International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 6, 2023

Original Article

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

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Received: 03 Aug 2023, Revised and Accepted: 12 Sep 2023

ABSTRACT

Objective: The combination of dutasteride (DTRE) plus silodosin (SLDN) is used for treating acute urine retention brought upon by benign prostatic hyperplasia in men. The contents of DTRE and SLDN in capsules and tablets must be monitored for quality. In this research, a quick, selective and robust stability indicating HPLC method has been developed for concurrent assay of DTRE and SLDN in capsules and tablets. Also, the stabilities of DTRE and SLDN under several types of applied stress were determined.

Methods: Analysis performed using Xterra Symmetry type column C18 ("4.6 mm x 150 mm, 5 µm" dimensions) and mobile phase having 0.1N strength, 20% volume fraction of dipotassium hydrogen phosphate and 80% volume fraction of pure form acetonitrile; PDA analysis was made at 265 nm. Stabilities of DTRE and SLDN were determined under several types of applied stress, including thermal, basic, oxidative, photo, and acid.

Results: The elution times for DTRE and SLDN were 2.003 min and 3.377 min, respectively. DTRE and SLDN linear ranges were 20–120 µg/ml and 1.25–7.5 µg/ml, respectively. Method is precise with 0.2498% (DTRE) and 0.0773% (SLDN) RSD values. Method is accurate with 98.913-101.049% (DTRE) and 100.023-100.162% (SLDN) recovery values. In degradation investigation, the degradant's peaks elution times are different from the elution times of DTRE and SLDN. Thus, proved specificity and stability, indicating the power of the method. DTRE and SLDN were found relatively stable in thermal and were found sensitive in oxidation. In overall, SLDN found more sensitive to applied stress, including thermal, basic, oxidative, photo, and acid, compared to DTRE.

Conclusion: Finally, this developed analytical approach was efficaciously applied to commercial capsule and tablet formulations containing fixed dose of DTRE and SLDN, demonstrating its usefulness for quality control and degradation investigations on DTRE and SLDN.

Keywords: Prostatic hyperplasia, Dutasteride, Silodosin, Quality control, Capsule, Tablet, Stability

DOI: https://dx.doi.org/10.22159/ijap.2023v15i6.49036. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Men's lower urinary tract problems are frequently brought on by benign prostatic hyperplasia, which is the non-cancerous growth or prostate tissue's hyperplasia [1, 2]. According to research, disease frequency rises as people get older. In fact, the histopathological prevalence of benign prostatic hyperplasia following autopsy is as elevated as 50%–60% for men approaching their 60s, and it rises to 80%–90% among individuals over age of 70 y [3, 4].

Hagiwara *et al.* demonstrated the effectiveness of dutasteride (DTRE, fig. 1) plus silodosin (SLDN, fig. 1) combination treatment in treating those who have acute urine retention brought upon by benign prostatic hyperplasia [5]. Both tablet as well as capsule formulations of the DTRE and SLDN combination are marketed [6, 7]. An alpha-blocker is SLDN. To facilitate the simple flow of urine, SLDN acts by soothing the muscles surrounding the prostate gland and bladder exit [8]. DTRE, a 5-alpha-reductase antagonist, aids in prostate gland growth reduction by lowering the quantity of the hormone, which fosters prostate gland progression [9].

The quality of medications and pharmaceutical items is crucial to maintaining human health and wellness [10, 11]. Poor quality control procedures can have disastrous effects when dealing with consumable goods that people depend on. The identity, potency, strength, and stability of a of medications and pharmaceutical items are some crucial qualities that must be examined [12]. The identification test is performed to verify that the active medicinal component listed on the label actually exists. Another essential quality is strength or potency. The specified levels of active medicinal component must be included in the final product. Only a few techniques were documented by Nataraj *et al.* [13] Kattempudi and Ramarao [14], and Hardik *et al.* [15] to find the specified quantities of DTRE and SLDN in the finished goods.

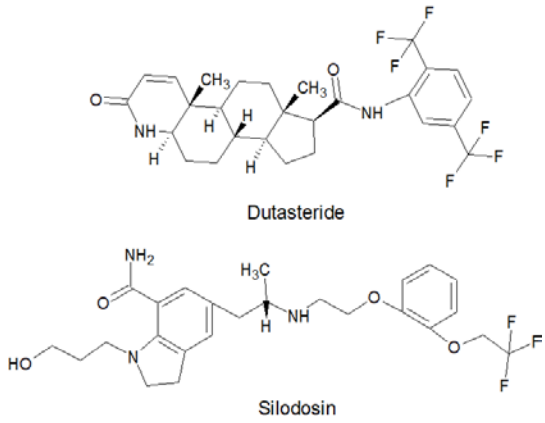

Fig. 1: Structures of dutasteride and silodosin

It's important to check the storage conditions since they must be followed for the pharmaceutical medication product to maintain its qualities. Inadequate storage conditions can cause the pharmaceutical medication product to physically and chemically deterioration, which might reduce its efficacy or possibly result in an

accumulation of hazardous substances [16-20]. The drug product's stability may be affected by the drug's active ingredient, which in turn is affected by the way it is made and packaging.

Nataraj *et al.* [13] method and Hardik *et al.* [15] method is applied to quantify the specified quantities of DTRE and SLDN in capsule doses while Kattempudi and Ramarao [14] method is applied only to bulk forms of DTRE and SLDN. None of these methods [13-15] were applied to bulk form, tablet form and capsule form of DTRE and SLDN. Furthermore, Nataraj *et al.* [13] method and Kattempudi and Ramarao [14] method did not report about DTRE and SLDN stabilities. In this study, we sought to measure specific concentrations of DTRE and SLDN in their bulk, tablet, and capsule forms by developing and validating a reliable RP-HPLC technique. Additionally, we looked at the stabilities of DTRE and SLDN under several types of applied stress, including thermal, basic, oxidative, photo, and acid.

