RESEARCH ARTICLE

Simultaneous Quantification of Dutasteride and Silodosin Using a Stability-Indicating RP-HPLC Approach

Parag Pathade, Madhuri Harde*, Bhaskar Aher, Vinod Bairagi

Department of Pharmaceutical Quality Assurance, KBHSS Trust Institute of Pharmacy, Nashik, Maharashtra, India.

Received: 06th December, 2023; Revised: 14th April, 2024; Accepted: 25th May, 2024; Available Online: 25th June, 2024

ABSTRACT

An alternative stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) approach remained established to quantify silodosin (SLD) and dutasteride (DTS) in the mixture, validated per International Council of Harmonization (ICH) recommendations. A Shimadzu Model LC-2030 PLUS (IND) HPLC system, 231 nm detection wavelength, and 10 minutes run duration were used to create the method. A 75:25 mobile phase of methanol and water with 0.05% OPA was used at 1-mL/min. The concentrations of SLD (8–40 µg/mL) and DTS (0.5–2.5 µg/mL) were shown to be linearly related, with R2 values of 0.9998 for SLD and 0.9993 for DTS.

Both of the drugs had accuracy and precision within 2% RSD. SLD and DTS mean recoveries were 97.5 to 102%. According to ICH criteria, degradation trials with acid, alkali, oxidizing agent, and heat assessed the approach's stability. The established approach is fast, accurate, sensitive, and precise for routine SLD and DTS analysis from bulk as well as pharmaceutical dosage forms.

Keywords RP-HPLC, ICH, Stability, Accuracy.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.2.44

How to cite this article: Pathade P, Harde M, Aher B, Bairagi V. Simultaneous Quantification of Dutasteride and Silodosin Using a Stability-Indicating RP-HPLC Approach. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):825-830.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A compound with the chemical formula (-) is silodosin (SLD).-1-(3-hydroxypropyl)[(2R)] -5-3-[2-(2,2,2-trifluoroethoxy) phenoxy]ethyl amino]propyl]2, 3-dihydro-1H-indole7-carboxamide, as seen in Figure 1. Men mostly take this medicine for benign prostatic hyperplasia (BPH), a benign swelling of the prostate gland that is not malignant. Alpha-1 adrenergic receptor antagonist silodosin primarily targets the bladder, neck, prostate, and urethra.

Chemically known as (1S,3aS,3bS,5aR,9aR,9bS,11aS), dutasteride (DTS) is another medication that is commonly used to treat mild prostatic hyperplasia. Anhydride 3-[2,5-bis(trifluoromethyl)phenyl]ninety-alpha, eleventh-methyl Figure 2 shows the compound -7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f] quinoline-1-carboxamide. Unlike silodosin, dutasteride blocks alpha-1 receptors in the prostate, bladder, neck, and urethra, making it an effective treatment for benevolent BPH caused by an inflamed prostate.

Another medicine that is mainly used is to treat BPH and androgenetic alopecia (male-pattern hairlessness) by blocking this conversion.[1]

The discovery and development of pharmaceutical drugs, along with human and animal investigations, are contingent on high-performance liquid chromatography (HPLC) technique development and validation.[2] These analytical methods compare drug compounds or products to specified acceptability criteria. HPLC is a method excellent for many applications, including measuring the peak concentration of novel chemical compounds, monitoring reaction variations throughout synthesis or scaling up, evaluating novel formulations, and checking the quality of pharmaceutical products.[3,4]

A literature survey indicated that various HPLC techniques had been documented for SLD and DTS in its pure and tablet forms.[5,6]

We set out to create and evaluate the reverse-phase high-performance liquid chromatography (RP-HPLC) technique in this study. This method of analysis is designed to simultaneously analyze the quantification of silodosin and dutasteride is extremely valuable due to their medicinal significance and predicted future demand in the pharmaceutical sector.[7,8]

