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HPLC METHOD DEVELOPMENT AND VALIDATION OF DEFLAZACORT AND TAMSULOSIN HYDROCHLORIDE IN COMBINED DOSAGE FORM

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ABSTRACT

The HPLC method was also developed and validated for simultaneous estimation of Deflazacort and Tamsulosin hydrochloride in combined dosage form. For the HPLC method, the linearity range lies between 37.5-112.5 µg/ml for Deflazacort and 0.5-1.5 µg/ml for Tamsulosin hydrochloride with co-relation coefficients of 0.9995 and 0.9924 respectively for both drugs. The percentage recovery was found to 99.51-99.97% for Deflazacort and 99.55-99.72% for Tamsulosin hydrochloride. LOD value was found to 2.61µg/ml and 0.132µg/ml for Deflazacort and Tamsulosin hydrochloride respectively. LOQ value was found to 7.90µg/ml and 0.40µg/ml for Deflazacort and Tamsulosin hydrochloride respectively. The assay result for Deflazacort and Tamsulosin hydrochloride was found to be 102.37 and 93.09% comparable indicating the good agreement with the label claim.

KEYWORDS: Deflazacort, Tamsulosin hydrochloride, HPLC, Validation.

INTRODUCTION

- HPLC is a physical separation technique conducted in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, polymers, and other organic compounds.[1,2,3,4]

- The deflazacort is an oxazoline derivative of prednisolone of anti-inflammatory and immunosuppressive activity.[5] It acts by blocking the secretion producing immune and allergic conditions, as a result of inflammation.[6] FDA approval of Deflazacort in February, 2017.[7]
- Tamsulosin, sold under the trade name Urimax, Veltam and Flomax is a medication used to treat symptomatic benign prostatic hyperplasia (BPH), help with the passage of kidney stones, and for urinary retention along with other measures.[8]
- Tamsulosin was developed by Yamanouchi Pharmaceuticals (now part of Astellas Pharma) and was first marketed in 1996. The U.S. patent expired in October 2009. The U.S. Food and Drug Administration (FDA) approved generics in March 2010.[9-12]

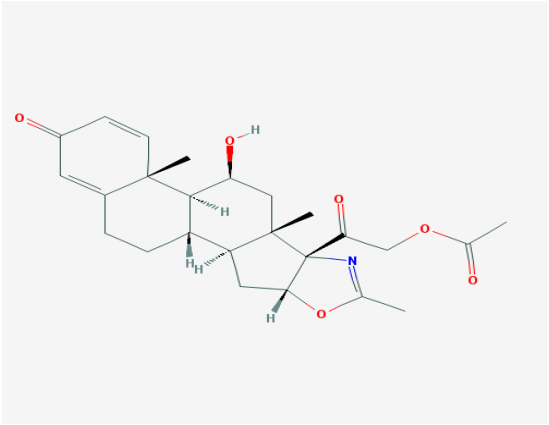

Figure 1: Deflazacort.

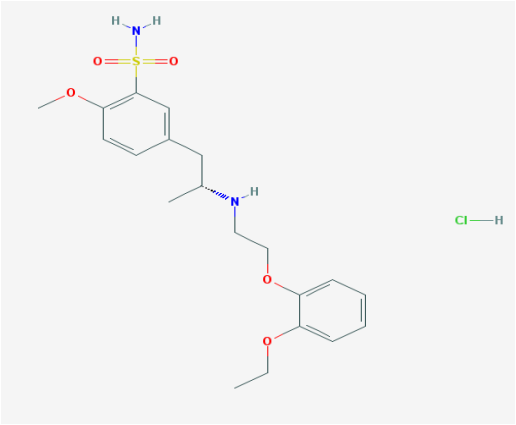

Figure 2: Tamsulosin hydrochloride.

- Literature search reveals that there are very methods reported for the determination of Deflazacort and Tamsulosin hydrochloride in single and combination with other drug by different instrumental techniques like RP-HPLC-PDA[13], RP-HPLC[14], UV[15], HPLC[16], and HPTLC[17] methods.

MATERIALS AND METHODS

Apparatus and Instrument

- HPLC was used Shimadzu LC-AT in which detector is SPD-20T. Software is Spinchrom Software. Thermo Hypersil Silica 5 µ, (250mm x 4.6mm) Column and Hamilton syringe 20 µl syringe was used.