MATERIALS AND METHODS

HPLC apparatus

The investigation of DTRE and SLDN analysis made use of Waters 2695HPLC equipment (USA) that has a quaternary pump, solvent degasser, autosampler, and a diode array type detector. For instrument control, collecting information, and operation, "Empower 2 software" was put to use. Xterra Symmetry type column C18 ("4.6 mm × 150 mm, 5 µm" dimensions) was utilized in investigation of DTRE and SLDN analysis.

HPLC-based DTRE and SLDN analysis conditions

The mobile phase was supplied into the column in the isocratic mode and included 0.1N strength, 20% volume fraction of dipotassium hydrogen phosphate (reagent grade Merck chemicals) and 80% volume fraction of pure form acetonitrile (HPLC grade Merck chemicals). The pH of which had been set to 2.5 employing orthophosporic acid (reagent grade Merck chemicals). The 10 µl of injection volumes was deployed for the DTRE and SLDN analyses. The detection wavelength for the DTRE and SLDN analyses was configured at 265 nm, and the isocratic elution run rate in the column was tuned at 1.0 ml/min. The temperature in column was tuned at ambient.

Solutions of DTRE and SLDN

The DTRE (5 mg) and SLDN (80 mg) were dissolved in K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) at 50 µg/ml and 800 µg/ml, respectively, to create a stock DTRE and SLDN solution. A working DTRE and SLDN solution with quantity 80 µg/ml of SLDN and 5 µg/ml of DTRE was made from diluting stock DTRE and SLDN solution (1.0 ml) with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*, 9.0 ml). The calibration DTRE and SLDN standards were generated by combining related portions of stock DTRE and SLDN solutions with K_2HPO_4 buffer and acetonitrile (2:8, *v/v*) to create six solutions of DTRE and SLDN with concentrations that ranged from 20-120 µg/ml for SLDN and 1.25-7.5 µg/ml for DTRE.

Tablet DTRE and SLDN solution

The powdered components of 10 Silofast-8D tablets (DTRE-0.5 mg; SLDN-8 mg per tablet) were precisely put into a flask with a volume measurement of 100 ml, and 25 ml of K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) was then included while the flask was being continuously stirred in a sonicator for around 30 min, adding K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) successively every 10 min. After allowing the flask to cool, the volume was filled with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) and filtered using centrifugation. One millilitre (1.0 ml) of the final produced solution (containing 800 µg/ml of SLDN and 50 µg/ml of DTRE) was put into a 10 ml measuring flask and K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) was added to the final volume to get test Silofast-8D solution with 80 µg/ml of SLDN and 5 µg/ml of DTRE theoretical concentration.

Capsule DTRE and SLDN solution

The contents of ten Siloros-8D capsules (DTRE-0.5 mg; SLDN-8 mg per capsule) were combined to form a homogenous powder. The Siloros-8D powder was weighed and dissolved in K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) for closest to 30 min using sonication, yielding a concentration of 800 µg/ml of SLDN and 50 µg/ml of DTRE. The prepared sample of Siloros-8D was filtered using centrifugation. A test Siloros-8D solution with threotical quantity 80 µg/ml of SLDN and 5 µg/ml of DTRE was made from a diluting produced solution containing 800 µg/ml of SLDN and 50 µg/ml of DTRE (1.0 ml) with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*, 9.0 ml).

Analysis of DTRE and SLDN in tablet and capsule doses

The proposed "HPLC based DTRE and SLDN analysis conditions" was used to analyse the pharmaceutically prepared test Silofast-8D solution and test Siloros-8D solution, and the concentrations of the DTRE and SLDN in Silofat-8D tablets and siloros-8D capsules were evaluated exploiting the regression equations of DTRE and SLDN, respectively.

Stability studies

Stability studies of DTRE and SLDN were investigated on stock DTRE and SLDN solution (800 µg/ml of SLDN and 50 µg/ml of DTRE) by abiding ICH guidelines [21].

Stability in 0.1N HCl

Acid degradation investigations were performed by combining 10 ml of 0.1N HCl (reagent grade, Sd fine chemicals) with 10 ml of stock DTRE and SLDN solution and agitated in a sonicator for around 30 min at near-room temperature. Before diluting to 100 ml with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*), the degradation DTRE and SLDN samples were neutralised with a sufficient quantity of sodium hydroxide (reagent grade, Sd fine chemicals).

Stability in 0.1N NaOH

Base degradation investigations were performed by combining 10 ml of 0.1N NaOH (reagent grade, Sd fine chemicals) with 10 ml of stock DTRE and SLDN solution and agitated in a sonicator for around 30 min at near-room temperature. Before diluting to 100 ml with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*), the degradation DTRE and SLDN samples were neutralised with a sufficient quantity of hydrochloric acid (reagent grade, Sd fine chemicals).

Stability in 30% peroxide

Peroxide oxidation investigations were performed by combining 10 ml of peroxide (reagent grade, Sd fine chemicals) with 10 ml of stock DTRE and SLDN solution and agitated in a sonicator for around 30 min at near-room temperature. The oxidized DTRE and SLDN samples were diluting to 100 ml with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*).

Stability at 105 °C

DTRE (5 mg) and SLDN (80 mg) in their solid forms were heated in an oven at 105 °C for six hours. The degraded DTRE (5 mg) and SLDN (80 mg) solid forms were dissolved in volume of 100 ml K_2HPO_4 buffer/acetonitrile (2:8, *v/v*). For analysis, a working degraded DTRE and SLDN solution was made from diluting above degraded DTRE and SLDN solution (1.0 ml) with K_2HPO_4 buffer/acetonitrile (2:8, *v/v*, 9.0 ml).

Stability in light

DTRE (5 mg) and SLDN (80 mg) in their solid forms were heated in sun for 6 h. The degraded DTRE (5 mg) and SLDN (80 mg) solid forms were dissolved in volume of 100 ml K2HPO4 buffer/acetonitrile (2:8, v/v). For analysis, a working degraded DTRE and SLDN solution was made from diluting above degraded DTRE and SLDN solution (1.0 ml) with K2HPO4 buffer/acetonitrile (2:8, v/v, 9.0 ml).

