.

CHROMATOGRAPHY

Among the many scientific fields that rely on chromatography are pharmaceutical analysis, environmental monitoring, food safety, and forensic sciences. Various physicochemical properties are used to separate sample components based on differential interactions with the stationary phase. UHPLC and HPLC (High-Performance Liquid Chromatography) are two common chromatographic techniques.

UHPLC (Ultra-High-Performance Liquid Chromatography)

In recent years, UHPLC has gained immense popularity due to its improved performance compared to conventional HPLC. Typically, smaller particle size columns (typically 1.7 to 2.6 m) and higher pressures (generally over 15,000 psi) facilitate faster separation times, higher resolution, and increased sensitivity.[5] Increasing pressure and reducing particle size result in narrower peaks and improved peak capacity, which makes it possible to analyze complex samples more efficiently.[6] It has been widely used in pharmaceutical analysis for determining drug concentrations, profiling impurities, and determining drug stability. In addition to saving time and resources, the technique ensures high precision and accuracy while analyzing large samples. UHPLC is also particularly suitable for analyzing trace components in complex matrices due to its enhanced resolution and sensitivity.

HPLC (High- Performance Liquid Chromatography)

Separating and analyzing sample components is made possible by HPLC, a chromatographic technique using a liquid mobile phase. Based on their interactions with the stationary phase's surface chemistry[7], analytes are partitioned between the stationary phase (usually a packed column) and the mobile phase. A wide range of analytes can be analyzed through HPLC, making it a popular choice in a variety of industries. Drug assay, impurity detection, stability studies, and pharmacokinetic studies are all routinely conducted using HPLC in pharmaceutical analysis. Pharmaceutical manufacturing routinely uses the method for quality control as it provides accurate and reliable quantitative data.[8]

The definition of method validation as per ICH is "Establishing documented evidence that a specific activity or product will consistently meet the quality characteristics and specifications it was designed for".[9]

MATERIALS AND METHOD

Chemicals and Reagent Used

Methanol, water belongs to HPLC grade and formic acid was belonging to AR grade were used for this methodology. Tamsulosin and Deflazacort comes in combination of 0.4mg and 30mg as brand name Tamfil DS.

Formic acid, methanol, water, and methanol are some examples of solvents used to solubilize drug preparations. Tamsulosin and Deflazacort are soluble in Methanol at a concentration of 0.1% formic acid. (78:22). The compound partially dissolves in methanol at 0.1% formic acid. (70:30). The ratio of methanol to formic acid is 75 to 25 (0.1%). Methanol: 0.1% formic acid. (80:20). So, we were concluded for the solvent selected in Methanol: 0.1% formic acid. (78:22).
Mobile Phase: Methanol: Formic acid (0.1%) (78:22)

Preparation of buffer solution

0.1ml of formic acid mixed with 100 ml of H_2O

Preparation of standard stock solution

Precisely weigh approximately 40 mg of Tamsulosin standard and 30 mg of Deflazacort standard individually and transfer each into separate 100 ml volumetric flasks. Dissolve the contents of each flask in the appropriate volume of the mobile phase and bring to the mark to prepare the individual stock solutions. Subsequently, take 1 ml of the Tamsulosin standard stock solution and carefully add it to the volumetric flask containing the Deflazacort standard stock solution. Make up the combined solution to the mark with the mobile phase.

Preparation of sample solution

Precisely weigh the average weight of one tablet from the sample and transfer it into a 100 ml volumetric standard flask. Subsequently, dissolve the tablet in the flask using the appropriate mobile phase, and then adjust the volume of the solution to the mark on the flask using the same mobile phase.

Wavelength selection

The chosen solvent for the UHPLC (Ultra-High-Performance Liquid Chromatography) analysis is a mixture of Methanol and 0.1% formic acid in a ratio of 78:22. This solvent blend has demonstrated optimal performance for the detection of both compounds at a wavelength of 282nm. Hence, this solvent composition is suitable for accurately estimating and quantifying the compounds through UHPLC analysis.