Materials and Chemicals

- Deflazacort and Tamsulosin hydrochloride give as a gift sample by Macleods pharma, Vapi, Gujarat.
- All the Reagents were HPLC Grade

Chromatographic Condition

- Stationary Phase is C18, Hypersil BDS (4.6 mm × 150 mm, 5µm) and Mobile phase is Buffer (pH-4.0): Methanol (50:50). Detection Wavelength was 227 nm and Run Time was 10 min. Temperature was 25 ± 2 ºC and flow rate 1 ml/min. Injection volume was 20µl.

Methodology of HPLC

Preparation of Standard solution

1) Preparation of Deflazacort stock solution

- Accurately weighed Deflazacort (75 mg) was transferred into 100 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol to give a stock solution having strength 750 µg/ml.

2) Working standard solution of Deflazacort

- 75 µg/ml of Deflazacort working standard solution was prepared by diluting 10 ml of stock solution with methanol in 100 ml volumetric flask up to the mark.

3) Preparation of Tamsulosin hydrochloride stock solution

- Accurately weighed Tamsulosin hydrochloride (10 mg) was transferred into 100 ml volumetric flask and dissolved in methanol and further 10 ml this solution diluted up to 100 ml with methanol to give a stock solution having strength 10µg/ml.

4) Working standard solution of Tamsulosin hydrochloride:

- 1 µg/ml of Tamsulosin hydrochloride working standard solution was prepared by diluting 10 ml of stock solution with methanol in 100 ml volumetric flask up to the mark.

Wavelength Selection for UV Detection

- 1 ml stock solution of Deflazacort dilute up to 10 ml of methanol (75 µg/ml)
- 1 ml stock solution of Tamsulosin hydrochloride dilute up to 10 of methanol (1 µg/ml)
- Take UV spectra of above two solutions individually between the ranges of 200nm-400nm using methanol as a blank.

Optimization of Mobile Phase

- **Working standard preparation (combine standard preparation):** Pipette out 1ml from Deflazacort standard stock solution (750 µg/ml) and 1ml from Tamsulosin hydrochloride standard stock solution (10 µg/ml) make up volume up to 10 ml with Mobile phase (mobile phase which used for trial) (Deflazacort-75 µg/ml, Tamsulosin hydrochloride-10 µg/ml).
- Inject above working standard preparation for mobile phase selection.

System Suitability Studies

- A standard solution for Deflazacort and Tamsulosin hydrochloride were prepared as per the test method and was injected six times into HPLC System.
- The system suitability parameters were evaluated from standard chromatogram by calculating %RSD from six replicate injections for both drugs.

Validation of HPLC Method

1. Linearity and range

- The linearity response was determined by analysing independent levels of Calibration curve in the range of 37.5-112.5 µg/ml for Deflazacort and 0.5-1.5 µg/ml for Tamsulosin hydrochloride. Plot the calibration curve of peak area vs. concentration and determine Correlation coefficient and regression line equations for Deflazacort and Tamsulosin hydrochloride.

2. Accuracy (% Recovery)

- Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Deflazacort and Tamsulosin hydrochloride were found out. Recovery between 98- 102 % justifies the accuracy of the method.

3. LOD & LOQ

- **LOD:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
- **LOQ:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

- A sensitivity of analytical method was evaluated by lowest limit of detection and lowest limit of quantitation.
- The LOD and LOQ were measured by the mathematical equation given below.
- LOD = 3.3 × SD/slope
- LOQ = 10 × SD /slope

4. Precision

- The precision expressed as standard deviation or relative standard deviation. Deflazacort and Tamsulosin hydrochloride were taken in a ratio was analyzed at three levels of concentration for three times. For Precision study intermediate concentrations were selected from linearity range for both drugs, i.e. 37.5, 75, 112.5 µg/ml and 0.5, 1, 1.5 µg/ml concentration range were taken in a ratio for Deflazacort and Tamsulosin hydrochloride.

A. Repeatability

- Measured area of solution containing Deflazacort (75µg/ml) and Tamsulosin hydrochloride (1µg/ml) at 227 nm. The peak area of solution was measured 6 times and % RSD was calculated.

B. Intraday precision

- Deflazacort and Tamsulosin hydrochloride were taken in a ratio was analyzed at three levels of concentration for three times in a day.
- Both the drugs Deflazacort and Tamsulosin hydrochloride content equivalent to 37.5µg/ml Deflazacort and 0.5 µg/ml Tamsulosin hydrochloride, 75 µg/ml Deflazacort and 1 µg/ml Tamsulosin hydrochloride, 112.5µg/ml Deflazacort and 1.5µg/ml Tamsulosin hydrochloride were analysed three times on the same day at 1hr. interval. Peak area of the solutions was measured and %RSD was calculated.
- % RSD should be less than 2%.