The proposed "HPLC-based DTRE and SLDN analysis conditions" was used to analyse the degraded DTRE and SLDN solutions, and the stabilities of the DTRE and SLDN in applied conditions of stress were evaluated.

RESULTS

Optimization: HPLC-based DTRE and SLDN analysis conditions

The intent of this project was to accomplish good enough separation of all analysed ingredients (DTRE and SLDN) with permissible critical characteristics of the chromatographic system (resolution factors,

asymmetry factor of DTRE and SLDN, analysis time) in minimal duration of analysis. Xterra Symmetry type column C18 ("4.6 mm x 150 mm, 5 m" dimensions) tuned at ambient temperature has been opted as the most effective stationary phase for DTRE and SLDN analysis amongst the columns evaluated. In preliminary experiments, acetonitrile was chosen as the organic portion of the mobile phase and K_2HPO_4 (0.1N, 2.5 pH units) was opted for as the buffer, and the relative percentage of the two was examined. For better separation with permissible critical characteristics of the chromatographic system, the mobile phase was supplied into the column at 1.0 ml/min flow speed in the isocratic mode and included 0.1N strength, 20% volume fraction of K_2HPO_4 (0.1N, 2.5 pH units) and 80% volume fraction of pure form acetonitrile. The detection wavelength for the DTRE and SLDN analyses was configured at 265 nm. Chromatogram of DTRE and SLDN with optimized HPLC-based DTRE and SLDN analysis conditions was given in fig. below (fig. 2).

Validation

Validation of optimized HPLC-based DTRE and SLDN analysis conditions was done by abiding ICH guidelines [22, 23].

System suitability

By infusing 5 repetitions of standard SLDN (800 µg/ml) and DTRE (50 µg/ml) solution, a system compatibility test was conducted and parameters were computed. Theoretical plates for DTRE and SLDN, peak symmetry for DTRE and SLDN, resolution among DTRE and SLDN and relative standard deviations were all calculated (table 1).

Selectivity

Four solutions were injected to Xterra Symmetry type column C18 ("4.6 mm x 150 mm, 5 m" dimensions) to assess HPLC-based DTRE and SLDN analysis condition's selectivity under ideal chromatographic circumstances: blank K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) mix solution, standard SLDN (80 µg/ml) and DTRE (5 µg/ml) solution, test Siloros-8D solution (SLDN-80 µg/ml; DTRE-5 µg/ml) and test Silofast-8D solution (SLDN-80 µg/ml; DTRE-5 µg/ml). The retention periods in chromatograms (fig. 3A-3D) corresponding to DTRE and SLDN were examined to gauge selectivity.

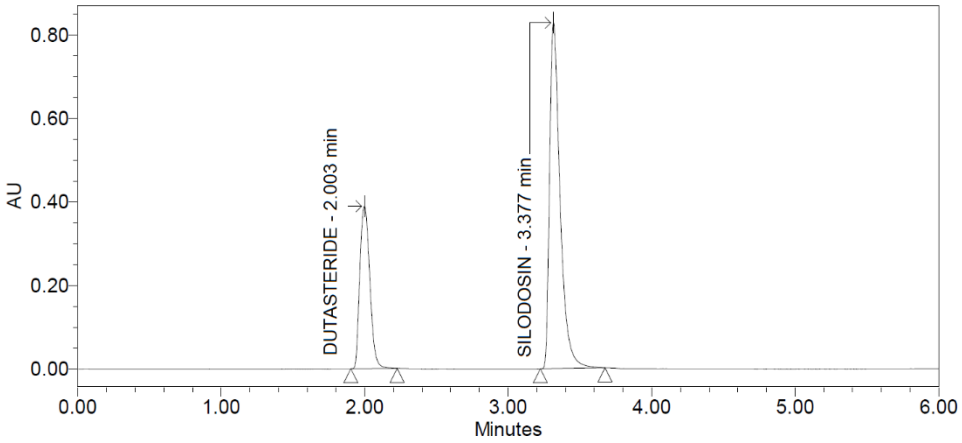

Fig. 2: Chromatogram of DTRE and SLDN with optimized HPLC-based DTRE and SLDN analysis conditions

Table 1: DTRE and SLDN system compatibility parameters

Parameter	Values for DTRE[1]	SD[2]/RSD%[3]	Values for SLDN[1]	SD[2]/RSD%[3]
Retention time	2.035	0.0017/0.0822%	3.380	0.0032/0.0936%
Peak area	183045	501.6837/0.2741%	4221075	4077.1171/0.0966%
Theoretical plate	2808.200	37.5327/1.3215%	8180.600	71.4444/0.8733%
Asymmetry	1.064	0.0195/1.8321%	1.168	0.0084/0.7163%
Resolution	-	-	9.520	0.1536/1.6137%

1= mean of five obtained values for DTRE/SLDN; 2= standard deviation of five obtained values for DTRE/SLDN; 3= relative deviation in percentage for DTRE/SLDN