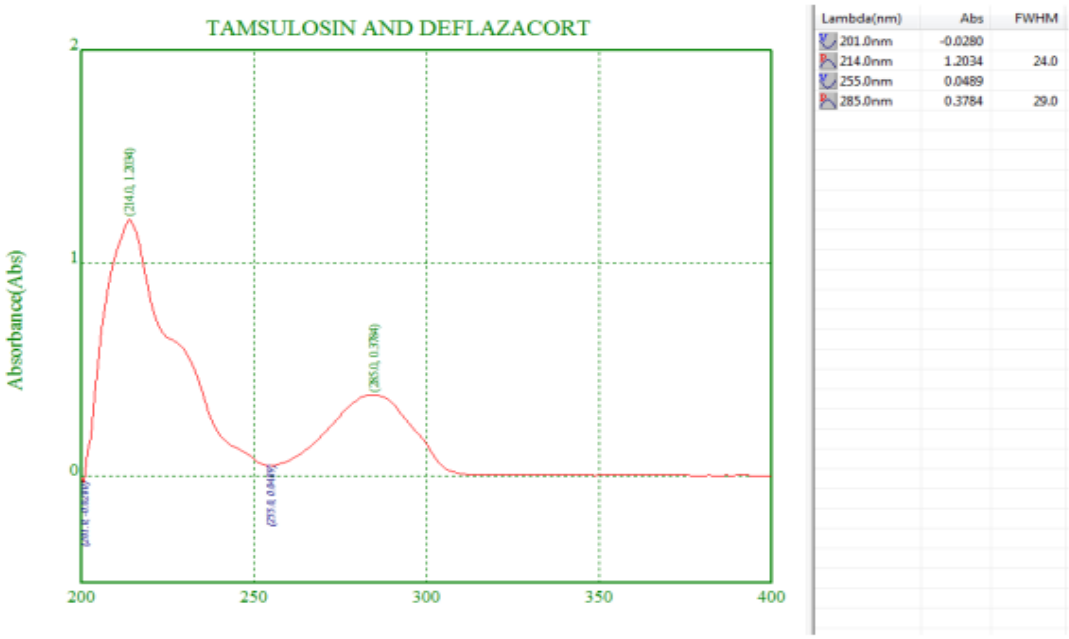

Figure 1: Wavelength Suitability.

UHPLC Chromatographic condition

The following optimized conditions were utilized in this chromatographic study to achieve a highly accurate and efficient separation of compounds. Methanol and 0.1% Formic acid were mixed in a (78:22) ratio to form the mobile phase. Target compounds were excellently eluted and resolved using this well-tailored mobile phase. To facilitate the separation, an INERTSIL C18 column with dimensions of 4.6mm x 50mm and a particle size of 3µ was employed. Using C18 as the stationary phase enhanced the overall chromatographic performance by providing exceptional selectivity and retention. It was found that a wavelength of 282nm provided excellent sensitivity and specificity for detection of the analytes of interest. In addition, repeated injections of 2µL samples were conducted to validate the method's robustness and reliability. All analytes in the sample were effectively separated and quantified during the chromatographic run, which lasted 10 minutes. As a result of the 0.7ml/min flow rate, the sharpness of chromatographic peaks was optimized while the analysis time was minimized. In addition to ensuring the chemical stability of the sample, the mobile phase was used as the diluent, which prevented potential interference. Reproducibility and accuracy were greatly enhanced by this step.[10] It was found that all compounds retained their retention times consistently and well, indicating a stable chromatographic system. The repeatability and precision of the method were further demonstrated by the symmetrical peak shapes observed.

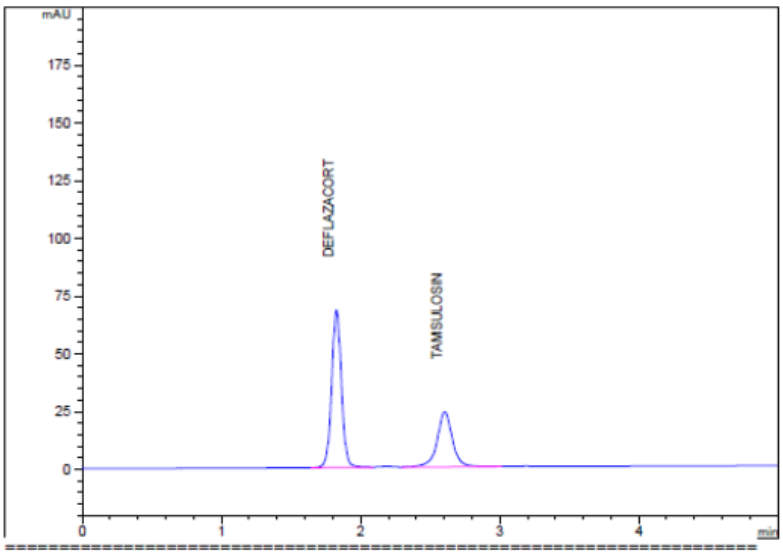

Figure 2: Optimized chromatogram of Tamsulosin and Deflazacort.

