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Original Research Article

Spectroscopic method development and validation of drug used in treatment of benign prostatic hyperplasia complicated with over active bladder

Shivangee B. Pithadiya [1,*], Amitkumar J. Vyas [2]

[1] *Department of Pharmaceutical Science, Faculty of Health Sciences, Marwadi University, Rajkot-Morbi Highway, Rajkot, Gujrat, 360003, India*

[2] *B.K. Mody Government Pharmacy Collage, Rajkot, Gujrat, India*

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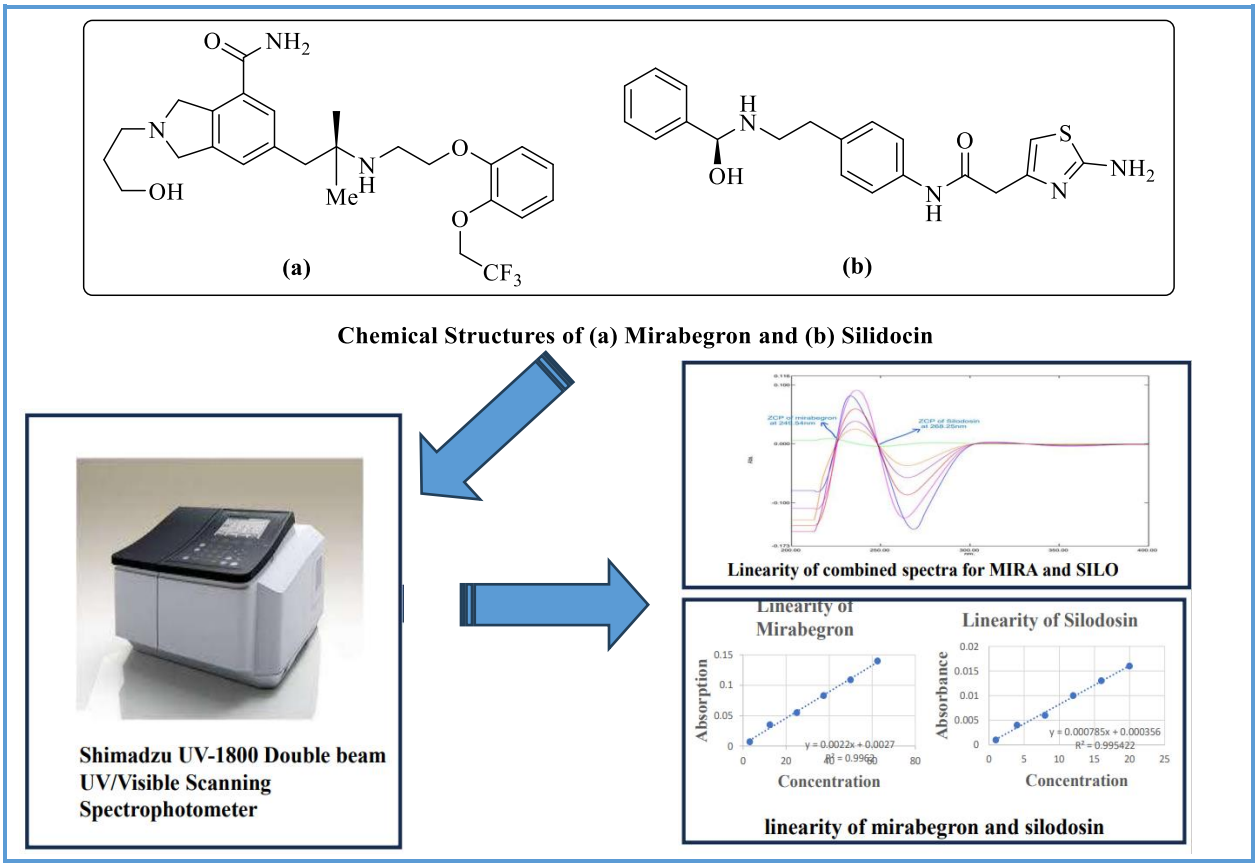
ABSTRACT