MATERIALS AND METHODS

Reagents and Chemicals

Reference standard and highly purified silodosin and dutasteride were received as gift samples from Sun Pharmaceutical Industries Ltd India. Silodosin and dutasteride are carefully extracted and synthesized into a marketable drug under the

Author for Correspondence: paragapathade@gmail.com

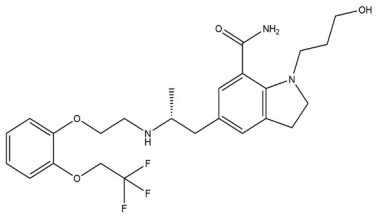

Figure 1: Silodosin

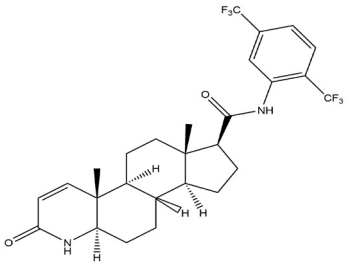

Figure 2: Dutasteride

brand name "Silodal-D4 tablet," local medical stores sell silodosin at 4 mg also dutasteride at 0.5 mg. Other investigative reagents, including methanol (HPLC grade), potassium hydrogen orthophosphate, and orthophosphoric acid, were graded analytically. Merck (India) supplied high-purity Milli-Q water. AR-grade compounds comprised the remainder of the chemicals.

Chromatographic Conditions and Instrument

Using a UV detector and a Thermo-Hypersil ODS, C18 4.6 x 250 mm, 5 µ columns, the analytical method was developed and validated using the HPLC system (Shimadzu Model LC-2030 PLUS (IND)). A constant temperature of 25ºC was applied to the column. Whole time machine is run is 10 minutes, with 20 µL being the injection volume and 1.0 mL/min being the fixed flow rate. HPLC system that enabled subtle identification of analytes consisted of a 940D pump, a 20 µL injection loop, and a UV 740D Absorbance detector. pH meter, ultra Sonicator, and electronic balance were all calibrated by Mettler Toledo during the system validation.

Stock Solution

Silodosin (80 mg) and dutasteride (5 mg) were measured with great precision. A precise amount of 80 mg of silodosin and 5 mg of dutasteride were dissolved separately in an adequate amount of mobile phase in two separate 10 mL calibrated volumetric flasks. Both solutions underwent sonication for a duration of 10 minutes in order to liquefy the medication. By including the mobile phase, the final volume of solutions was brought to 100 mL. It was determined that the resulting solution included 800 µg/mL of silodosin and 50 µg/mL of dutasteride, making it a stock solution.

Standard Solution

Preparation of std. silodosin solution

The final concentration was 8 to 40 µg/mL, achieved by adding the mobile phase to 0.1 to 0.5 mL of a freshly prepared standard stock solution (800 µg/mL) and adjusting the volume to 10 mL.

Preparation of std. dutasteride solution

To get 0.5 to 2.5 µg/mL, add 10 mL of mobile phase to 0.1 to 0.5 mL of newly made standard stock solution (50 µg/mL) pipetted into a 10 mL volumetric flask.

Preparation Solution for Assay

Dosage of silodosin and dutasteride is 4 and 0.5 mg, respectively, according to the label. After mixing it with 10 mL of methanol, a sample was prepared. After 10 minutes of sonication, the mixture was able to be extracted. The remaining 0.1 mL was diluted to 10 mL using the mobile phase. It was prudent to examine drug peak area after adding the solution. This investigative process, involving stages,[9,10] accurately measured silodosin and dutasteride in the marketed product to ensure dependability and label claims.