ASSAY OF MARKETED FORMULATION

Precisely weigh the average weight of one tablet from the sample and transfer it into a 100 ml volumetric standard flask. Proceed to dissolve the tablet in the flask using the appropriate mobile phase, and then bring the solution up to the mark on the flask with the same mobile phase, achieving the desired volume.[11]

Procedure

In this study, five replicates of the standard preparation were separately injected into the equilibrated UHPLC system, each with a volume of 2 µl. The response of the major peak attributed to Deflazacort and Tamsulosin was measured for each injection. Additionally, two replicates of the sample preparation were injected separately, also with a volume of 2 µl. The response of the major peak corresponding to Deflazacort and Tamsulosin was measured for each sample injection. The amount of drug samples was calculated by using formula,

$$\%\ labeled\ amount = \frac{Amount\ of\ \text{Tamsulosin/ Deflazacort}\ (\frac{mg}{tablet})}{Label\ claim} x100$$

Validation Parameters[12]

System suitability

System suitability of the method was assessed by performing six replicate injections of the standard preparation. The parameters evaluated were resolution, tailing, and number of theoretical plates, based on the analysis of the five replicate injections of the standard solution.

Linearity

In order to determine the linearity of a detector response, a graph with concentrations versus area of 30mg of Deflazacort & 40mg of Tamsulosin is drawn and then the correlation coefficient is determined. Multiple solutions of Deflazacort and Tamsulosin were prepared, containing concentrations ranging from 50%, 75%, 100%, 125% and 150%. These solutions were subsequently injected into the HPLC system for analysis.

ACCURACY

Standard solution

Deflazacort and Tamsulosin standard should be weighed accurately and transferred to a volumetric flask of 100 ml. Mobile phase should be added to the makeup volume.

Preparation of 80%, 100% and 120% solution

Accurately weigh and transfer powdered samples equivalent to approximately 24 mg, 30 mg, and 36 mg of Deflazacort, and 0.32 mg, 0.40 mg, and 0.48 mg of Tamsulosin into separate 100 ml volumetric standard flasks. Proceed to dissolve each sample in the flasks using the appropriate mobile phase and then adjust the volume to the mark on the flasks. Take 5 ml of each of these solutions and dilute them to a total volume of 100 ml using the mobile phase.

Procedure

A standard solution, an accuracy solution of 80%, an accuracy solution of 100%, and an accuracy solution of 120% were injected. Deflazacort and Tamsulosin mean recovery values were calculated and summarized.

Precision

Standard solution preparation

Precisely weigh and transfer approximately 30 mg of Deflazacort standard into a 100 ml volumetric standard flask. Dissolve the Deflazacort in the flask, and then add 1 ml of Tamsulosin standard stock solution. Finally, make up the volume in the flask using the mobile phase.

Sample solution preparation

Precisely weigh the average weight of one tablet from the sample and transfer it into a 100 ml volumetric standard flask. Subsequently, dissolve the tablet in the flask using the appropriate mobile phase, and then adjust the volume of the solution to the mark on the flask with the same mobile phase, achieving the desired volume.

Specificity

In the optimized method, the interference was carefully examined to ensure the absence of any interfering peaks in both the blank and placebo samples at the retention times of the drugs analyzed.

Robustness

Minor deliberate modifications were introduced to the method, such as varying the flow rate and wavelength settings. Surprisingly, these changes did not produce any significant deviations in the results, and all measurements remained within the acceptable range according to the guidelines provided by the ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) (13). To assess the method's robustness, specific conditions were employed, including flow rate adjustments (both minus and plus) and variations in wavelength (decreasing and increasing). During this robustness evaluation, samples were injected in duplicate to ensure reliable and consistent outcomes.