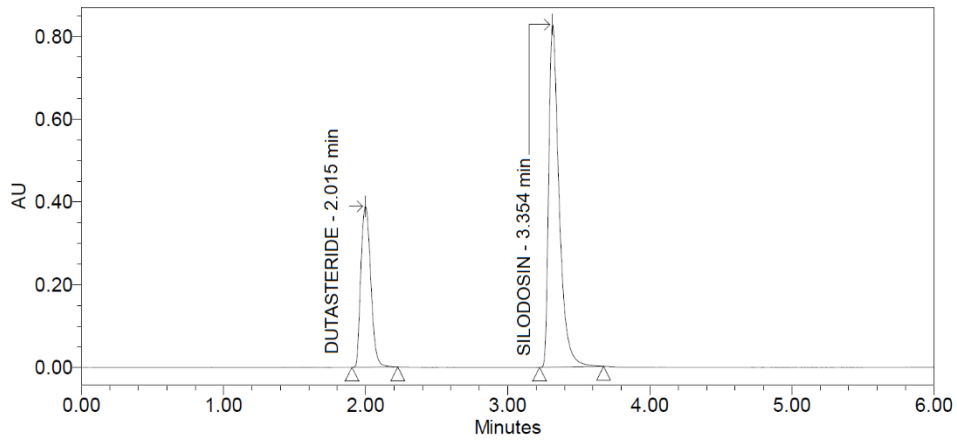

Fig. 3A: Standard SLDN (80 µg/ml) and DTRE (5 µg/ml) solution chromatogram

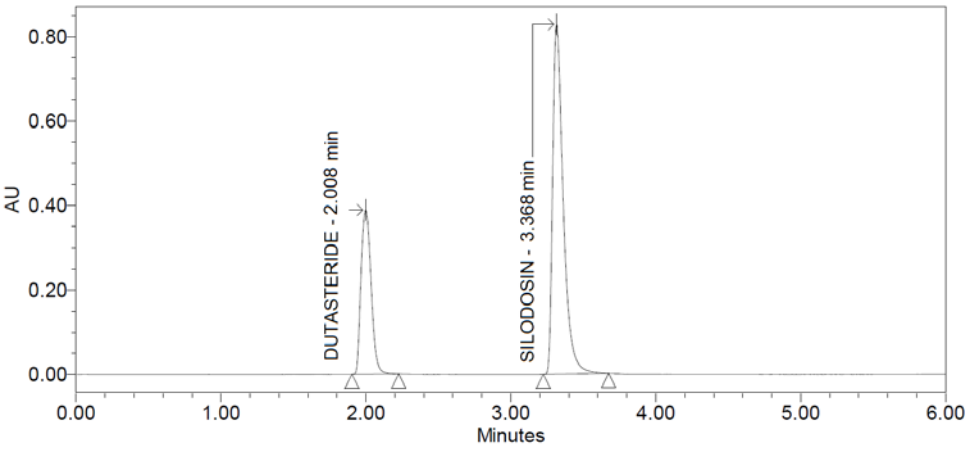

Fig. 3B: Test siloros-8D solution (SLDN-80 µg/ml; DTRE-5 µg/ml) chromatogram

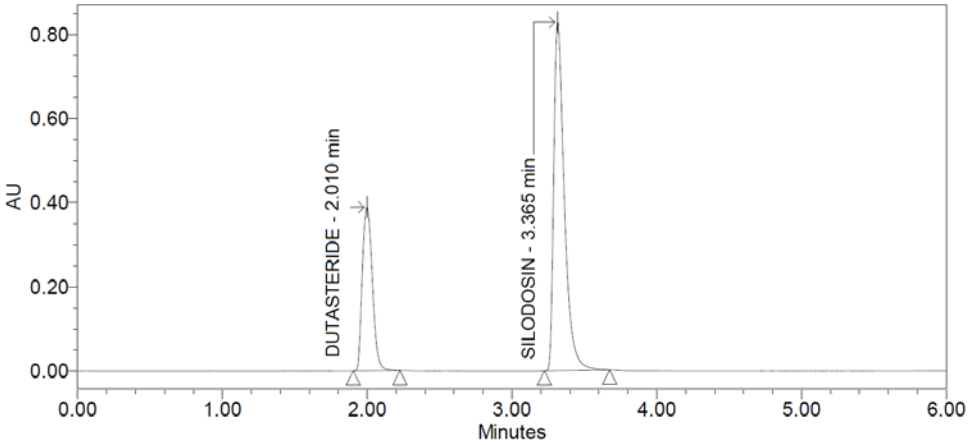

Fig. 3C: Test silofast-8D solution (SLDN-80 µg/ml; DTRE-5 µg/ml) chromatogram

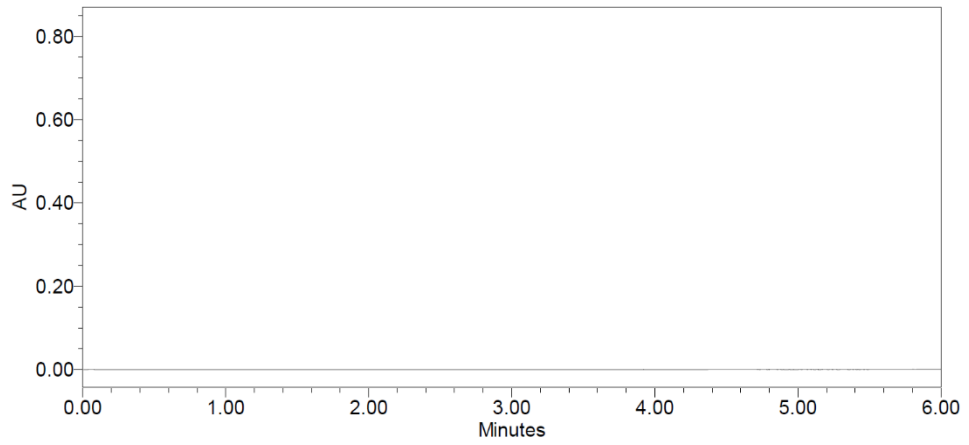

Fig. 3D: Blank K_2HPO_4 buffer/acetonitrile (2:8, *v/v*) mix solution chromatogram

Linearity

By examining six concentrations of DTRE and SLDN ranging from 20–120 µg/ml and 1.25–7.5 µg/ml, respectively, the method's (HPLC based DTRE and SLDN analysis) linearity was assessed. The test was carried out under the previously described chromatography experimental conditions. A summary of the linear equations for DTRE and SLDN were provided. The linearity graphs of DTRE and SLDN were also provided (fig. 4).