System Suitability Study

The system suitability study used two HPLC systems under identical settings at different periods to evaluate system-to-system variability. Five samples were made and tested according to the method. For this test, various parameters remained examined. This set of parameters verified repeatability and resolution against the protocol. The approval criteria for the system suitability research were determined. The RSD for principal peak retention durations as the sum of the five replicate doses of each regular solution must not surpass 1%. Five separate injections of every conventional solution should yield results with respect to the primary peak area having an RSD of no more than 2.0%. SLD and DTS peaks should have at least 2000 theoretical plates, while SLD and Dutasteride peaks should have NMT 2.0 tailing factors.[11]

Method Validation

System relevance, specificity, accuracy, linearity, ruggedness, LoD, and LoQ were among the tests that were carried out to confirm the validity of the established analytical techniques. These tests were conducted in accordance with guidelines set by ICH.[12]

Linearity

Before OPA by baseline, the mobile and stationary phases were allowed to equilibrate. In a 10 mL volumetric flask, transfer 0.1, 0.2, 0.3, 0.4, and 0.5 mL of the solution from the standard stock solutions of silodosin and dutasteride, respectively, to reach final concentrations of 8, 16, 24, 32, and 40 µg/mL for SLD and 0.5, 1, 1.5, 2, and 2.5 µg/mL for DUT. When the mixture is ready, add the mobile phase. Drug concentration versus peak area graphs show peaks at 231 nm after injection.

Precision

The precision research proved the method's repeatability. Method and system precision were tested. In system precision, standard SLD and DUT solutions were injected five times per procedure. Peak area RSD should be NMT over 2.0%.

Accuracy

The experiment was performed three times for both the medication SLD and DUT at concentrations of 80, 100, and

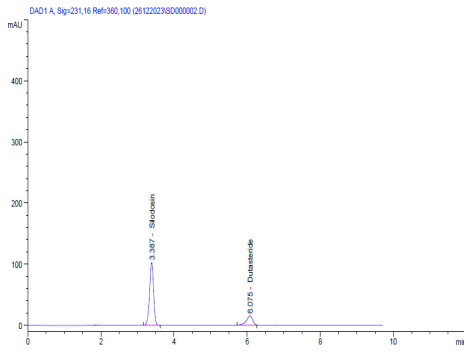

Figure 3: Chromatogram of silodosin and dutasteride

120% of the prescribed amount in order to evaluate accuracy. Accuracy was evaluated in relation to the standard solution based on strength. The average recovery for both medications was computed. At any spike level, the mean recovery for both drugs is between 90 and 110.0%.

Robustness

A deliberate fluctuation in flow rate was used to verify the suggested method's robustness. The HPLC instrument was supplied with the prepared standard solution at three distinct flow rates as per the evaluation procedure. The appropriateness parameters of the system were assessed.

Specificity

By injecting both standard and test SLD and DUT solutions, the analytical technique was tested. These samples were examined for chromatogram interference peaks. Standards and tests should have identical chromatograms with close retention time.[12,13]

LoD/LoQ

LoD is the least concentration the system can detect. LoQ, on the other hand, is the lowest quantifiable amount. Linearity data was used to derive LoD and LoQ using the formula.[14]

RESULTS & DISCUSSION

Method Development and Optimized Chromatographic Conditions

Experiments were conducted with a number of different mobile phase compositions; however, after a number of tests, it was determined that the most efficient and dependable mobile phase composition was a combination of methanol and water (0.05% OPA) at a ratio of 75:25. In order to ensure that the chromatographic analysis was carried out correctly throughout the entire investigation, this particular mobile phase composition was carefully preserved. Table 1 includes the various mobile phase combinations that were attempted, the results that were observed, and the ultimate mobile phase. (Figure 3) provides a visual representation of the chromatographic profile that was observed as a consequence of the utilization of this improved mobile phase. Taking all

Table 1: Different mobile phase compositions

Mobile phase	*Retention time (min)*		*Remark*
	SLD	*DTS*	
Methanol+ Water (80:20% v/v) Acetic Acid 0.1% 0.7 mL	12.005	16.262	Broad peaks were obtained.
Methanol+ Water (45:55% v/v) Acetic Acid 0.1% 1-mL in methanol	3.659	4.666	Splitting is observe
Acetonitrile + Water (45:55% v/v) Acetic acid 0.1% 0.7 mL	2.695	3.100	No sharp peak
Methanol+ Water OPA 0.05% (75:25% v/v)	3.387	6.075	Resolve peak and sharp