RESULTS AND DISCUSSION

The assay of the formulation was performed and recorded in table 1, the average percentage purity for Deflazacort and Tamsulosin was found to be 100.24% and 100.50%, respectively, with % RSD values of 0.71 and 0.27, respectively. All validation parameters were within acceptable limits in table 2, according to ICH guidelines. The plate count was greater than 1000, and the tailing factor was less than 2. For the linearity study, five linear concentrations of Tamsulosin and Deflazacort were injected in duplicate. The obtained average areas were used to construct linearity equations, which were y = 0.2195x + 0.06119 for Tamsulosin and y = 0.903x - 0.0634 for Deflazacort, with correlation coefficients (R^2) of 0.9999 for both compounds. Linearity graph was plotted in the figure 3&4. The accuracy of the method

was evaluated through standard addition method, where three levels of accuracy samples were prepared. Triplicate injections were given for each level, and the mean % recovery was calculated as 99.63% to 100.90% for Tamsulosin, and 99.80% to 100.90% for Deflazacort, respectively. System precision was determined by injecting six sample injections, and the average area, standard deviation, and % RSD were calculated for Tamsulosin (0.50%) and Deflazacort (0.21%). The obtained % RSD values were well within the limit of "2," indicating the method's excellent precision. The retention time for Tamsulosin was found to be 2.61 minutes, and for Deflazacort, it was 1.822 minutes. Furthermore, no interfering peaks were detected in blank and placebo samples at the retention times of these drugs, confirming the method's specificity. In the robustness study, variations were made in flow rate (Flow minus: 0.8 ml/min, Flow plus: 1.2 ml/min), and wavelength (Wavelength minus and Wavelength plus). Wavelength of standard and sample chromatogram was mentioned in the figure 5 & 6. Duplicate injections were performed, and the system suitability parameters were evaluated. It was observed that the robustness conditions did not significantly affect the system suitability parameters, and all parameters were within the acceptable range.

Table 1: Assay of marketed formulation.

Sample	Labelclaim	Peakarea*	Amount obtained*	Percent label claim%w/w*	SD	%RSD
Deflazacort	30 mg	331.262	30.072	100.24%	0.71	0.71
Tamsulosin	0.4mg	180.671	0.402	100.50%	0.49	0.27

*values are expressed as Mean of six readings

Table 2: Validation Parameters.

Parameters		Tamsulosin	Deflazacort	Limit
System suitability parameters Tailing factors Theoretical plates		 0.942 3012	 0.888 3345	-
Linearity: Regression equation (Y=mx+c)		y = 0.2195x + 0.06119 (R^2 =0.99)	y = 0.903x - 0.0634 (R^2 = 0.99)	R<1
Assay (% mean assay)		100.54%	100.24 %	98-102%
Specificity		Specific	Specific	No interference of any peak
Method precision %RSD		0.50	0.21	NMT 2.0%
Accuracy %		99.63% to 100.90%	99.80% to 100.90%	98-102%
Robustness	FM	0.33	0.35	%RSD NMT 2.0
	FP	0.34	0.39	

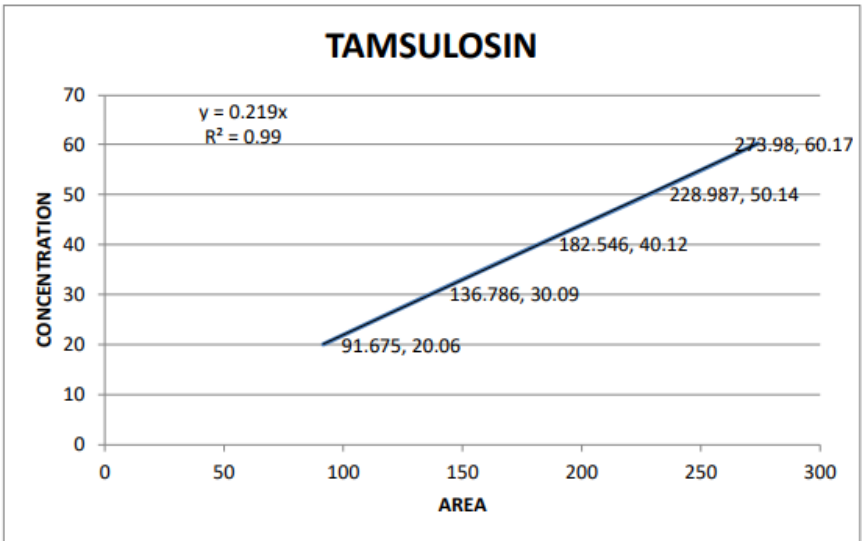

Figure 3: Linearity study of Tamsulosin.

