IJMPR 2021, 5(2), 205-213

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Review Article
SJIF Impact Factor: 5.273

DISSOLUTION METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TAMSULOSIN AND DUTASTERIDE IN ITS COMBINED MODIFIED RELEASE DOSAGE FORM USING RP-HPLC METHOD

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Received on: 20/03/2021
Revised on: 10/04/2021
Accepted on: 30/04/2021

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ABSTRACT

The research work was aimed to develop a dissolution method for simultaneous estimation of Dutasteride and Tamsulosin from extended-release capsule. The formulation is having Dutasteride in immediate release and Tamsulosin in modified release form. A change over dissolution media was developed in this study. 0.1N HCl with 0.2% SLS was selected as dissolution medium to enable complete dissolution of Dutasteride for initial two hours, and continued by changing the dissolution medium to pH 7.2 phosphate buffer for 8 hours, using USP Apparatus 1, at 100RPM. Working standards were prepared by using the respective dissolution media, for specific sample. Chromatographic separation was achieved by analysing filtered sample, using Agilent's high performance liquid chromatograph and X bridge C_{18}, 5µm, 4.6 x 150mm column, with solvent-A of 0.05M phosphate buffer (pH 6.8), and solvent-B of acetonitrile by gradient elution technique. The flow rate was maintained at 1.5 ml/min and the detection wavelength was 225nm, with sample run time of 18 minutes. In acid stage Tamsulosin was eluted at 5.7 minutes, and Dutasteride was eluted at 9.4minutes. Buffer stage was developed only for Tamsulosin and eluted at 2.3minutes. Analytical method was validated and complies to the regulatory guidelines. The effect of RPM on dissolution profile was evaluated, which does not have any significance on drug release, and F_2 value of 65 & 84 with 75 RPM & 91 and 71 with 125RPM against 100RPM for Dutasteride and Tamsulosin, respectively. Hence, the developed method is suitable for estimating drug release of both components simultaneously.

KEYWORDS: Dutasteride (DUT), Tamsulosin HCl (TSL), simultaneous estimation, RP-HPLC.

INTRODUCTION

Dutasteride and Tamsulosin extended-release capsules is a fixed dose combination product. Each hard capsule contains Tamsulosin hydrochloride modified release pellets and one Dutasteride soft gelatin capsule as immediate release part. Dutasteride belongs to a group of medicines called 5-alpha reductase inhibitors and Tamsulosin belongs to a group of medicines called alpha-blockers. The fixed dose combination product is indicated for the treatment of symptomatic Benign Prostatic Hyperplasia in men.[1]

Being a combination of immediate release and modified release component in one product, developing a simultaneous estimation method, with precision at lesser ppm is a challenge. From Dutasteride solubility data it was observed that, the API is insoluble throughout the pH range in absence of surfactant. Upon addition of sodium lauryl sulphate (SLS) in dissolution media, Dutasteride exhibited pH dependent solubility; maximum solubility was observed in 0.1N HCL with 0.2% SLS. The target is an immediate release product, so dissolution in the stomach and absorption in the upper small intestine is expected suggesting the use of dissolution medium with low pH.[2-4]

Tamsulosin being modified release product, and enteric coated, the drug release was targeted in intestine. The office of generic drug recommended to have the dissolution using apparatus -2 for capsule product. For a multiparticulate drug delivery system having USP Apparatus 2 is difficult for the analyst to handle the loss of pellets during media transfer. Hence, the method has been aimed to develop using USP Apparatus 1, and simultaneous estimation method for Dutasteride and Tamsulosin, using one sample. Dissolution time was continued, to get the complete drug release form product.[5-8]

The literature survey reveals, few research works has been carried out for simultaneous estimation of assay,

where the drug concentration has been achieved by increase in sample size. Dissolution method development was not performed by any researcher, and method should be capable of detecting lowest concentration of 0.02ppm, using HPLC method. Hence, the scope for sample dilution or sample solution preparation is very low. The complete development was focused on mobile phase development & suitable chromatographic condition selection. Dutasteride is insoluble in water and having the pKa value of 2.17 at acidic condition, and 12.56 at basic condition. Hence, buffers are not required for elution of Dutasteride. Tamsulosin is sparingly soluble in water, and having the pKa value around 9, in both acidic and basic condition, which recommends having buffer for proper elution of Tamsulosin. A gradient elution method was selected to elute both components together, in a precise manner.[9-12]

Chromatographic column selection: HPLC columns are designed to eliminate the compromise of sorbent selection and deliver the flexibility to work under any mobile phase, temperature and pH chromatography conditions, which is a requirement for method development. Even the most challenging sample mixtures can be quickly resolved with a sample method development protocol consisting of two mobile phase pH and two organic solvents. The C_{18} columns showed very little degradation in chromatographic performance, exceeding the performance of the best silica column. Hence, C_{18} column has been selected for analysis.