Table 2: Optimised chromatographic conditions

Parameters	*Details*
Column	Schimadzu LC-2030 C18, 4.6 x 250 mm, 5 µ column
Detector wavelength	231 nm
Flow rate	1.0 mL/min
Mobile phase	MEOH : Water 75:25 0.05% OPA
Column temp	25ºC
Run time	10 minutes
Injection volume	20 µL
Retention time	SLD 3.38 minutes DTS 6.07 minutes

Table 3: Data of system suitability study

Injection	*RT*	*RT*	*Peak area*	*Peak area*	*Theoretical plates*	*Theoretical plates*	*Tailing factor*	*Tailing factor*
	SLD	*DTS*	*SLD*	*DTS*	*SLD*	*DTS*	*SLD*	*DTS*
1	3.387	6.075	3355554	4121613	6485	4949	1.1	1.34
2	3.38	6.055	3362551	4111013	6478	4889	1.08	1.39
3	3.386	6.065	3355453	4122683	6472	4922	0.98	1.24
4	3.387	6.075	3285556	4121619	6505	4907	1.101	1.14
5	3.387	6.075	3335252	4151613	6544	4940	1.1	1.09
Mean	3.3854	6.069	3338873.2	4125708	6496.8	4921.4	1.07	1.24
SD	0.0030	0.0089	31502.4363	15245.0945	…			…
%RSD	0.090	0.147	0.944	0.370	…			…

Table 4: Linearity study of SLD

Concentration (ppm)	*Area*	*Statistical analysis*
8	777.9009	
16	1401.1045	y = 80.57X+119.5 Correlation Coefficient (R^2) =0.9998
24	2032.2508	
32	2707.8492	
40	3347.4800	

Table 5: Dutasteride linearity study

Concentration (ppm)	*Area*	*Statistical analysis*
0.5	182.7254	
1	364.1038	y = 343.0x+10.45 Correlation Coefficient (R^2) =0.9993
1.5	515.4371	
2	691.1130	
2.5	874.8866	

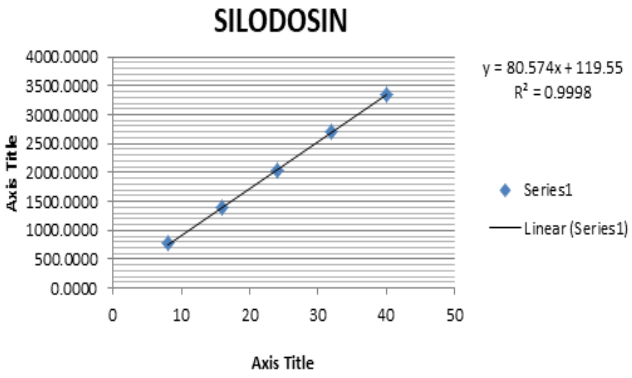

Figure 4: Silodosin calibration curve

Table 6: Outcome of recovery data for silodosin and dutasteride

Drug	*Level (%)*	*Amt. taken (µg/mL)*	*Amt. added (µg/mL)*	*area Mean* ± S.D.*	*Amt. recovered Mean * ± S.D.*	*%Recovery Mean * ± S.D.*	*Mean % recovery*	*Statistical analysis of %recovery (Mean)*
SLD	80	8	6.4	14.44 ± 0.016	6.44 ± 0.016	100.70 ± 0.25	100.70	0.25
	100	8	8	16.15 ± 0.103	8.15 ± 0.103	101.94 ± 1.29	98.37	0.71
	120	8	9.6	17.61 ± 0.040	9.61 ± 0.040	100.06 ± 0.42	101.94	1.27
DTS	80	0.5	0.4	7.18 ± 0.023	3.18 ± 0.023	99.39 ± 0.70	99.26	0.05
	100	0.5	0.5	8.01 ± 0.002	4.01 ± 0.002	100.17 ± 0.05	100.06	0.42
	120	0.5	0.6	8.78 ± 0.030	0.05 ± 0.05	99.66 ± 0.61	98.83	0.62