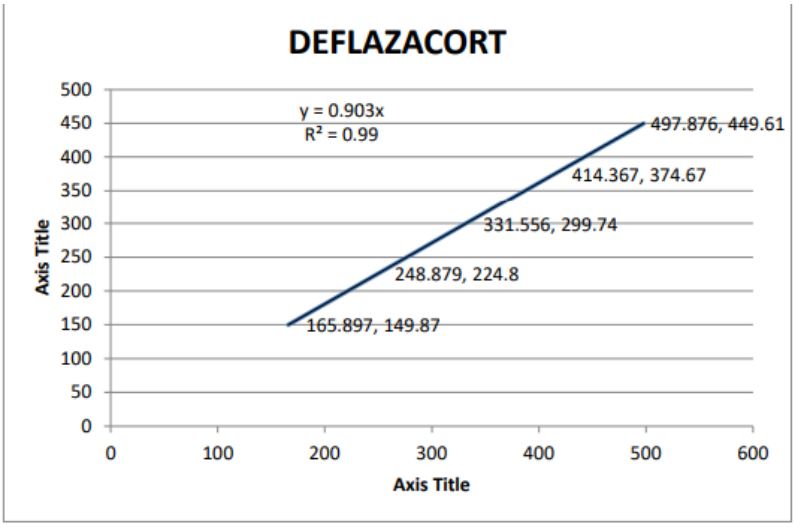

Figure 4: Linearity study of Deflazacort.

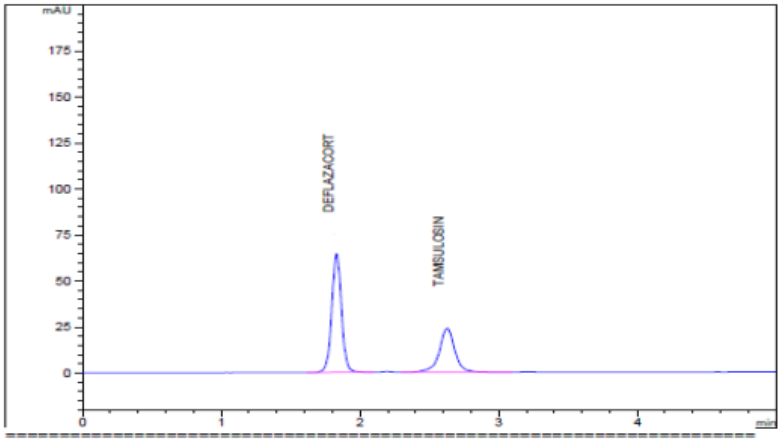

Figure 5: Wavelength plus standard Chromatogram of Tamsulosin and Deflazacort.

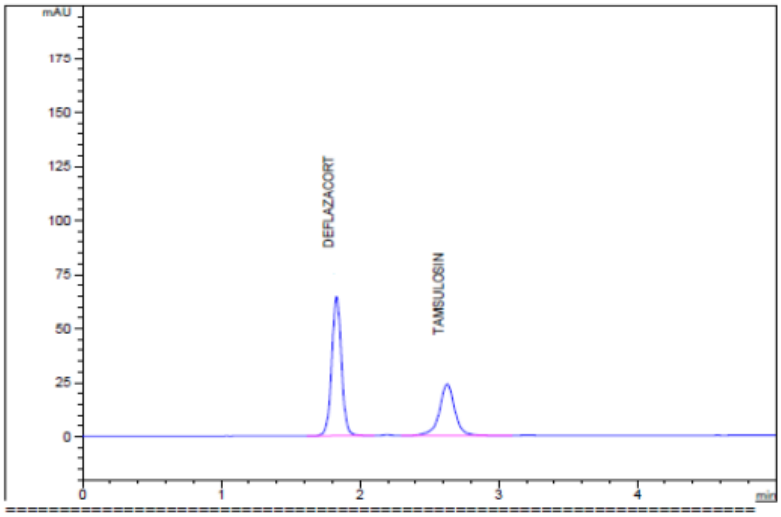

Figure 6: Wavelength plus sample Chromatogram of Tamsulosin and Deflazacort.