MATERIALS AND METHODS

Materials: Chemicals and reagents were procured were Merck India. Dutasteride and Tamsulosin were received as gift sample from Dr.Reddy's laboratories pvt. limited. Dissolution was performed using USP Apparatus 1(Electrolab). The analysis was carried out using Agilent HPLC. Other instruments used for analysis were Analytical Balance, Ultrasonic Bath, Centrifuge, pH meter, Oven and Mechanical shaker. Polytetra fluoro ethylene (PTFE) filter used for sample filtration were purchased from Rankem, India.

Methods

Analytical method: The finished product is having the strength of 0.5mg Dutasteride and 0.4mg of Tamsulosin HCl, the dissolution was performed in 900ml of media. The scope for sample dilution is very low, since 10% drug release in 900ml would have the concentration 0.04ppm. Various trials were conducted, to select the suitable combination of mobile phase. Standards were injected, to evaluate the separation of peaks and elution. Based on the literature survey, two solvents were selected. Solvent A -0.05M pH6.8 Phosphate buffer & solvent B-Acetonitrile, and the ratios evaluated were presented in table-1.

Table 1: Ratio of Solvent A& B for gradient programme selection.

Time	0-5 minutes	5-10 minutes	10-15 minutes	15-20 minutes
Trial-1	100:0	0:100	50:50	100:0
Trial-2	75:25	25:75	50:50	75:25
Trial-3	25:75	50:50	75:25	25:75
Trial-4	75:25	50:50	25:75	75:25
Trial-5	65:35	50:50	20:80	65:35
Trial-6	35:65	50:50	80:20	35:65

Preparation of mobile phase (Solvent A): 0.05M pH 6.8 phosphate buffer was prepared by transferring 17.5 g of dibasic potassium phosphate to a suitable container containing 2000 mL of water and dissolved. The pH was adjusted to 6.8± 0.05 using orthophosphoric acid. The solution was filtered through 0.45 µm PTFE filter.

Preparation of Tamsulosin standard stock solution: TSL stock solution was prepared by adding 17.8mg of TSL into volumetric flask containing 10ml of methanol. The solution was sonicated for 10minutes to get a clear solution. Volume was made upto 200ml with purified water and used as stock solution for preparing standard for acid stage and buffer stage. (concentration: 89µg/ml).

Preparation of Tamsulosin standard solution (Acid stage): 15ml of Tamsulosin standard stock solution was transferred to 100ml volumetric flask, and volume was made upto 100ml of acid stage dissolution medium. 10ml of resulting solution was transferred into 100ml volumetric flask, and volume was made upto 100ml with acid stage dissolution medium (concentration: 1.3µg/ml).

Preparation of Tamsulosin standard solution (Buffer stage): 5ml of Tamsulosin standard stock solution was transferred to 100ml volumetric flask, and volume was made upto 100ml of buffer stage dissolution medium. 10ml of resulting solution was transferred into 100ml volumetric flask, and volume was made upto 100ml with buffer stage dissolution medium (concentration: 0.44µg/ml).

Preparation of Dutasteride standard solution: Dutasteride stock solution was prepared by adding 22.4mg of Dutasteride into 200ml volumetric flask containing 8ml of methanol. The solution was sonicated for 10minutes to get a clear solution. Volume was made upto 200ml with acid stage dissolution medium. 5ml of resulting solution was transferred into 100ml volumetric flask, and volume was made upto 100ml with acid stage dissolution medium. (concentration: 5.6µg/ml).

Preparation of Dutasteride/Tamsulosin working standard solution (Acid stage): 10ml of Dutasteride standard solution and 10ml of Tamsulosin standard solution acid stage were transferred to 100ml volumetric flask. Volume was made upto 100ml with acid stage dissolution medium (concentration- 0.56µg/ml of DUT & 0.13µg/ml of TSL).

Sample solutions withdrawn from dissolution apparatus were filtered and directly injected into HPLC.

Chromatographic condition: HPLC analysis was performed on Agilent HPLC system with UV detector. Chromatographic separation of DUT and TSL were carried on X Bridge C_{18} column with 150 x 4.6 mm and 5 µm particle size. Gradient condition with the mobile phase containing solvent-A and solvent-B with a flow rate of 1.5ml/minute was used for analysis, with a run time of 18 minutes. The detection wavelength was set at 225 nm, with the sample volume of 200µL. HPLC column was maintained at a temperature of 30°C and the sample was maintained at the temperature of 25°C.

Dissolution method development: Dissolution apparatus-1 was used for evaluation, to enable the ease of transfer during dissolution media change over, with 900ml of media volume. Dissolution was performed with 0.1%, 0.2% & 0.3% concentration of sodium lauryl sulphate, to achieve complete release of Dutasteride, and require ensuring the enteric coating was not disturbed by sodium lauryl sulphate concentration. Initial 2 hrs dissolution was performed in 0.1N HCl, followed by pH 7.2 phosphate buffer for 8hrs. The effect of RPM on drug release profile was evaluated at 75 RPM, 100RPM & 125RPM. Calculations were applied to quantify the percentage of drug release in comparison to peak area of standard.