The simultaneous measurement of Mirabegron and Silodosin in a synthetic mixture was made possible by the development and validation of a rapid and precise ultraviolet-visible (UV-Vis) spectrophotometric method. The simultaneous Estimation of Mirabegron and Silodosin was carried out at specific wavelengths, specifically 268.25 nm (Zero Crossing Point of Silodosin) and 249.54 nm (Zero Crossing Point of Mirabegron), using UV detection for the first-order derivative method. It was observed that there was no interference from the diluents at these wavelengths. The method demonstrated linearity within the concentration range of 3.125-62.5 µg/mL for mirabegron and 1-20 µg/mL for silodosin. The results indicated that the correlation coefficients for mirabegron and silodosin were 0.996 and 0.995, respectively. The accuracy ranges for the Silodosin and Mirabegron were determined to be 99.84-100.61% and 98.54-102.09%, respectively. The limits of detection and quantitation for mirabegron were determined to be 1.35 µg/mL and 4.10 µg/mL, respectively. The results showed that silodosin has a limit of quantitation of 19.71 µg/mL and a limit of detection as 6.50 µg/mL. It was found that the precision and repeatability analysis's RSD was less than 1. The approach was verified in accordance with the ICH Q_2R_1 standard. The results demonstrated that the suggested method involves the mixing of Mirabegron and silodosin in a synthetic mixture. A simple, specific, accurate, exact, and reliable UV-visible spectroscopic Method has been developed and validated in this study.

Corresponding author, email: shivangipithadiya1999@gmail.com (S.B. Pithadiya).

Graphical Abstract



Introduction

Mirabegron (MIRA) is a 2-(2-Amino-1,3-thiazol-4-yl)N-[4-(2-[(2R)-2hydroxy-2phenylethyl]aminoethyl]phenyl]acetamide with a molecular weight of 396.509 g/mol [1]. Silodosin (SILO) is Known by IUPAC name (3-hydroxypropyl) 5[(2R)-2[2-[2-[2-(2,2,2-trifluoroethoxy) phenoxy] ethylamino] propyl] -2,3-dihydroindole-7-carboxamide [2]. Mirabegron activates the β3-adrenoceptors in the detrusor muscle of the bladder, promoting bladder filling and urine storage without impacting contractions during voiding. Moreover, silodosin specifically blocks the activity of alpha(α)-1A adrenergic receptors and exhibits the strongest attraction to the alpha(α)-1A subtype. Therefore, a combination of these two medications is employed to address both overactive bladder (OAB) and benign prostatic hyperplasia (BPH) [3].

The combination of MIRA and SILO in individuals with complex OAB who also had BPH was well tolerated and no significant adverse effects were identified [3]. This fixed-dose combination undergoing a clinical trial phase three and in this conventional tablet used in the strength of 3.2 : 1 dosing ratio of MIRA and SILO, respectively [4]. Figures 1 and 2 display the chemical structures of MIRA and SILO.

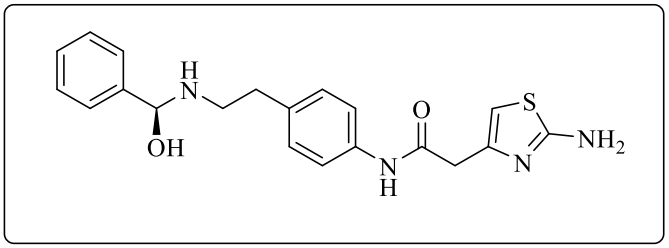


Figure 1. Chemical structure of Mirabegron.

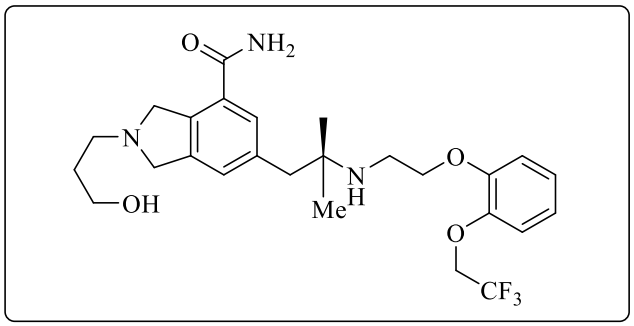


Figure 2. Chemical structure of Silodosin.

Based on the literature review, it has been determined that SILO is acknowledged in JP (Japanese Pharmacopoeia), while MIRA is not acknowledged in any pharmacopoeia [5]. Several spectroscopic methods, such as UV spectroscopy, were detailed to determine the levels of MIRA and SILO individually and in combination with other medications [6-10] and also other study were reported [11-15]. Hence, it is logical to create a method that may be employed with a fixed-dose combination that is adaptable, and based on scientific principles. The pharmaceutical industry and research labs can use existing methods for regular analysis.