CONCLUSION

Deflazacort and Tamsulosin together in a single dose form offer several advantages by using UHPLC. Simple, precise, accurate, and reliable are the characteristics of the method. It eliminates the need for tedious extraction procedures for standard and sample preparation. Chromatographic analysis is more efficient due to the short run time of less than 10 minutes. As well as being suitable for analysis of raw materials, dissolution studies, and uniformity studies, the method is also suitable for

many other applications. As a result of its rapidity, specificity, sensitivity, and overall applicability, the UHPLC method developed is well suited for routine analyses in research institutions, quality control departments in industries, and approved testing laboratories, as well as for clinical pharmacokinetic studies. As well as ensuring regulatory compliance, it can contribute significantly to pharmaceutical product development and formulation analysis.

REFERENCES

1. Boltia, S.A., Abdelkawy, M., Mohamed, T.A. et al. Eco-friendly RP-HPLC and HPTLC Methods for Simultaneous Determination of Tamsulosin Hydrochloride and Deflazacort in the Presence of 21-Hydroxy Deflazacort and Testing the In-vitro Dissolution of the Combined Dosage Form via RP-HPLC Method. Chromatographia, 2021; 84: 285–295. https://doi.org/10.1007/s10337-021-04009-y
2. Gómez-Caballero A, Abad-García B, Berrueta LA, Gallo B, Vicente F. UV-visible spectrophotometry in pharmaceutical analysis. J Pharm Biomed Anal., 2000; 21(5): 731-55.
3. Jain PS, Satheshbabu PR, Nagesh HN, Srinivas K, Vasantharaju SG, Karthik A. Development and validation of a stability-indicating HPLC method for Tamsulosin hydrochloride. J Chromatogr Sci., 2011; 49(8): 646-51.
4. Yang W, Bai Y, Xie H, Zhang L, Tang L. Development and validation of an UHPLC-MS/MS method for simultaneous determination of Deflazacort and its two active metabolites in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci., 2015; 990: 64-71.
5. Broeckling CD, Reddy IR, Duran AL, Zhao X, Sumner LW. METLIN: A Metabolite Mass Spectral Database. Ther Drug Monit, 2006; 28(6): 747-51.
6. Guiochon G. The early days of high-performance liquid chromatography. J Chromatogr A., 2010; 1217(44): 6708-19.
7. Gritti F, Guiochon G. Theoretical and practical aspects of gradient elution in comprehensive two-dimensional liquid chromatography. J Chromatogr A., 2013; 1292: 2-25.
8. Wilson AJ, Arcoria A, Dreher D, van de Goor T. The use of UHPLC and MS in pharmaceutical analysis. J Pharm Biomed Anal., 2007; 44(2): 430-8.
9. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd edition. Hoboken, New Jersey: John Wiley & Sons, Inc., 2010.
10. Porpiglia F, Vaccino D, Billia M, Renard J, Cracco C, Ghignone G et al. Corticosteroids and Tamsulosin in the medical expulsive therapy for symptomatic distal ureter stones: single drug or association. EurUrol, 2006; 50(2): 339–344.
11. TyagiNitin Sharma, Mittal K, Mashru R, Bhardwaj T, Malik J. HPTLC densitometric and RPHPLC method development and validation for determination of salbutamol sulphate, bromhexine hydrochloride, etofylline in tablet dosage forms. Pharmaceutical AnalyticaActa, 2015; 6(3): 350.
12. RP-HPLC Method development and validation for simultaneous determination of Deflazacort and tamsulosin Hydrochloride. Kanna Laksmi Sidram,International journal of pharmaceutical research, 2021; 13(3): 817-825.
13. Patel SA and Patel NJ, "High performance thin layer chromatographic method for estimation of deflazacort in tablet." J. Pharma. Sci., 2011; 01(07): 94-98. 18. ICH Q2 (R1); Validation of Analytical Procedures; Text and Methodology. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, ICH Harmonised Tripartite Guideline, 2005.