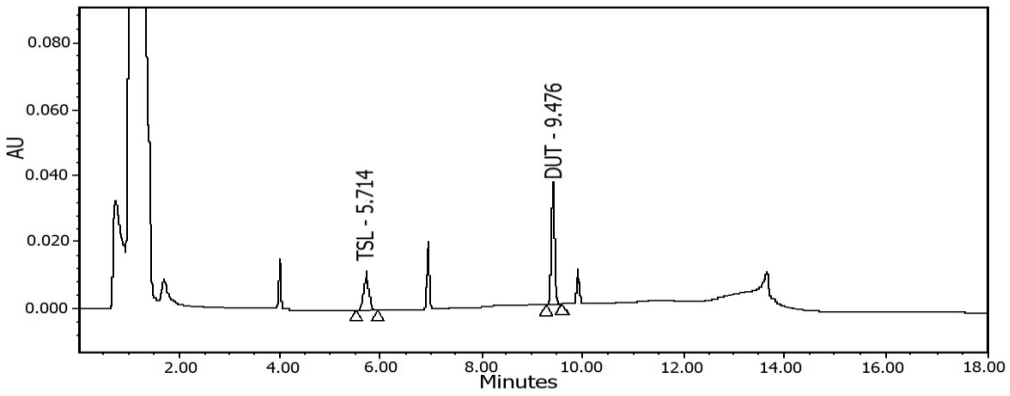
Filter interference study: Interference from the filter of the dissolution system was evaluated in acid medium and buffer medium using Whatman PVDF filter paper of 0.45µm, with 2.5cm diameter. Dutasteride equivalent to 100% concentration and Tamsulosin equivalent to 10% of concentration was added to 900ml acid stage medium, mixed for 10minutes. Sample were withdrawn from vessel before and after filtration and analyzed for content of DUT & TSL. Tamsulosin equivalent to 100% concentration was added to 900ml buffer stage medium, mixed for 10minutes. sample were withdrawn from vessel before and after filtration and analyzed for content of TSL.

Linearity: DUT in acid stage medium was evaluated by analyzing a series of 6 standard solution containing DUT reference standard ranging from 0.0222µg/ml to 0.666µg/ml, which approximately equivalent to 4% to 120% of 100% dissolved DUT concentration of 0.56 µg/ml. TSL in acid stage medium was evaluated by analyzing a series of 5 standard solution containing reference standard ranging from 0.0178µg/ml to 0.1778µg/ml, which approximately equivalent to 4.3% to 43% of 100% dissolved TSL concentration of 0.44µg/ml. TSL in buffer stage medium was evaluated by analyzing a series of 6 standard solution containing reference standard ranging from 0.0178µg/ml to 0.5333µg/ml, which approximately equivalent to 4% to 120% of 100% dissolved TSL concentration of 0.44µg/ml.

Accuracy: For DUT in acid stage medium was evaluated by spiking sample with known amount of DUT at levels of 10%, 100% & 120% of 100% DUT release (equivalent to 0.5mg DUT). For TSL in acid stage medium was evaluated by spiking sample with known amount of TSL at levels of 4%, 10% and 40% TSL release (equivalent to 0.4mg TSL). For TSL in buffer stage medium was evaluated by spiking sample with known amount of TSL at levels of 10%, 100% and 120% TSL release (equivalent to 0.4mg TSL).

DISCUSSION

The analytical method was evaluated with different combination and sequence of gradient system. The gradient system of trial -4 eluted two separate distinct peaks with appropriate peak shape. The standard chromatograms of Dutasteride and Tamsulosin in both acid medium and buffer medium were presented in figure 1.



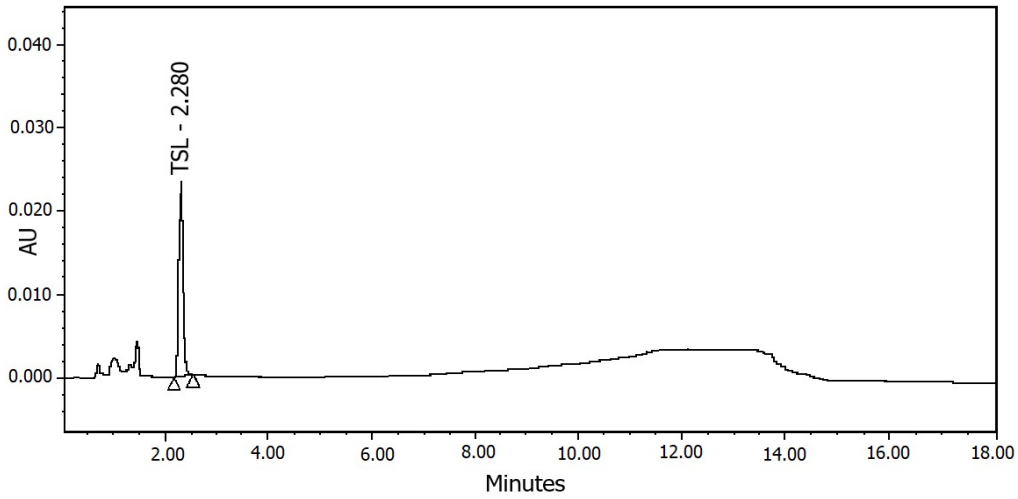


Figure 1: (a)-Standard chromatogram of Tamsulosin (TSL) and Dutasteride (DUT) in acid medium, (b)-Standard chromatogram of Tamsulosin (TSL) in buffer medium.