Martials and Methods

Materials, reagents, and software

Prudence Life Science supplied SILO, and CTX Life Science supplied MIRA. Microcrystalline cellulose, lactose, magnesium stearate, and sorbitol were used as excipients in the preparation of the synthetic combination, while HPLC-grade methanol and acetonitrile were used as the diluent. A UV-1800 Shimadzu visible UV spectrophotometer was used, and UV probe software (version 2.6) was used to analyze data.

Procedure for determining the sampling wavelength

primarily for the zero order spectra of the 200-400 nm UV scans for MIRA and SILO, to determine the wavelength for the first-order derivative method. After obtaining the zero-order absorption spectra of both medications superimposed, first-order derivatives for MIRA and SILO were created using scaling factor 1 and 16 delta lambda (Δλ). The wavelengths at which silodosin (ZCP) and mirabegron (ZCP) zero crossing points are located are 268.25 and 249.54 nm, respectively. Thus, the wavelength for SILO is 249.54 nm, whereas the wavelength for MIRA estimation is 268.25 nm. Zero order and the 1st order spectra overlay is observed in Figures 3 and 4 for the drug MIRA and SILO.

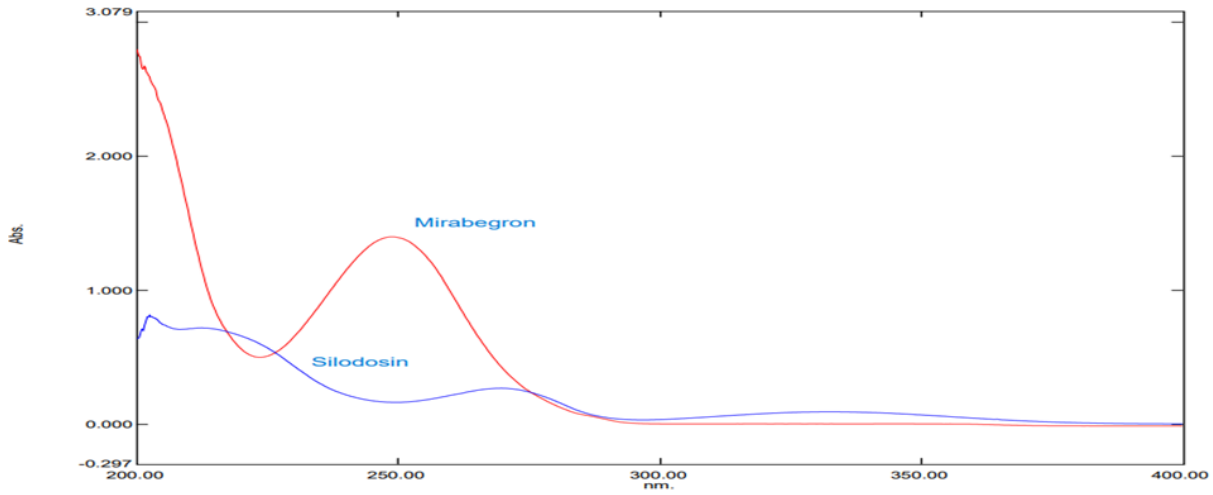


Figure 3. Zero order spectra overlay of MIRA and SILO.