C. Interday precision

- Deflazacort and Tamsulosin hydrochloride were taken in a ratio was analyzed at three levels of concentration for three different consecutive days.
- Both the drugs Deflazacort and Tamsulosin hydrochloride content equivalent to 37.5µg/ml Deflazacort and 0.5µg/ml Tamsulosin hydrochloride, 75µg/ml Deflazacort and 1 µg/ml Tamsulosin hydrochloride, 112.5µg/ml Deflazacort and 1.5µg/ml Tamsulosin

hydrochloride were analysed three times on the consecutive days. Peak area of the solutions was measured and %RSD was calculated.

- % RSD should be less than 2%.

5. Robustness

- The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The introduction of small changes like change in the mobile phase composition and flow rate. The effects of these changes on the results were examined.

Assay Analysis

Preparation of synthetic mixture

- (Label claim: 75 mg Deflazacort and 1 mg Tamsulosin hydrochloride)
- Weighed accurately 750 mg of Deflazacort and 10 mg of Tamsulosin hydrochloride and transferred to a 10 ml volumetric flask. Add 6 ml methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with methanol. Filter it using whatman filter paper. (Deflazacort-75 µg/ml and Tamsulosin hydrochloride -1 µg/ml)

Preparation of Working Sample Solution

- Pipette out 1ml from synthetic mixture (Deflazacort-75µg/ml and Tamsulosin hydrochloride – 1 µg/ml) and make up volume up to 10ml with mobile phase (water: methanol) (70:30) (Deflazacort-4µg/ml, Tamsulosin hydrochloride 30µg/ml).
- Inject working standard preparation and working sample preparation for assay analysis.

RESULT AND DISCUSSION

Wavelength Selection for UV Detection

- Overlay Spectra of Deflazacort and Tamsulosin hydrochloride are overlain at 227 nm. So this wavelength was selected for detection

Optimization of mobile phase

- Various mobile phase are like water with Methanol, acetonitrile and Different pH of Buffer was used but proper peak was not observed. Finally mobile phase consisting of Phosphate Buffer (pH-4.0): Methanol (50:50) showed good resolution and sharp peak.

System Suitability Studies

Table 1: System suitability studies.

Parameter	Deflazacort	Tamsulosin hydrochloride
Number of Theoretical Plates (N)	4361	7978
Retention Time	3.273	4.807
Asymmetry	1.259	1.310
Resolution	-	7.416

Method Validation of Hplc

Linearity and range

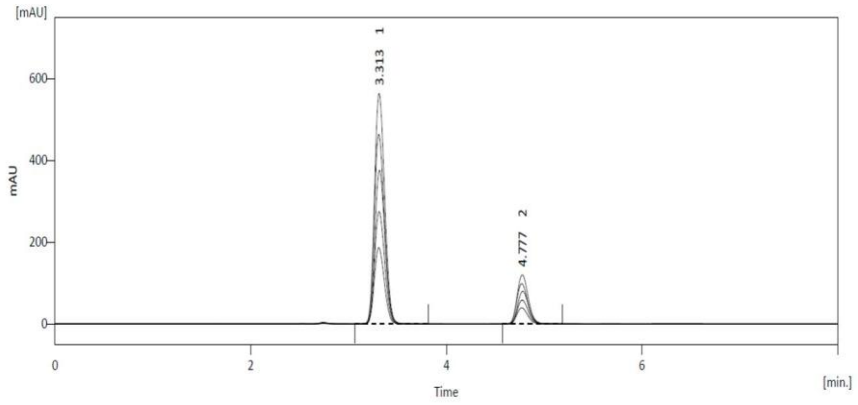

Figure 3: Chromatogram of linearity for 37.5-112.5 µg/ml for Deflazacort (Retention time) and 0.5-1.5 µg/ml for Tamsulosin hydrochloride.

Table 2: Calibration Data For Deflazacort.