Dissolution method development

Dissolution method was evaluated with different concentration of sodium lauryl suphate (SLS) for completeness of dissolution of Dutasteride, and percentage drug release of Tamsulosin the dissolution data is presented in table-2, and the dissolution profile graph is presented figure-2. The dissolution data reveals, 0.2% w/v of sodium lauryl sulphate in 0.1N HCl is required to completely dissolve Dutasteride, and drug release of Tamsulosin was not altered by SLS concentration. The concentration of sodium lauryl sulphate is not having impact on enteric coated Tamsulosin. The same has been presented in figure- 3.

Table 2: Dissolution profile of dutasteride & tamsulosin from combodart with different concentration of sodium lauryl sulphate in 0.1n hcl for 2 hrs. Usp-i, 900ml.

Time (hrs)	Batch Number: 111834					
	0.1% SLS		0.2% SLS		0.3% SLS	
	DUT	TSL	DUT	TSL	DUT	TSL
0.5hrs	25.9 ± 0.6	3.3 ± 0.4	46.7 ± 0.9	4.4 ± 0.2	82.9 ± 0.4	3.8 ± 0.4
1 hr	45.7 ± 0.5	8.1 ± 0.5	76.1 ± 0.8	8.7± 0.5	94.0 ± 1.2	8.3 ± 0.6
1.5 hrs	68.9 ± 1.0	8.8 ± 0.4	91.5 ± 0.1	9.1 ± 0.4	100.5 ± 0.3	9.6 ± 0.5
2 hrs	73.8 ± 1.5	13.8 ± 0.7	100.3 ± 0.1	13.6 ± 0.5	100.4 ± 0.5	14.3 ± 0.7
Note: mean ± SD, n=3						

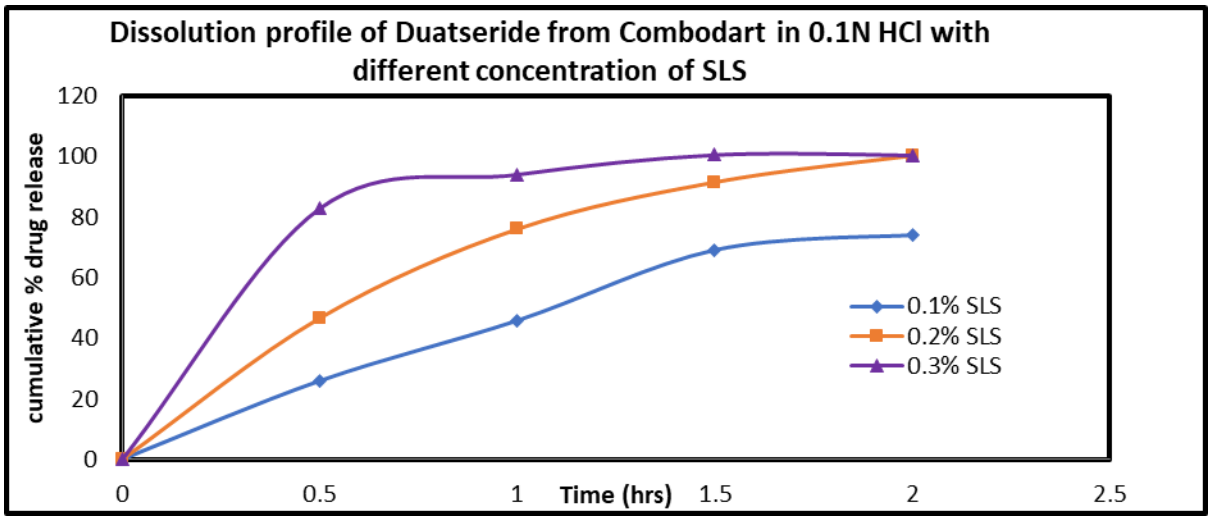


Figure-2: Dissolution profile of Dutasteride from Combodart at different concentration of sodium lauryl sulphate in 0.1N HCl.