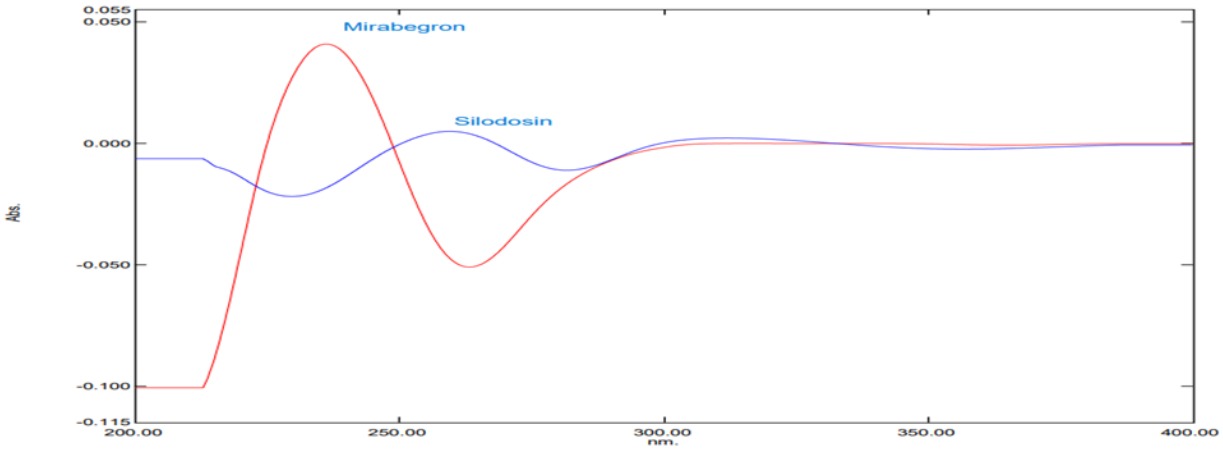


Figure 4. The 1st order derivative spectra overlay of MIRA and SILO.

Preparation of solutions

Preparation of standard stock solution of mirabegron

25 mg of Mirabegron was precisely measured out and transferred into a 100 mL volumetric flask, and after adding around 50 mL of methanol, it was thoroughly mixed. Next, methanol was exactly added to the mark. The ultimate solution had 250 µg/mL of concentration.

Preparation of standard stock Solution of silodosin

8 mg of silodosin was weighed precisely into a 100 mL volumetric flask. About to the mark, the HPLC-grade methanol was added and fully combined. The final solution had 80 µg/mL of concentration.

Preparation of standard solution of the mixture of both drugs

50 mL volumetric flask was prepared with precisely 8 milligrams of silodosin and 25 mg of mirabegron. The methanol was appropriately amounted and thoroughly mixed in. 500 parts per million of mirabegron and 160 parts per million of silodosin were presented in the final solution. 12.5 mL of the

aforementioned mixture's solution was transferred into a 25-millilitre volumetric flask, it was topped off with the necessary quantity of methanol, and was stirred well. The final mixture had 250 ppm of mirabegron and 80 ppm of silodosin.

Preparation of synthetic mixture

The following common tablet excipients were added: lactose, mannitol, magnesium stearate, and microcrystalline cellulose, which were introduced as gliding, diluent, dissolving, and binder, respectively, to a 100 mL volumetric flask containing 50 mg of MIRA and 16 mg of SILO. Subsequently, roughly 50 mL of methanol were introduced, thoroughly dissolved using a sonicator, and adjusted to achieve 500 micrograms per mL for MIRA and 160 micrograms per mL for SILO. 10 mL of the aforementioned solution was pipetted out as needed, and then dilute with methanol to produce the final concentrations of SILO at 32 µg/mL and MIRA at 100 µg/mL, respectively.

Assay of synthetic mixture

To determine the concentrations of MIRA and SILO in a synthetic mixture, in a 100 mL volumetric flask, 8 mg of SILO and 25 mg of MIRA are weighed and measured and it was completely incorporated by adding common excipients. Once the solution has been filtered through a 0.45-m membrane filter, the first few millilitres of the filtrate should be discarded. After dilution, it was mixed thoroughly: 1 mL of filtrate solution to 10 mL of diluents. In this experiment, the final concentrations of MIRA and SILO that were examined were 25 ppm and 8 ppm, respectively.

Method validation [16]

Linearity

The proper quantity of drug solution was made using standard stock solutions, and it was subsequently split among several 10 mL volumetric flasks. HPLC-grade methanol was used to alter the volume of the MIRA and SILO drugs until the desired concentrations were achieved. These were 3.125, 12.5, 25, 37.5, 50, and 62.5 ppm as well as 1, 4, 8, 12, 16, and 20 ppm, respectively. The absorbance spectra of each solution were recorded against Methanol blank at 268.25 nm for MIRA and 249 nm for SILO medications, respectively, after the absorbance spectra were plotted against concentration.

Accuracy

Three different drug concentration levels of 50, 100, and 150% (12.5, 25 and 37.5 µg/mL) were spiked to assess the accuracy of the method in triplicate for MIRA and (4, 8, and 12 µg/mL) SILO. The percentage of the recovery range was used to assess the method's accuracy.