Table 7: Result of intra and inter day precision studies

Drug	*Conc^n (µg/mL)*	*Precision*			
		Intraday		*Interday*	
		Mean ± SD	*%Amt found*	*Mean ± SD*	*%Amt found*
SLD	16	1405.2 ± 5.67	99.74	1402.9 ± 0.96	99.56
	24	2031.4 ± 1.53	98.88	2031.9 ± 2.16	98.90
	32	2697.9 ± 18.3	100.01	2711.3 ± 1.24	100.53
DTS	1	358.48 ± 1.32	101.47	357.8 ± 0.56	101.27
	1.5	519.64 ± 5.36	98.97	521.82 ± 6.49	99.39
	2	694.4 ± 7.45	99.71	690.75 ± 4.96	99.17

*Mean of every 2 analyses for RP-HPLC method

Table 8: Result of robustness study of silodosin and dutasteride

Parameters (Flow rate) (mL/min)	*SLD*			*DTS*		
	Conc.(µg/mL)	*(Mean ± SD)*	*%RSD*	*Conc.(µg/mL)*	*(mean ± SD)*	*%RSD*
0.9	24	2191.47 ± 1.92	0.09	1.5	584.75 ± 0.76	0.13
1	24	2072.47 ± 1.89	0.08	1.5	531.75 ± 0.58	0.11
1.1	24	1870.80 ± 2.47	0.13	1.5	494.71 ± 1.61	0.33

of these data into consideration, it is clear that a methodical approach was followed in order to ascertain the mobile phase that was the most appropriate for the chromatographic investigation of SLD and DTS. The chromatographic variables that have been optimized are provided in Table 2.

Table 9: Result of LoD and LoQ for silodosin and dutasteride

Parameter	*Silodosin*	*Dutasteride*
LoD (µg/mL)	0.98	0.2682
LoQ (µg/mL)	1.2	0.8128

Table 10: Silodosin and dutasteride forced degradation study

Degradation parameter	*Area of degraded Sample silodosin*		*Area of degraded sample futasteride*		*Degradation of silodosin*		*Degradation of dutasteride*	
	1 hour	*2 hours*	*1 hour*	*2 hours*	*1 hour*	*2 hours*	*1 hour*	*2 hours*
Alkali 0. 1 N NaOH	453.21	403.56	321.11	308.19	3.43	4.02	14.83	30.05
Acid 0. 1 N HCl	420.26	401.34	367.25	245.65	2.40	3.92	13.37	19.8
3% H_2O_2	412.9	389.8	343.90	311.34	1.36	6.09	4.12	7.42
Neutral	--	365.85	--	325.56	1.82	2.31	0.27	1.33
Photolytic 24 hours	--	--	--	--	0.34		1.22	