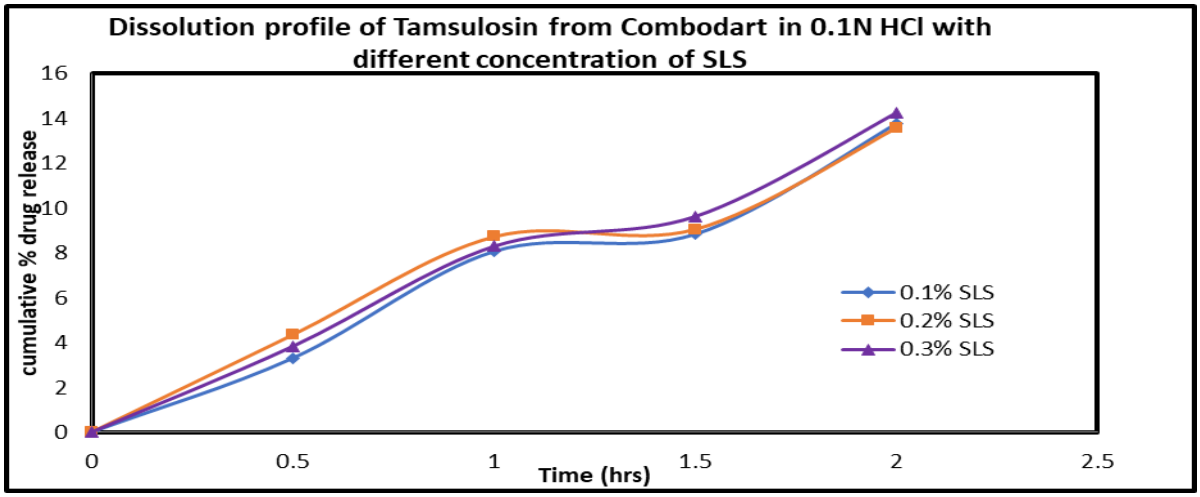


Figure-3: Dissolution profile of Tamsulosin from Combodart at different concentration of sodium lauryl sulphate in 0.1N HCl.

Based on the dissolution results obtained with 0.2% SLS in 0.1NHCl, the dissolution was evaluated for complete release of Tamsulosin by changing the dissolution media to 0.05M pH 7.2 Phosphate buffer for 8hrs.The dissolution Tamsulosin is observed with 100%. The effect of RPM on dissolution profile was evaluated, and dissolution profile is presented in table-3, figure-4 &5.

Table 3: Dissolution profile of dutasteride & tamsulosin from combodart with different rpm in 0.1n hcl with 0.2% sls for 2 hrs, followed by ph 7.2 phosphate buffer for 8hrs. Usp-i, 900ml.

Time (hrs)	Batch Number: 111834					
	75RPM		100RPM		125 RPM	
	DUT	TSL	DUT	TSL	DUT	TSL
0.5hrs	40.6 ± 0.6	5.9 ± 0.4	46.7 ± 0.9	6.2 ± 0.4	47.5 ± 0.8	5.1 ± 0.2
1 hr	69.0 ± 0.6	8.8 ± 0.4	76.1 ± 0.8	11.6 ± 0.3	75.2 ± 0.7	10.2 ± 0.3
1.5 hrs	89.8 ± 0.4	10.3 ± 0.7	91.5 ± 0.1	9.7 ± 0.2	93.5 ± 0.8	10.7 ± 0.5
2 hrs	100.2 ± 0.1	13.4 ± 0.6	100.3 ± 0.1	13.7 ± 0.4	100.1 ± 0.1	14.1 ± 0.5
3hrs		42.3 ± 0..5		45.4 ± 0.5		47.6 ± 0.2
4hrs		61.0 ± 0.7		62.0 ± 0.7		70.7 ± 0.5
6hrs		83.2 ± 0.6		85.8 ± 0.3		90.6 ± 0.5
8 hrs		99.1 ± 0.2		100.3 ± 0.5		98.7 ± 0.2
10hrs		99.6 ± 0.1		100.6 ± 0.2		101.1 ± 0.6
F_2 (Against 100 RPM)	**65**	**84**			**91**	**71**
Note: mean ± SD, n=3						