Precision

Six duplicates of each dose- 25 µg/mL for MIRA and 8 µg/mL for SILO- were used to test repeatability. Intraday and interday precision in MIRA and SILO were measured in triplicate at three different concentration levels: MIRA at 50, 100, and 150% (12.5, 25, and 37.5 µg/mL) and SILO at (4, 8, and 12 µg/mL). The RSD was computed to evaluate precision.

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

LOD and LOQ of MIRA and SILO were determined using the average slope and standard deviation of intercepts.

Specificity

Six repetitions at doses of 25 µg/mL and 8 µg/mL, respectively, were used to test the specificity of MIRA and SILO. To assess any potential interference that the excipients might have created, this was done both with and without their addition.

Robustness

By purposely altering the experimental conditions, such as the wavelength, the method's robustness was verified. The wavelength adjustments were performed within an accuracy of 0.5 nm. The wavelengths that were used for MIRA were 267.25 nm, 268.25 nm, and 269.25 nm. The wavelengths that were employed for SILO were 248.54 nm, 249.54 nm, and 250.54 nm. The Standard Deviation (RSD) was computed to assess the robustness of the method.

Results and Discussion

Linearity

For SILO and MIRA, the calibration curve ranges were 2-20 µg/mL and 3.125-62.5 µg/mL, respectively. The correlation coefficients of the MIRA and SILO were found to be 0.9962 and 0.9954, respectively. The method is linear. Figures 5 and 6 demonstrate the linearity graph of mirabegron and silodosin, respectively.

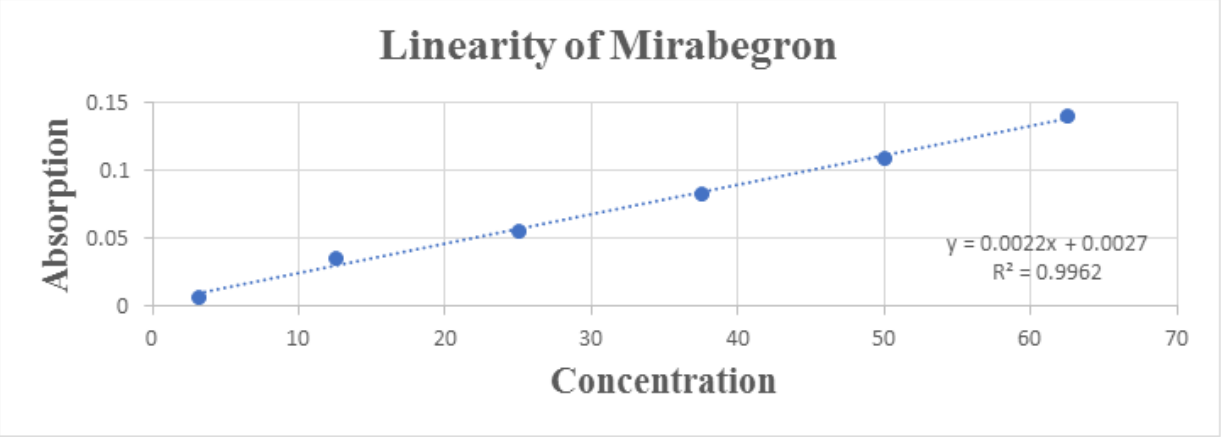


Figure 5. Linearity of Mirabegron.