- Linear equation for DTRE: DTRE peak area = 36882.7428 × quantity of DTRE (µg/ml)-600.6666; R^2/correlation coefficient = 0.99970
- Linear equation for SLDN: SLDN peak area = 53242.7428 × quantity of SLDN (µg/ml)-23618.3214; R^2/correlation coefficient = 0.99992

Limit of detection and limit of quantification

Ensuing ICH rules, these two parameters for DTRE and SLDN were calculated by applying the slope of DTRE/SLDN curves of calibration

and the standard deviation observed for DTRE/SLDN response. Limits of detection for DTRE and SLDN were 0.0449 µg/ml and 0.2527 µg/ml for DTRE and SLDN, respectively. While Limits of quantification for DTRE and SLDN were 0.1360 µg/ml and 0.7658 µg/ml for DTRE and SLDN, respectively.

Precision

This was verified by analysing a typical SLDN (80 g/ml) and DTRE (5 g/ml) solution for six times in one day following the suggested methodology. Calculations were made for the relative deviations (table 2).

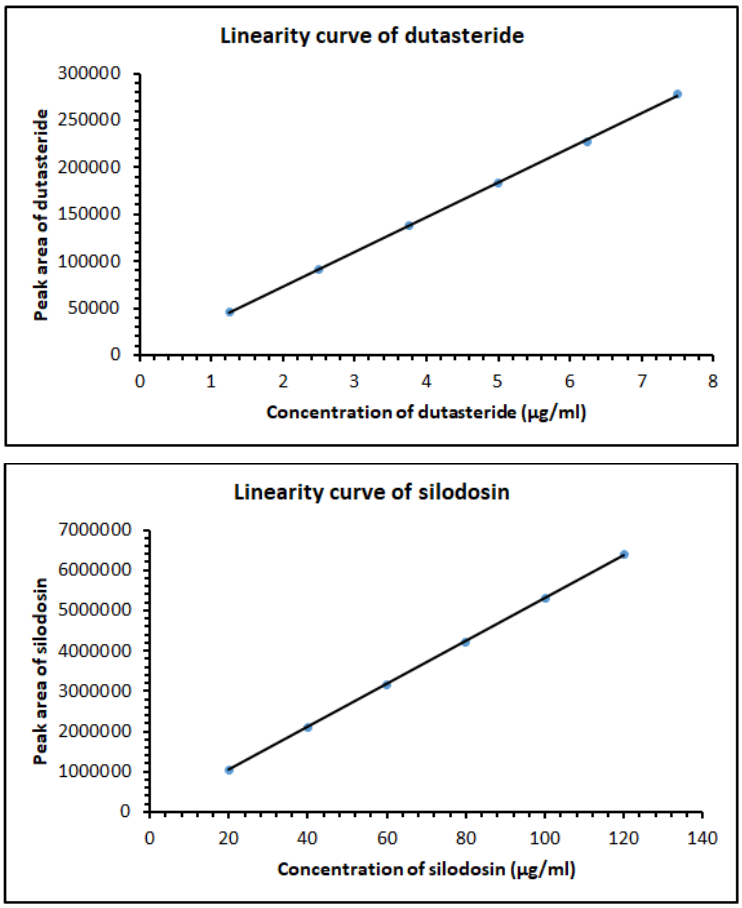

Fig. 4: Graphs proving linearity for DTRE and SLDN

Table 2: Precision evaluation for DTRE and SLDN analysis

Precision for DTRE				**Precision for SLDN**			
Peak area obtained	**Quantity taken (µg/ml)**	**Quantity analyzed (µg/ml)**	**Statistical assessment**	**Peak area obtained**	**Quantity taken (µg/ml)**	**Quantity analyzed (µg/ml)**	**Statistical assessment**
183541	5	4.999	**Mean[1]:**	4218614	80	80.051	**Mean[1]:**
183951	5	5.010	5.013	4212695	80	79.939	80.032
184935	5	5.037	**SD[2]:**	4214863	80	79.980	**SD[2]:**
183984	5	5.011	0.0125	4218375	80	80.046	0.0618
184001	5	5.011	**RSD[3]:**	4221574	80	80.107	**RSD[3]:**
184015	5	5.012	0.2498	4219634	80	80.070	0.0773

1= mean of five analysed values (µg/ml) for DTRE/SLDN; 2= standard deviation of six analysed values (µg/ml) for DTRE/SLDN; 3= relative deviation in percentage for DTRE/SLDN analysed values (µg/ml)

Ruggedness

Applying the HPLC-based DTRE and SLDN analysis method with two distinct equipments in two diverse analytical laboratories across a range of time intervals allowed for an evaluation of the suggested methodology's (HPLC based DTRE and SLDN analysis method) robustness. Calculations were made for the relative deviations (Tables 3, 4).

Accuracy

The illustration of an accurate technique is the successful recovery of the DTRE and SLDN. Pre-analyzed tablet/capsule samples were added in triplicate with the specified concentrations of DTRE and SLDN at three distinct concentrations levels (50% = 2.5 µg/ml DTRE and 40 µg/ml SLDN, 100% = 5.0 µg/ml DTRE and 80 µg/ml SLDN, and 150% = 7.5 µg/ml DTRE and 120 µg/ml SLDN), in order to test the methodology's (HPLC based DTRE and SLDN analysis method) accuracy. Calculations were made for the percent recoveries of DTRE and SLDN (Tables 5, 6).

Robustness

This parameter for the methodology had been assessed by analysing the impact of slight variations in the experimental chromatography variables (proportion of acetonitrile; flow rate; temperature) on the

method's analytical performance. The recovery % (table 7, 8) for DTRE and SLDN was computed repeatedly when one experimental chromatography parameter was altered and every other parameter maintained the same.