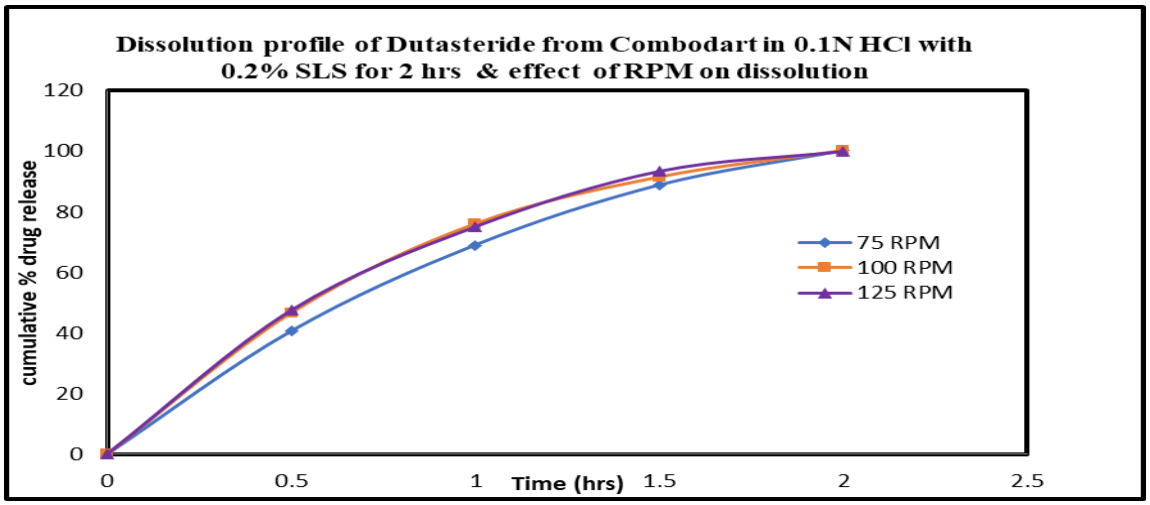


Figure-4: Dissolution profile of Dutasteride from Combodart in 0.1N HCl with 0.2% SLS for 2 hrs & effect of RPM on dissolution.

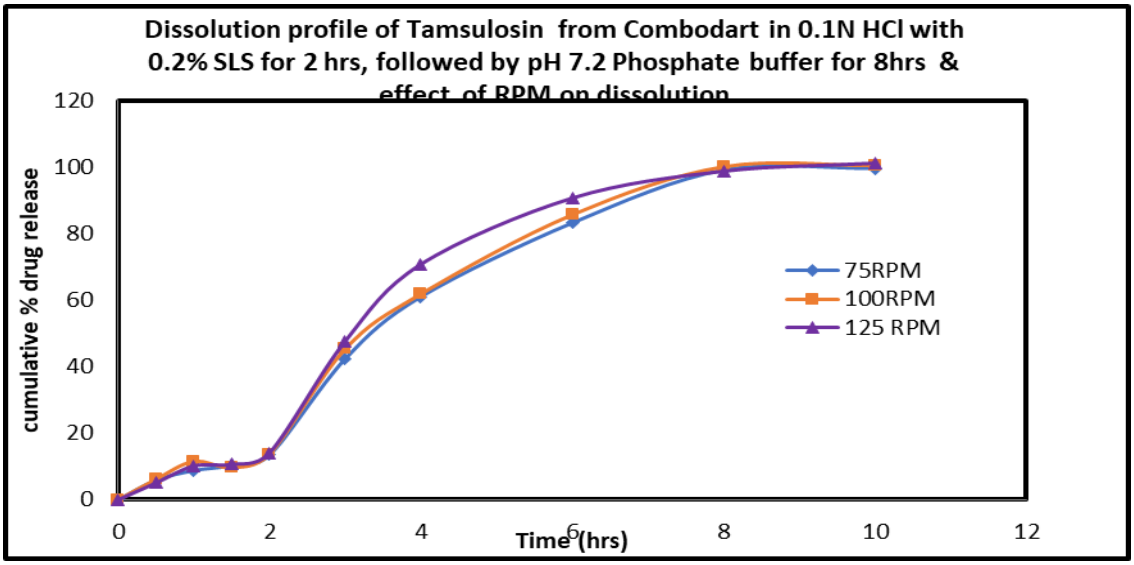


Figure-5: Dissolution profile of Tamsulosin from Combodart in 0.1N HCl with 0.2% SLS for 2 hrs, followed by pH 7.2 Phosphate buffer for 8hrs & effect of RPM on dissolution.

The dissolution data reveals, the complete release of Dutasteride was observed in acid medium, and complete release of Tamsulosin was observed in pH 7.2 phosphate buffer. The agitation speed is not having significant impact on drug release, observed with F_2 value of more than 50% at 75 RPM and 125RPM, in comparison to 100RPM.

Method validation[13]: Analytical method validation was performed for filter interference, linearity and accuracy.

Filter interference study: The peak response before filtration and after filtration were presented in table-3 for both acid and buffer medium. The observed percentage difference was below 1.0%, which concluded there is no interference of filter.

Table-4: Peak response before and after filtration.

Medium	Active drug	Peak response		% Difference
		Before filtration	After filtration	
Acid medium	DUT	85936.6 ± 32.0	85871.8 ± 55.8	0.08%
	TSL	12824 ± 30.5	12864.8 ± 37.3	0.32%
Buffer medium	TSL	129451.2 ± 385.8	129003.4 ± 156.8	0.35

Linearity: Linearity graph for Dutasteride in acid dissolution medium was established for the range of 4-120%, presented in fig-6a and table-5. The correlation coefficient (R^2) is 1.000, meet the acceptance criteria of ≥0.995 & within ±5% of the response of 100% of DUT working standard concentration (0.56 µg/ml).

Linearity graph for Tamsulosin in acid dissolution medium was established for the range of 4-43%, presented in fig-6b and table-5. The correlation coefficient (R^2) is 1.000, meet the acceptance criteria of ≥0.990 & within ±10% of the response of 30% of TSL working standard concentration (0.44 µg/ml).