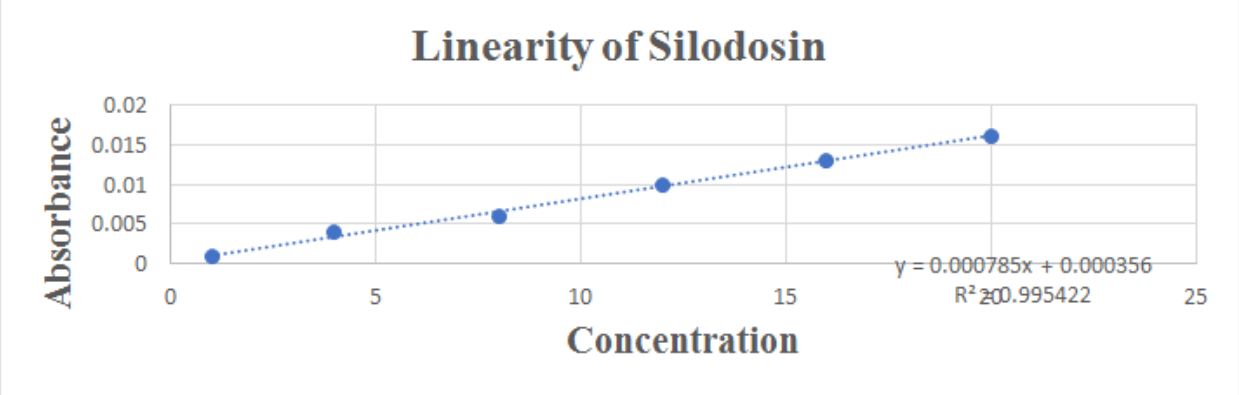


Figure 6. Linearity of Silodosin.

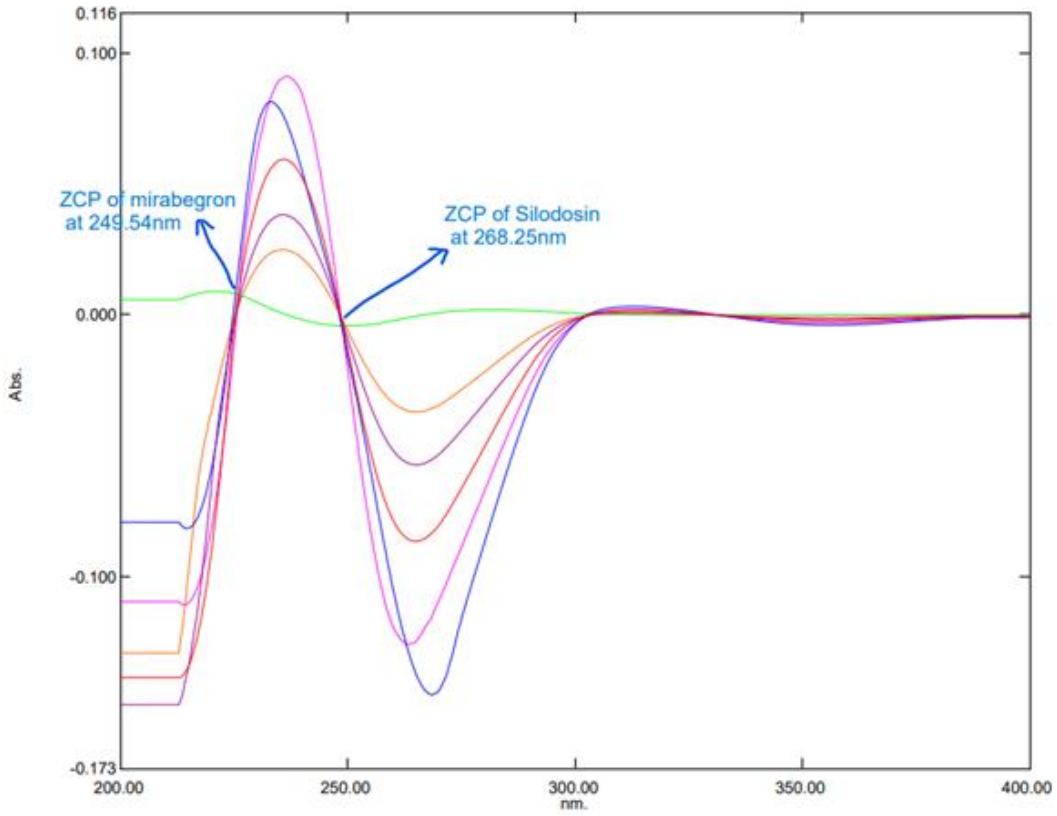


Figure 7. Linearity of combined spectra for MIRA and SILO.

Specificity

The excipient interference was determined to be less than 0.5% at the working wavelength of 249.54 nm for SILO and 268.25 nm for MIRA, indicating that the method is specific.

LOD and LOQ

LOD and LOQ of MIRA and SILO were determined by formula. LOD and LOQ were found to be 1.35 µg/mL and 4.10 µg/mL for MIRA and 6.50 µg/mL and 19.71 µg/mL for SILO, respectively.