Table 3: Ruggedness evaluation for DTRE analysis

Day 1; Equipment 1; Lab 1; Analyst 1				Day 2; Equipment 2; Lab 2; Analyst 2			
Peak area obtained	**Quantity taken (µg/ml)**	**Quantity analyzed (µg/ml)**	**Statistical assessment**	**Peak area obtained**	**Quantity taken (µg/ml)**	**Quantity analyzed (µg/ml)**	**Statistical assessment**
184986	5	5.038	Mean[1]:	181964	5	4.956	Mean[1]:
182967	5	4.983	4.988	183491	5	4.997	4.992
183648	5	5.002	SD[2]:	184018	5	5.012	SD[2]:
181547	5	4.944	0.0326	182963	5	4.983	0.0208
182259	5	4.964	RSD[3]:	183518	5	4.998	RSD[3]:
183548	5	4.999	0.6540	183942	5	5.010	0.4161

1= mean of five analysed values (µg/ml) for DTRE; 2= standard deviation of six analysed values (µg/ml) for DTRE; 3= relative deviation in percentage for DTRE analysed values (µg/ml).

Table 4: Ruggedness evaluation for SLDN analysis

Day 1; Equipment 1; Lab 1; Analyst 1				Day 2; Equipment 2; Lab 2; Analyst 2			
Peak area obtained	**Quantity taken (µg/ml)**	**Quantity analyzed (µg/ml)**	**Statistical assessment**	**Peak area obtained**	**Quantity taken (µg/ml)**	**Quantity analyzed (µg/ml)**	**Statistical assessment**
4218624	80	80.051	Mean[1]:	4222641	80	80.127	Mean[1]:
4214629	80	79.975	80.071	4219534	80	80.068	80.085
4213928	80	79.962	SD[2]:	4220157	80	80.080	SD[2]:
4215896	80	79.999	0.1213	4219358	80	80.065	0.0287
4225482	80	80.181	RSD[3]:	4221869	80	80.113	RSD[3]:
4229517	80	80.258	0.1515	4218869	80	80.056	0.0358

1 = mean of five analysed values (µg/ml) for SLDN; 2 = standard deviation of six analysed values (µg/ml) for SLDN; 3 = relative deviation in percentage for SLDN analysed values (µg/ml).

Table 5: Accuracy evaluation for DTRE analysis

Level of addition (%)	**Peak area obtained**	**Quantity added (µg/ml)**	**Quantity analyzed (µg/ml)**	**Recovered (%)**	**Statistical assessment**	
					Mean[1]	**SD[2]/RSD[3]**
50	92567	2.5	2.519	100.763	101.049	1.1159/1.1049
50	91962	2.5	2.503	100.105		
50	93961	2.5	2.557	102.280		
100	179987	5	4.902	98.036	98.913	1.3026/1.3169
100	184345	5	5.021	100.410		
100	180459	5	4.915	98.293		
150	276348	7.5	7.452	99.360	99.810	0.4438/0.4447
150	278816	7.5	7.519	100.247		
150	277638	7.5	7.487	99.824		

1= mean of three analysed values (%recovery) for DTRE; 2= standard deviation of three analysed values (%recovery) for DTRE; 3= relative deviation in percentage for DTRE three analysed values (%recovery).

Table 6: Accuracy evaluation for SLDN analysis

Level of addition (%)	**Peak area obtained**	**Quantity added (µg/ml)**	**Quantity analyzed (µg/ml)**	**Recovered (%)**	**Statistical assessment**	
					Mean[1]	**SD[2]/RSD[3]**
50	2115928	40	40.120	100.301	100.031	0.2632/0.2631
50	2104837	40	39.910	99.775		
50	2109928	40	40.007	100.017		
100	4229381	80	80.255	100.319	100.162	0.1542/0.1539
100	4216384	80	80.009	100.011		
100	4222584	80	80.126	100.158		
150	6389354	120	119.960	99.966	100.023	0.0584/0.0584
150	6396817	120	120.100	100.083		
150	6392815	120	120.025	100.021		

1= mean of three analysed values (%recovery) for SLDN; 2= standard deviation of three analysed values (%recovery) for SLDN; 3= relative deviation in percentage for SLDN three analysed values (%recovery).

Degradation study

Stability studies of DTRE and SLDN were investigated in 0.1NHCl media, 0.1N NaOH media, 30% peroxide media, thermal and sun light. Calculated in terms of DTRE and SLDN recoveries, and DTRE and SLDN deteriorated, respectively, was the extent of stabilities.

Over 30 min of being exposed to 0.1N HCl medium, DTRE and SLDN showed degradation of up to 0.829% and 1.827%, respectively. This demonstrates that SLDN is better stable than DTRE in 0.1N HCl media. Three more peaks (RT's–1.325 min; 4.626 min; and 5.227 min) in the chromatogram (fig. 5A) showed the existence of degradation products.

Table 7: Robustness evaluation for DTRE analysis

Parameter studied	Peak area obtained	Quantity taken (µg/ml)	Quantity analyzed (µg/ml)	Recovered (%)	Statistical assessment	
					Mean[1]	SD[2]/RSD[3]
75% vol. acetonitrile	184862	5	5.035	100.692	101.470	1.6898/1.6653
80% vol. acetonitrile	184162	5	5.016	100.310		
85% vol. acetonitrile	189851	5	5.170	103.409		
0.9 ml/min flow speed	182659	5	4.975	99.492	100.153	0.5744/0.5735
1.0 ml/min flow speed	184562	5	5.026	100.528		
1.1 ml/min flow speed	184398	5	5.022	100.439		
25 °C temperature	182298	5	4.965	99.295	99.820	0.7447/0.7461
27 °C temperature	184826	5	5.034	100.672		
29 °C temperature	182659	5	4.975	99.492		

1= mean of three analysed values (% recovery) for DTRE; 2= standard deviation of three analysed values (% recovery) for DTRE; 3= relative deviation in percentage for DTRE three analysed values (% recovery)