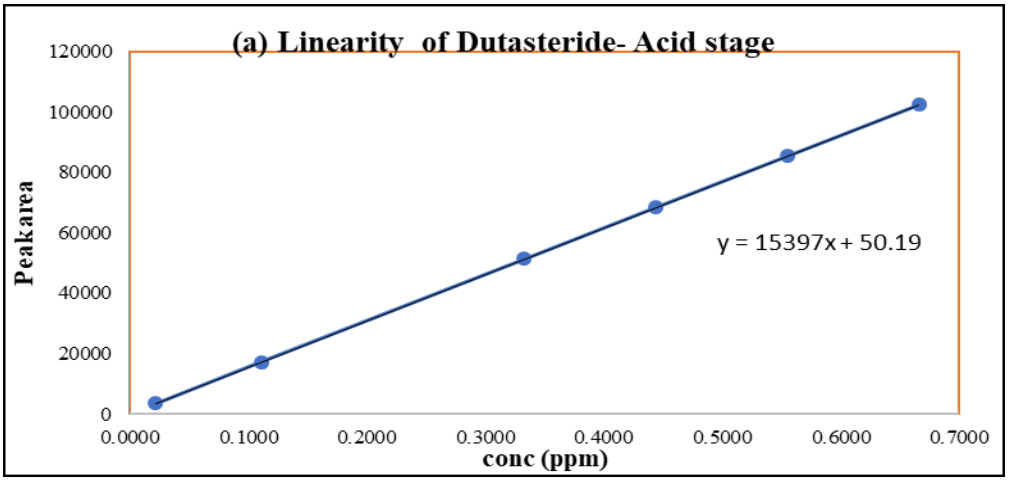
Linearity graph for Tamsulosin in buffer dissolution medium was established for the range of 4-120%presented in fig-6c and table-5. The correlation coefficient (R^2) is 1.000, meet the acceptance criteria of ≥0.995 & within ±5% of the response of 100% of TSL working standard concentration (0.44 µg/ml).

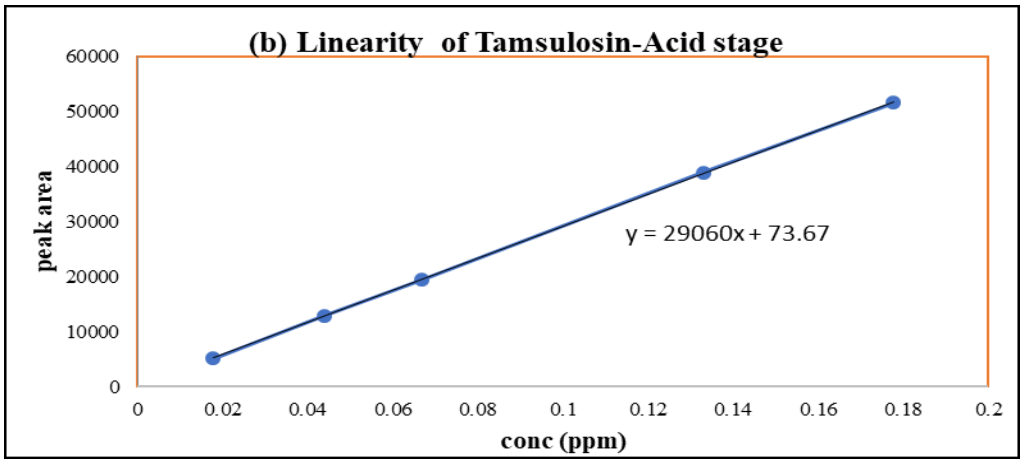
Table 5: linearity study for dutasteride and tamsulosin in acid medium, tamsuloin in buffer medium.

	Linearity in acid medium				Linearity in buffer medium	
	Dutasteride		Tamsulosin		Tamsulosin	
	Concentration (ppm)	Peak Area	Concentration (ppm)	Peak Area	Concentration (ppm)	Peak Area
1	0.0222	3426	0.0178	5152	0.0178	5162
2	0.1110	17126	0.044	12949	0.0889	25825
3	0.3330	51385	0.0667	19425	0.2667	77529
4	0.4440	68525	0.133	38878	0.3556	103625
5	0.5550	85425	0.1778	51629	0.4444	129121
6	0.6660	102564			0.5333	154785

Table 6: Validation Parameters Established By Linearity.