Accuracy

By introducing a known quantity of a standard medication into the assay concentration at three levels in three duplicates, the suggested method's accuracy was assessed. For MIRA and SILO, respectively, accuracy was found to range between 98.54-102.09 µg/mL and 99.84-100.61 µg/mL. Tables 1 and 2 indicate the recovery study's findings. The recovery study indicates that the procedure is accurate.

Precision

RSD is used to represent repeatability and intermediate precision. After absorbance was measured, the repeatability study and intra-day and inter-day precision RSD < 2 were found to be adequate. Tables 3 and 4 present the precision results, which indicate that the approach was determined to be precise.

Robustness

When a deliberate wavelength change occurs and the RSD of absorbance is less than 2, it can be concluded that the method is robust. The Results are provided in Table 5.

Table 1. Accuracy study for Mirabegron

Target Conc.	Level	Amt. Added	Total Amt	Mean absorbance	Mean concentration	Mean %Recovery
25	50%	12.5	37.5	0.084	36.95	98.54
25	100%	25	50	0.115	51.04	102.09
25	150%	37.5	62.5	0.14	62.40	99.85

Table 2. Accuracy study for Silodosin

Target Conc.	Level	Amt. Added	Total Amt.	Mean absorbance	Mean concentration	Mean %Recovery
8	50%	4	12	0.01	11.98	99.84
8	100%	8	16	0.013	16.05	100.32
8	150%	12	20	0.016	20.12	100.61

Table 3. Intraday and Interday precision of Mirabegron and Silodosin

Precision		Intraday precision		Interday precision	
Wavelength	(%)	Mean abs ± SD	RSD	Mean abs ± SD	RSD
At 268.25 nm (MIRA)	50	0.035±0.26	1.61	0.035±0.26	0
	100	0.055±0.26	1.03	0.055±0.26	0
	150	0.083±0.26	0.69	0.083±0.26	0
At 249.54 nm (SILO)	50	0.004±0.81	1.61	0.004±0.79	0
	100	0.006±0.79	1.03	0.006±0.079	0
	150	0.01±0.0	0.69	0.01±0	0

Table 4. Repeatability of Mirabegron and Silodosin

Sr NO.	Mirabegron		Silodosin	
	Concentration	Absorbance	Concentration	Absorbance
1	25	0.055	8	0.006
2	25	0.055	8	0.006
3	25	0.055	8	0.006
4	25	0.055	8	0.006
5	25	0.055	8	0.006
6	25	0.055	8	0.006
MEAN	0.0555		0.006833333	
SD	0.00054772		0.000408248	
RSD	0.000986887		0.059743652	

Table 5. Robustness study for Ranolazine and Metformin hydrochloride

Drugs	Wavelength	Mean abs ± SD	%RSD
MIRA	267.25 nm	0.059 ± 0.26	1.01
	268.25 nm	0.055 ± 0.026	1.03
	269.25 nm	0.054 ± 0.26	0.26
SILO	248.54 nm	0.001 ± 0.00	0.00
	249.54 nm	0.007 ±0.00	0.00
	250.54 nm	0.012 ± 0.00	0.00

Conclusion

Using the first-order derivative method, a new technique has been created to simultaneously determine the concentrations of Silodosin (SILO) and Mirabegron (MIRA) in a synthetic combination. The ICH Q2R1 standard was followed in the verification of this methodology. Within the concentration range of 3.125-62.5 µg/mL for mirabegron and 1-20 µg/mL for silodosin (SILO), respectively, the linearity of both compounds was verified. It was shown that the correlation coefficients for silodosin (SILO) and mirabegron were 0.995 and 0.996, respectively. For the Mirabegron and Silodosin, respectively, accuracy was found to be between 98.54 and 102.09% and 99.84 and 100.61%. Mirabegron's limits of quantification (LOQ) and detection (LOD) were found to be 4.10 µg/mL and 1.35 µg/mL, respectively. Silodosin was found to have a LOD of 6.50 µg/mL and a LOQ of 19.71 µg/mL. RSD for the precision and repeatability analysis was determined to be less than 1. As a result, this method can be used to analyze dose forms effectively and independently. It takes less time and is simple, precise, accessible, robust, and accurate.