Table 8: Robustness evaluation for SLDN analysis

Parameter studied	Peak area obtained	Quantity taken (µg/ml)	Quantity analyzed (µg/ml)	Recovered (%)	Statistical assessment	
					Mean[1]	SD[2]/RSD[3]
75% vol. acetonitrile	4226167	80	80.194	100.243	100.147	0.0840/0.0838
80% vol. acetonitrile	4219627	80	80.070	100.088		
85% vol. acetonitrile	4220548	80	80.088	100.109		
0.9 ml/min flow speed	4214268	80	79.968	99.960	100.104	0.1457/0.1455
1.0 ml/min flow speed	4220116	80	80.079	100.099		
1.1 ml/min flow speed	4226548	80	80.201	100.252		
25 °C temperature	4214395	80	79.971	99.963	100.061	0.0960/0.0959
27 °C temperature	4218634	80	80.051	100.064		
29 °C temperature	4222483	80	80.124	100.155		

1= mean of three analysed values (%recovery) for SLDN; 2= standard deviation of three analysed values (%recovery) for SLDN; 3= relative deviation in percentage for SLDN three analysed values (%recovery)

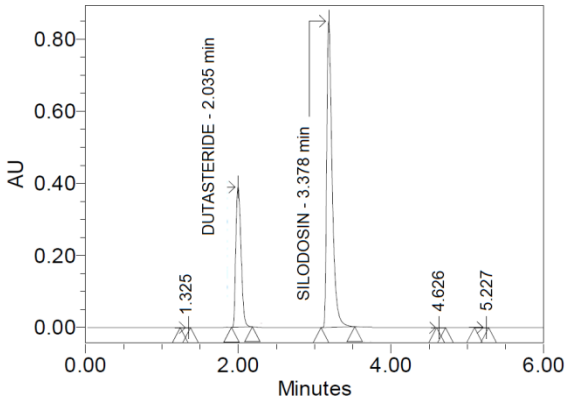

Fig. 5A: Acid-degraded DTRE and SLDN chromatogram

Over 30 min of being exposed to 0.1N NaOH medium, DTRE and SLDN showed degradation of up to 1.564% and 2.861%, respectively. In this case, DTRE is better stable than SLDN in 0.1N NaOH media. The presence of the degraded DTRE and SLDN products was shown by two additional peaks (RT's–0.998 min and 5.066 min) in the chromatogram (fig. 5B).

Following exposure to 30% peroxide medium, four degradation products (RT's–0.996 min; 4.651 min; 4.907 min; and 5.148 min) were found (fig. 5C), with DTRE degraded by 5.126% and SLDN degraded by 12.562%. Here found that SLDN is very sensitive to oxidation than DTRE.

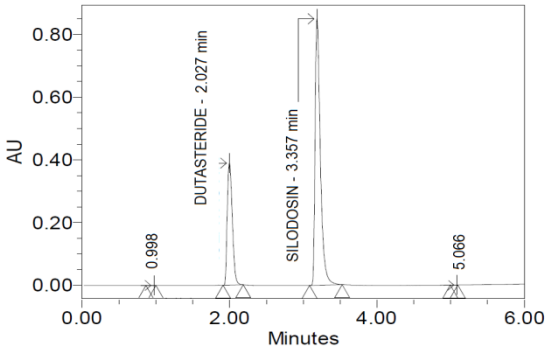

Fig. 5B: Alkaline degraded DTRE and SLDN chromatogram

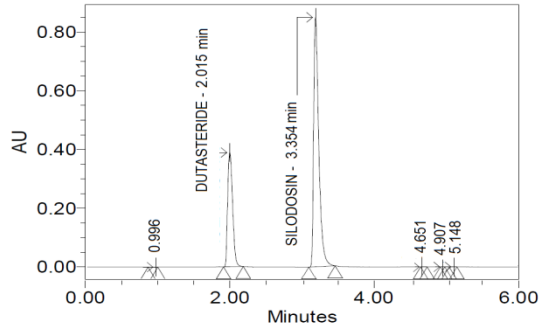

Fig. 5C: Peroxide-degraded DTRE and SLDN chromatogram

There were no superfluous peaks in the chromatogram (fig. 5D) of the DTRE and SLDN as an effect of the heat-stimulated stress, although there was a little degradation after 6 hr at high temperatures (105 °C), which was more noticeable in SDLN (0.981%) than in DTRE (0.518%).

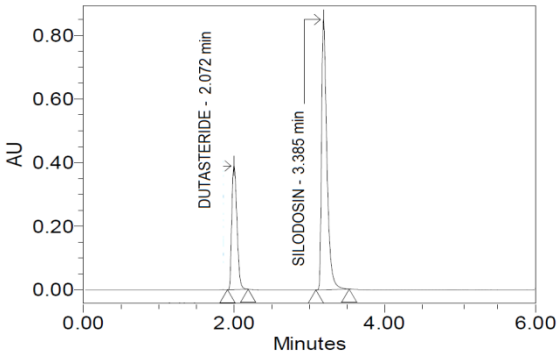

Fig. 5D: High temperature degraded DTRE and SLDN chromatogram

In case of DTRE and SLDN exposed to sunlight, degraded DTRE and SLDN products were noticed at retention times of 1.348 min, 4.395 min and 5.001 min (fig. 5E) with 2.428% degradation of SLDN and 1.340% degradation of DTRE. DTRE is more stable than SLDN in this scenario under direct sunlight.

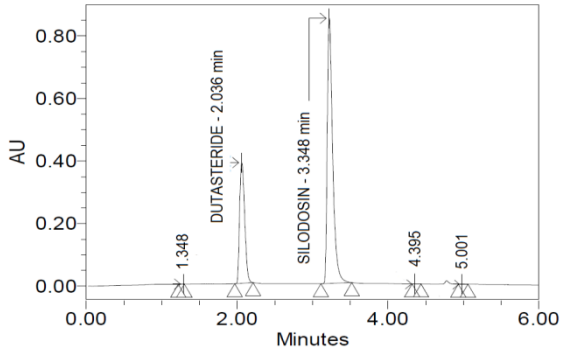

Fig. 5E: Sunlight degraded DTRE and SLDN chromatogram

Assessment of DTRE and SLDN pharmaceutical dosage form

By analysing Siloros-8D capsules and Silofast-8D tablets, two commercially obtainable dose forms for men's lower urinary tract issues, the suggested method's (HPLC-based DTRE and SLDN analysis conditions) suitability for routine use was evaluated. The contents of two analytes were 7.957 mg SLDN and 0.498 mg DTRE in Siloros-8D capsules, whereas 7.875 mg SLDN and 0.493 mg DTRE in Silofast-8D tablets. The mean value derived from the three individual assessments served as the basis for all outcomes (table 9). The contents of DTRE and SLDN are in accordance with Silofast-8D tablets (8 mg SLDN; 0.5 mg DTRE) specification and Siloros-8D capsule (8 mg SLDN; 0.5 mg DTRE) specification.