Parameters	DUT-in Acid medium	TSL-in acid medium	TSL-in buffer medium
Linearity (ppm)	0.022-0.666	0.0178-0.1778	0.0178-0.5333
correlation co-efficient (R^2)	0.999	1.000	0.999
Regression equation	y = 15397x + 50.19	y = 29060x + 73.67	y = 30407x + 101.3
LOD (ppm)	0.002	0.002	0.002
LOQ (ppm)	0.005	0.005	0.006





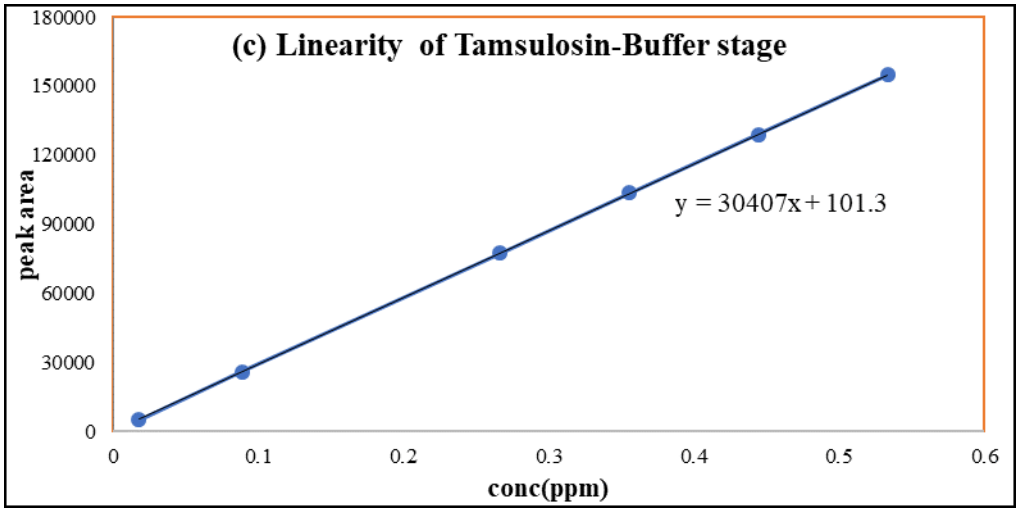


Figure 6: (a) Linearity of Dutasteride- Acid stage, 6(b)- Linearity of Tamsulosin-Acid stage & 6(c)-Linearity of Tamsulosin-Buffer stage.

The correlation coefficient (R^2) value met the acceptance criteria of more than 0.997. The linear regression data shows that the method is linear, and it is adequate for its intended concentration range. The LOD values for DUT in acid stage, TSL in acid stage and TSL in buffer stage were determined to be 0.002ppm, 0.002ppm and 0.002ppm, and the LOQ values were 0.005ppm, 0.005ppm and 0.006ppm respectively.'

Accuracy: Accuracy for Dutasteride in acid medium was evaluated by spiking known concentrations of Dutasteride at levels of 10%, 100% and 120% of the 100% Dutasteride release (equivalent to 0.5mg DUT) in triplicate. Accuracy for Tamsulosin HCl in acid medium was evaluated by spiking known concentrations of Tamsulosin at levels of 4%, 10% and 40% of the 100% Tamsulosin release (equivalent to 0.4mg TSL) in triplicate. Accuracy for Tamsulosin HCl in buffer medium was evaluated by spiking known concentrations of Tamsulosin at levels of 10%, 100% and 120% of the 100% Tamsulosin release (equivalent to 0.4mg TSL) in triplicate. The observed results were found to be less than 5% RSD and the results were presented in Table 7 &8.

Table 7: Accuracy (results of recovery study - acid medium).

Level of recovery (%)	concentration actual (µg/ml)		concentration added (µg/ml)		Mean % recovery ± SD (n=3)		% RSD	
	DUT	TSL	DUT	TSL	DUT	TSL	DUT	TSL
4	-	0.44	-	0.0176	-	94.4±0.1	-	0.1
10	0.56	0.44	0.056	0.044	104.4±1.8	98.0±0.4	1.7	0.4
40	-	0.44	-	0.176	-	99.1±0.2	-	0.2
100	0.56	-	0.56	-	100.7±0.3	-	0.3	
120	0.56	-	0.672	-	100.1±0.2	-	0.2	

Table 8: Accuracy (results of recovery study - buffer medium).

Level of Recovery (%)	concentration actual (µg/ml)	concentration added (µg/ml)	Mean % recovery ± SD (n=3)	% RSD
	TSL	TSL	TSL	TSL
10	0.44	0.044	101.2 ±0.4	0.3
100	0.44	0.44	99.2±0.8	0.8
120	0.44	0.528	99.1±.5	0.5

CONCLUSION

Novelty in this research work involves, establishing better isolation and elution of active ingredients without diluting further the sample solution, with very low concentration using HPLC. Gradient system sequence was developed in a combination of acetonitrile and pH 6.8 phosphate buffer to elute both drugs, with a run time of 18 minutes. Developing a dissolution method for simultaneous estimation of two active drugs, one in immediate release dosage form, and another in modified release dosage form using a single dissolution run, with the aid of changeover dissolution media. The method was validated in compliance with the ICH guidelines. Hence, this developed method can be conveniently adopted for routine quality control analysis of dissolution of DUT and TSL modified release capsules.

ACKNOWLEDGEMENT

The authors are thankful to Malla Reddy Pharmacy College for providing necessary facilities and also gratitude towards Dr.Reddy's laboratories, for providing gift sample.

Conflict of Interest: The author(s) declare(s) that there is no conflict of interest.

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