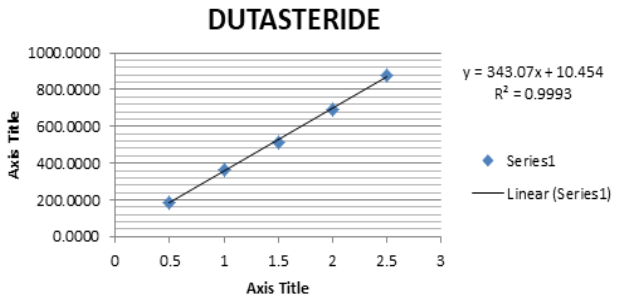

Figure 5: Dutasteride calibration curve

System Suitability

The findings of 5 duplicates put into the HPLC system were found to be within the permitted range. The SLD and DTS compounds were seen to have excellent retention and were successfully separated at 3.387 and 6.075 minutes, respectively. RSD values of 0.09 and 0.14% for SLD and DTS, respectively, met the acceptance criterion of not more than 2%, showing a high level of repeatability for the replicate injections. The tailing factor for SLD was determined to be 1.07, while for DTS, it was discovered to be 1.24. These values show that both substances exhibited excellent peak symmetry, meeting the acceptability criteria of not exceeding 2%. Over the minimum acceptable threshold of 2000, an actual number of theoretical plates was found to be over 4000, thus demonstrating exceptional column efficiency. In addition, the peak area responses of the main peaks for SLD and DTS were determined to be 3338873 and 4125708, respectively, with an RSD of 0.94 and 0.37% (within the acceptable limit of not more than 2%). The data for the system suitability research are displayed in Table 3.

Linearity

The calibration curve for SLD and DTS was determined to be linear within a concentration range of 8 to 40 µg/mL. However, for dutasteride, the investigation was conducted within the range of 0.5 to 2.5 µg/mL. (Figures 4 and 5) displays a graphical representation of SLD and DTS. The regression coefficient (R^2) was shown to be 0.9998 for SLD and 0.9993 for DTS. Tables 4 and 5 present concentration range and its corresponding area response for SLD and DTS.

Accuracy

The percentage recovery of SLD and DTS was examined to verify the suggested method. In this study, conventional medicines were added to the pill solution at 80, 100, and 120% concentrations. After that, recovery was examined. Table 6 shows that these recovery trial numbers are reliable. SLD and DTS always recovered 97.5 to 102%.

Precision

The method was developed carefully analyzing many duplicates of SLD and DTS standard solutions. To identify intraday and interday variations, each solution was analyzed three times. Table 7 shows the intraday and interday precision findings. All %RSD readings below 2% indicate the analytical method's precision.

Robustness

In particular, flow rate effects on drug peak retention time and tailing factor were examined. Flow rate regulated by ± 0.1 mL/min. Table 8 shows robustness studies' results, proving the approach can tolerate deliberate alterations. Flow rates of 0.9,1, and 1.1 mL/min were all part of the robustness study. With the peak area's relative standard deviation (%RSD) below 2%, the technique performed wonderfully under these altered conditions. The outcomes validate the assertion that the analytical approach is stable and consistently produces the same outcomes in different settings.

LoQ

Both silodosin and dutasteride have low detection and quantification limits that are displayed in Table 9. LoD and LoQ have been determined, proving that approach is suitable for measuring smaller levels of SLD and DTS reliably.

Stress Studies

An identical specimen of silodosin and dutasteride was tested in acidic, alkaline, oxidative, and hydrolytic conditions. Stress testing showed that the deterioration fulfilled acceptance requirements, proving the method's stability.[15,16] This shows the analytical method's reliability in identifying and describing breakdown products, revealing silodosin and dutasteride molecules' inherent stability. To further understand these data, Table 10 precisely displays the stress degradation experiments for silodosin and dutasteride.

These tables show the degree of deterioration under each stress condition.

CONCLUSION

In order to measure silodosin and dutasteride in both bulk and dose form, we created and tested a stability-indicating RP-HPLC technique. These pharmaceutical compounds can be distinguished and measured under diverse stress conditions, proving the method's resilience and stability. Approach validation results show precision, accuracy, linearity, and specificity, supporting its usage in regular analysis. The developed and tested RP-HPLC method accurately and sensitively measures silodosin and dutasteride in diverse samples. Effective application in the pharmaceutical sector has demonstrated this strategy to be a reliable analytical tool with broad drug development and manufacturing implications.