Table 9: Assessment of DTRE and SLDN in capsule doses and tablet doses

SDLN assay in siloros-8D capsules				
Claim value (mg)	**Found value (mg)**	**Assayed (%)**	**Mean found[1] (mg)**	**SD[2]/RSD[3]**
8	7.965	99.563	7.957	0.0599/0.7533
8	7.893	98.663		
8	8.012	100.150		
SDLN assay in Silofast-8D tablets				
8	7.944	99.300	7.875	0.1850/0.9449
8	8.015	100.188		
8	7.865	98.313		
DTRE assay in siloros-8D capsules				
0.5	0.498	99.600	0.498	0.0025/0.5050
0.5	0.501	100.200		
0.5	0.496	99.200		
DTRE assay in silofast-8D tablets				
0.5	0.502	100.400	0.493	0.0085/1.7240
0.5	0.493	98.600		
0.5	0.485	97.000		

1= mean of three found values (mg) for SLDN/DTRE; 2= standard deviation of three found values (mg) for SLDN/DTRE; 3= relative deviation in percentage for SLDN/DTRE three found values (mg) for SLDN/DTRE

DISCUSSION

In order to ensure the quality of active medicinal components and finished products (tablets, capsules, etc.), it is imperative to come up with a reliable and also sensitive chromatographic strategy that allows complete partition of active medicinal components in a shorter period of time, in addition to qualitative alongside quantitative analysis. In our research study, an HPLC approach was adopted to establish a procedure for quality control of DTRE and SLDN in tablets and capsules, as well as for evaluating the stabilities of DTRE and SLDN under stress environments.

The theoretical plates for DTRE and SLDN, peak symmetry for DTRE and SLDN, resolution between DTRE and SLDN, and relative standard deviations were computed as system suitability parameters (table 1). These findings point to the system's efficiency [24]. All analytes (DTRE and SLDN) are clearly shown to be well separated from one another in fig. 3A-3D, which also shows that no interrupting peaks were seen. As a result, the method's selectivity is verified [25].

The peak areas of DTRE/SLDN and concentration of DTRE/SLDN have a great linear association, as shown by the correlation coefficients (0.99970 for DTRE and 0.99992 for SLDN) got for the linearity equations. Thus, the method's (HPLC-based DTRE and SLDN analysis) linearity within specified concentration ranges, 20–120 µg/ml for DTRE and 1.25–7.5 µg/ml for SLDN was demonstrated [26]. Low detection and limit of quantification readings for DTRE and SLDN demonstrated sensitivity for HPLC based DTRE and SLDN analysis method [27].

The approach (HPLC-based DTRE and SLDN analysis method) was proven to be precise with relative variances (table 2) for DTRE and SLDN analysis under 2.0% [28]. The analytical procedure (HPLC-based DTRE and SLDN analysis method) proved accurate; all analysed samples had percentage recoveries of DTRE and SLDN in

ranges of 98.913% to 101.049% (table 5, 6) [28]. Since the relative variance wasn't higher than 0.20% (table 3, 4), it was determined that the ruggedness results from lab to lab, equipment to equipment, analyst to analyst and day-to-day fluctuation were repeatable [29]. Small alterations in each of the variables (proportion of acetonitrile; flow rate; temperature) under study had no appreciable impact on the outcomes (table 7, 8). This gave evidence of the suggested method's reliability in regular analysis of DTRE and SLDN [29].

The retention periods for DTRE and SLDN were unaffected by any stress situations applied. The separation of the degraded DTRE/SLDN products and quantification of the analytes (DTRE and SLDN) demonstrated the excellent specificity besides the stability-indicating potential of the suggested approach [30].

CONCLUSION

In this research, a quick, selective and robust RP-HPLC methodology has been developed for instantaneous assay of DTRE and SLDN. The process was evaluated in conformance with the ICH requirement and concluded useful for the expected application. This promotes their effective usage in DTRE and SLDN routinely in quality control laboratories. The method may be employed precisely and accurately for the assessment of DTRE and SLDN in formulations, as evidenced by the low precision values and good assay percentages for DTRE and SLDN in Siloros-8D capsule and Silofast-8D tablet. In stability investigation, the method demonstrated excellent specificity besides stability-indicating potentiality.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

K. Jagadeesh and K Ganesh Kadiyala made significant contributions to the detection of medication combinations. They created a High-Performance Liquid Chromatography (HPLC) approach using a type 18 column. The mobile phase used in their method consisted of a 0.1N strength solution with a volume fraction of 20% dipotassium hydrogen phosphate and 80% pure acetonitrile.

B. N. Suresh Varma Dendukuri and Rama Swamy Guttula have made significant contributions to the advancement of the High-Performance Liquid Chromatography (HPLC) technology, namely in the use of type column18 and the analysis using Photodiode Array (PDA) detection at a wavelength of 265 nm.

V. L. N. Balaji Gupta Tiruveedhi, Peddinti Vamsi, and Rajya Lakshmi Chavakula made significant contributions to the assessment of the stabilities of DTRE and SLDN. These stabilities were evaluated under many forms of applied stress, encompassing thermal, basic, oxidative, UV, and acid stressors.

CONFLICTS OF INTERESTS

Declared none

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