Sr. no.	Conc. (µg/ml)	Peak Area (Mean±SD)
1	37.5	1340.46
2	56.25	1979.13
3	75	2706.05
4	93.75	3332.04
5	112.5	4054.36
Correlation coefficient		0.999
Limit of correlation coefficient		NLT0.99

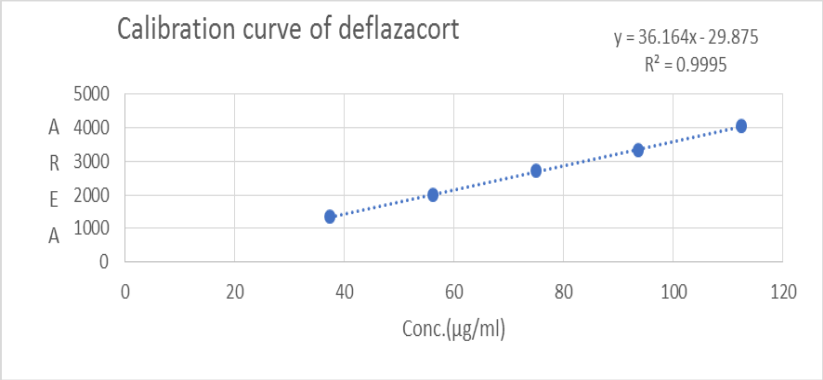

Figure 4: Calibration curve for Deflazacort (37.5-112.5 µg/ml).

Table 3: Calibration Data for Tamsulosin Hydrochloride.

Sr. no.	Conc. (µg/ml)	Peak area
1	0.5	307.93
2	0.75	454.70
3	1	621.79
4	1.25	725.65
5	1.5	931.65
Correlation coefficient		0.9924
Limit of correlation coefficient		NLT0.99

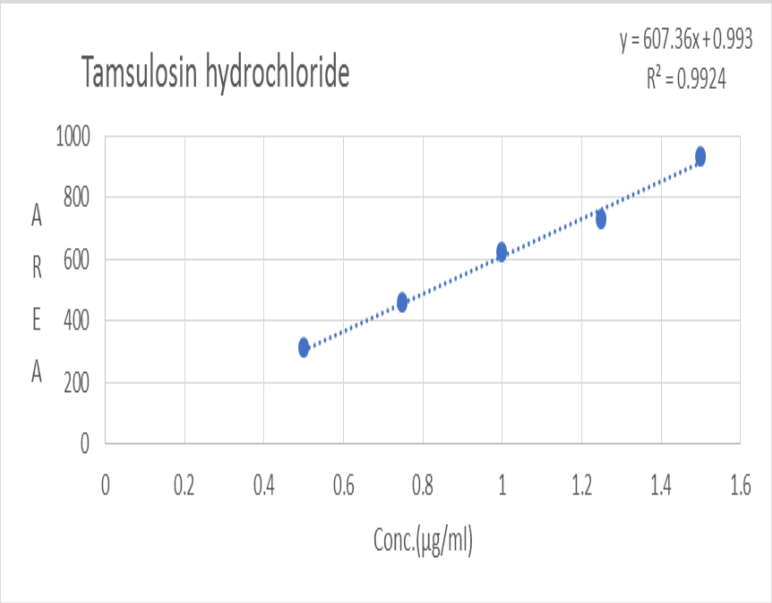

Figure 5: Calibration curve for Tamsulosin hydrochloride (0.5-1.5 µg/ml).

Table 4: Result of Linearity

Parameters	Deflazacort	Tamsulosin hydrochloride
Linearity range	37.5 to 112.5	0.5 to 1.5
Regression line equation	y = 36.164x - 29.875	y = 607.36x + 0.993
Correlation coefficient(R^2)	R^2 = 0.9995	R^2 = 0.9924

LOD and LOQ

- LOD of Deflazacort and Tamsulosin hydrochloride was found respectively 2.610 and 0.123. LOQ of Deflazacort and Tamsulosin hydrochloride was found respectively 7.909 and 0.40.

Accuracy (% Recovery)

Table 5: Result of Accuracy (% Recovery).