Acknowledgements

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Shivangee Bharatbhai Pithadiya : 0009-0006-5412-502X

References

[1]. Sankar P.R., Kishore K.P., Babji B., Sulthana S.Md. Analytical method development and validation for the determination of mirabegron in pharmaceutical dosage form by RP-HPLC, *International Journal of Pharmaceutical Sciences and Research*, 2020, **11**:2223 [Crossref], [Google Scholar], [Publisher]

[2]. Malode S.S., Wagh M.P. Formulation and Evaluation of Orodispersible Tablets Containing Taste Masked Mirabegron Resinate, *Research Journal of Pharmacy and Technology*, 2021, **14**:4736 [Crossref], [Google Scholar], [Publisher]

[3]. Surawase R.K., Baheti K.G. Box behnken design for optimization of mirabegron solid dispersion by fluidized bed processing. *Research Journal of Pharmacy and Technology*, 2022, **15**:1472 [Crossref], [Google Scholar], [Publisher]

[4]. CDSCO-Drug combination approval, December 2023. [Publisher]

[5]. Japanese Pharmacopoeia, 8th Edn, The ministry of Health, Labourand Welfare, 2017, 1573.

[6]. Nageswara R.R., Madhuri D., reddy L.S., Rani K., Tejaswini P., Kumara S.G. Development and validation of derivative spectrophotometric method for estimation of mirabegron in bulk and tablet dosage form. *World Journal of Pharmaceutical Research*, 2017, **6**:760 [Publisher]

[7]. Matsukawa Y., Takai S., Majima T., Funahashi Y., Sassa N., Kato M., Yamamoto T., Gotoh M. Comparison in the efficacy of fesoterodine or mirabegron add-on therapy to silodosin for patients with benign prostatic hyperplasia complicated by overactive bladder: A randomized, prospective trial using urodynamic studies. *Neurourology and Urodynamics*, 2019, **38**:941 [Crossref], [Google Scholar], [Publisher]

[8]. Suresh B.K., Anusha N., Sravani A.B. Analytical method development and validation for the estimation of Mirabegron in pure and its solid dosage form by UV spectrophotometric method. *International Journal of Research in Pharmaceutical Sciences and Technology*, 2020, **1**:146 [Crossref], [Google Scholar], [Publisher]

[9]. Ravisankar P., Vidya V.S., Nithya P.D., Babu P.S. Validated UV spectrophotometric method for quantitative determination of Mirabegron in bulk and Pharmaceutical dosage form. *Der Pharmacia Lettre*, 2016, **8**:96. [Google Scholar]

[10]. Boltia S.A., Abdelkawy M., Mohammed T.A., Mostafa N.N. Validated stability-indicating spectrophotometric methods for the determination of silodosin in the presence of its degradation products. *Spectrochimica Acta Part A: Molecular and Biomolecular spectroscopy*, 2018, **202**:131 [Crossref], [Google Scholar], [Publisher]

[11]. Peddagundam B., Abdul Ahad H., Chinthaginjala H., Ksheerasagare T., Ganthala A. K., Gummadisani G. R. The design of soft drugs: Basic principles, energetic metabolite methods, approved compounds, and pharmaceutical applications, *Journal of Chemical Reviews*, 2022, **4**:241 [Crossref], [Publisher]

[12]. Mezoughi A., Mohammed W., Ettarhouni Z.O. Recent biological applications and chemical synthesis of thiohydantoins. *Journal of Chemical Reviews*, 2020, **2**:189 [Crossref], [Publisher]

[13]. . Mezoughi A., Mohammed W., Ettarhouni Z.O. Recent biological applications and chemical synthesis of thiohydantoins, *Journal of Chemical Reviews*, 2020, **2**:189 [Crossref], [Publisher]

[14]. M. Sengar, S. Saxena, S. Satsangee, R. Jain, Silver nanoparticles decorated functionalized multiwalled carbon nanotubes modified screen printed sensor for voltammetric determination of butorphanol, *Journal of Applied Organometallic Chemistry*, 2021, **1**:95 [Crossref], [Google Scholar], [Publisher]

[15]. S. Sharma, A short review of past, present, and future of metallocene and its derivatives as an effective therapeutic agent, *Journal of Applied Organometallic Chemistry*, 2023, **3**:142 [Crossref], [Publisher]

[16]. Guideline I.H.T., Validation of analytical procedures: text and methodology, *Q2 (R1)*, 2005, **1**:05 [Google Scholar]

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