REFERENCES

1. Hagiwara K, Koie T, Iwamura H, Imai A, Hatakeyama S, Yoneyama T, Hashimoto Y, Ohyama C. Efficacy and Safety of Silodosin and Dutasteride Combination Therapy in Acute Urinary Retention due to Benign Prostatic Hyperplasia: A Single-Arm Prospective Study. BioMed Research International. 2016;2016(1):4975851.
2. Nataraj KS, Srinivasa RA, Harshitha S, Pravallika K. Analytical method development and validation for the estimation of silodosin and dutasteride in capsule dosage form by RP-HPLC method. J Drug Metab Toxicol. 2020;11(2):1-8.
3. Fischer K, Höffler S. RP-HPLC analysis of phenolic lignin monomers with dad and coulometric array detection. Journal of Chromatographic Science. 2021 Apr;59(4):312-24.
4. Choudhari VP, Nikalje AP. Stability-indicating TLC method for the determination of dutasteride in pharmaceutical dosage forms. Chromatographia. 2009 Jul;70:309-13.
5. Jagadeesh K, Kadiyala KG, Dendukuri BS, Swamy R, Guttula VB, Vamsi P, Chavakula RL. Quality Control Assessment of Dutasteride and Silodosin in Capsules and Tablets Employing A Novel Developed HPLC Technique; Evaluation of Stabilities of Dutasteride and Silodosin In Accelerated Degradation. International Journal of Applied Pharmaceutics. 2023; 15(6):98-107.
6. Yarra US, Gummadi S. Stability indicating RP-UPLC method for simultaneous quantification of bempedoic acid and ezetimibe in bulk and pharmaceutical formulations. Future Journal of Pharmaceutical Sciences. 2021 Dec;7:1-9.
7. Shah HP, Khandar A, Deshpande S, Bagade S. Novel RP-HPLC method for simultaneous estimation of Silodosin and Dutasteride in multiunit solid dosage form. Res. J. Pharma. Biol. Chem. Sci. 2014;5:801-10.
8. Wadie M, Abdel-Moety EM, Rezk MR, Marzouk HM. Sustainable and smart HPTLC determination of silodosin and solifenacin using a constructed two illumination source chamber with a smartphone camera as a detector: comparative study with conventional densitometric scanner. Sustainable Chemistry and Pharmacy. 2023 Jun 1;33:101095.
9. Er E, Erk N. An effective and sensitive stability-indicating chromatographic approach based on HPLC for silodosin assay. Journal of Analytical Science and Technology. 2016 Dec;7:1-8.
10. Bojanapu A, Subramaniam AT, Munusamy J, Dhanapal K, Chennakesavalu J, Sellappan M, Jayaprakash V. Validation and method development of tadalafil in bulk and tablet dosage form by RP-HPLC. Drug research. 2014 Apr 29:82-5.
11. Pund S, Changediya V, Rajurkar V. A Comprehensive Review of Deflazacort with Special Reference to Pharmacological Profile and Pharmaceutical Validation Processes. International Journal of Drug Delivery Technology. 2023;13(2):749-752.
12. Pathade P, Sumrao A, Sonawane B, Shirode D, Shewale S, Shinde V, Bairagi V. Development and Validation of Stability Indicating UV Spectrophotometric Method for Estimation of Resveratrol in Bulk and Tablet Dosage Form. International Journal of Drug Delivery Technology. 2024;14(1):385-388.
13. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.
14. Pund S, Nalawade S, Rajurkar V, Jayatpal S, Deshmukh N, Tare H. A brief review on recent advances in reverse phase HPLC. Multidisciplinary Reviews. 2024 Jan 18;7(4):2024072.
15. Patil K, Narkhede S, Nemade M, Rane S, Chaudhari R, Dhobale G, Tare H. A Validated Sensitive Stability Indicating HPLC Method for the Determination of Etoricoxib in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):352-7.
16. Burakale P, Sudke S, Bhise M, Tare H, Kachave R., Exploring Film Forming Ability of Newly Synthesized Rosin Esters. International Journal of Drug Delivery Technology. 2023;13(3):908-912.