Assay level	Tablet content taken eq. To (mg)		Standard added (mg)		Total drug recovered (mg)		% Recovery of standard added	
	Defla	Tam	Defla	Tam	Defla	Tam	Defla	Tam
Blank	37.5	0.5	-	-	37.48	0.52	-	-
			Mean±SD		**37.49 ± 0.01**	**0.50 ± 0.01**		
80%	37.5	0.5	30	0.4	29.62	0.39	98.75	99.79
			Mean±SD		**29.99 ± 0.34**	**0.39 ± 0.005**	**99.97**	**99.55**
100%	37.5	0.5	37.5	0.5	37.14	0.49	99.05	99.09
			Mean±SD		**37.32 ± 0.15**	**0.49 ± 0.005**	**99.51**	**99.72**
120%	37.5	0.5	45	0.6	45.07	0.59	100.17	99.86
			Mean±SD		**44.86 ± 0.19**	**0.60 ± 0.005**	**99.68**	**99.61**

Precision

A. Repeatability

- Measured area of solution containing Deflazacort-75 µg/ml and Tamsulosin hydrochloride-1 µg/ml at 227 nm. The area of solution was measured 6 times and % RSD was found 0.38% for PRS and 0.78% for ASP. The result was as shown in table.

Table 6: Result of Repeatability.

Parameter	Deflazacort	Tamsulosin hydrochloride
Average	2699.277	618.869
Standard Deviation	10.415	4.863
%RSD	0.385	0.785
Limit:%RSD for area NMT 2.0%		

B. Intraday precision

Table 7: Results of Intraday Precision.

Name of drug	Concentration (µg/ml)	Mean (n=3)	SD (n=3)	%RSD
Deflazacort	37.5	1327.72	14.18	1.068
	75	2686.12	18.68	0.695
	112.5	4026.22	25.15	0.624
Tamsulosin hydrochloride	0.5	306.21	0.964	0.315
	1.0	612.97	10.54	1.72
	1.5	923.52	7.21	0.780

C. Interday precision

Table 8: Results of Interday Precision.

Name of drug	Concentration (µg/ml)	Mean (n=3)	SD (n=3)	%RSD
Deflazacort	37.5	1330.72	5.52	0.414
	75	2684.97	13.48	0.502
	112.5	4024.98	16.56	0.411
Tamsulosin hydrochloride	0.5	304.81	2.21	0.725
	1.0	614.41	6.32	1.029
	1.5	920.22	10.11	1.098

Robustness

Table 9: Results of robustness for Deflazacort and Tamsulosin hydrochloride.

Sr. No	Parameter	Mean area (n=3)		SD (n=3)		%RSD	
		Defla	Tam	Defla	Tam	Defla	Tam
1	Flow Rate +0.2	2635.75	604.63	28.89	6.18	1.09	1.02
2	Flow Rate -0.2	2797.68	641.27	24.74	6.58	0.88	1.02
3	Mobile Phase +2	2634.69	602.35	24.55	8.35	0.93	1.38
4	Mobile Phase -2	2767.79	634.92	20.99	5.79	0.75	0.91
5	pH +0.2	2582.92	590.73	15.74	9.71	0.61	1.64
6	pH -0.2	2773.53	635.08	25.27	7.14	0.91	1.12

Assay Analysis

Table 10: Area of standard solution.

Sr. no	Drug name	Label claim (mg)	Area of standard
1	Deflazacort	75	2703.33
2	Tamsulosin hydrochloride	1	621.17

Table 11: Assay of Deflazacort and Tamsulosin hydrochloride.

Sr. no	Drug	Area of sample	% assay	Mean	SD (n=3)	%RSD
1	Deflazacort	2752.224	101.80	102.37	0.511	0.499
		2770.91	102.49			
		2779.201	102.80			
2	Tamsulosin hydrochloride	580.43	93.44	93.09	1.038	1.115
		571.03	91.92			
		583.37	93.91			

CONCLUSION

- From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Deflazacort and Tamsulosin hydrochloride was found to be simple, precise, accurate and high resolution. The developed method was validated in terms of specificity, linearity, precision, accuracy and robustness.

- For the HPLC method, the linearity range lies between 37.5-112.5µg/ml for Deflazacort and 0.5-1.5 µg/ml for Tamsulosin hydrochloride with co-relation coefficients of 0.9995 and 0.9924 respectively for both drugs.
- LOD value was found to 2.61µg/ml and 0.132µg/ml for Deflazacort and Tamsulosin hydrochloride respectively. LOQ value was found to 7.90µg/ml and 0.40µg/ml for Deflazacort and Tamsulosin hydrochloride respectively.
- Recovery studies shows that method is capable to recover analyte from the formulation. RSD of interday and intraday precision is within acceptable limit of 2% proves that method is precise.
- Hence, it is evident that developed method can be used in pharmaceutical industries for routine quality control of Deflazacort and Tamsulosin Hydrochloride in combined dosage form.

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