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UV-SPECTROSCOPY METHOD DEVELOPMENT AND VALIDATION OF DEFLAZACORT AND TAMSULOSIN HYDROCHLORIDE IN COMBINED DOSAGE FORM

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ABSTRACT

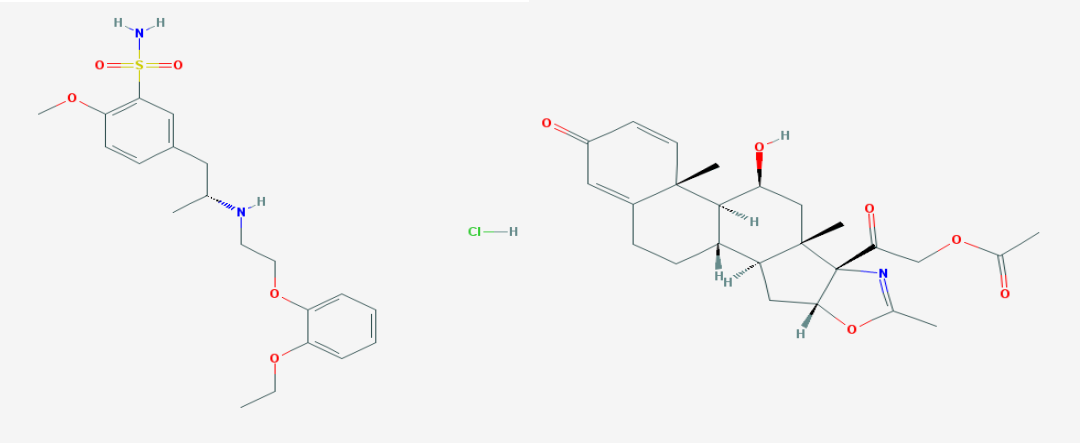
- A simple, specific, accurate and precise First order derivative spectrophotometric method was developed and validated for simultaneous estimation of Deflazacort and Tamsulosin hydrochloride in combined dosage form. The wavelength of estimation for Deflazacort was 266 nm and for Tamsulosin hydrochloride was 242 nm. The linearity range was found to be 8-40 µg/ml for Deflazacort and 1.6-8 µg/ml for Tamsulosin hydrochloride. The co-relation co-efficient was found to be 0.9916 and 0.9971 for Deflazacort and Tamsulosin hydrochloride respectively. The % recovery for Deflazacort and Tamsulosin hydrochloride were found to be 99.95-100.42% and 99.31-100.31% respectively. Intraday precision of Deflazacort and Tamsulosin hydrochloride were found to be 0.87-1.46 and 1.33- 1.87% RSD and Interday precision were found to be 0.86-1.86 and 1.42-1.71% RSD respectively.

KEYWORDS: Deflazacort, Tamsulosin hydrochloride, First order derivative UV-Spectroscopy method, Validation.

INTRODUCTION

- UV-visible spectrophotometry is one of the most frequently employed methods in pharmaceutical analysis. It involves the measurement of amount of ultraviolet (190-380nm) or visible (380-800nm) radiation absorbed by a substance in solution.[1,2]

- UV-visible spectrophotometry is widely used technique because many molecules have good absorption in UV/Visible region as they have conjugated double bonds and one can also make color complexes with various reagents and then can measure the absorbance in visible region if any compound is not absorbing in UV region.[3]
- The wavelength of absorbance depends on number of conjugated double bond, as the number increases the wavelength for absorbance shift towards the visible region.[4]
- The deflazacort is an oxazoline derivative of prednisolone of anti-inflammatory and immunosuppressive activity.[5,6] It acts by blocking the secretion producing immune and allergic conditions, as a result of inflammation.[7] FDA approval of Deflazacort in February, 2017.[8]
- 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.[9,10]
- 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.[11,12]



A. Tamsulosin hydrochloride **B. Deflazacort**

- 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.
- Spectrophotometry is considered as the most convenient analytical technique, because of its wide availability, simple instrumentation and less time consuming.

MATERIALS AND METHODS

Instrumentation: Double beam UV-Visible Spectrophotometer Shimadzu-1800.

Reagents and materials

- All the reagents and solvents used were of AR grades.
- Deflazacort and Tamsulosin hydrochloride give as a gift sample by Macleods pharma, Vapi, Gujarat.
- Marketed formulation DEFCORT-TM (Deflazacort- 30mg, Tamsulosin hydrochloride- 0.4 mg) tablet Manufacture by MACLEODS PHARMA. LTD.

Method Development

1. Standard stock solution of Deflazacort

Accurately weighed 100mg quantity of Deflazacort was transferred into different 100 ml volumetric flasks, dissolved and diluted up to mark with methanol to get 1000µg/ml solution for Deflazacort. This 1000µg/ml stock solution was further diluted to obtained 100µg/ml solution for Deflazacort.

2. Standard stock solution of Tamsulosin hydrochloride

Accurately weighed 100mg quantity of Tamsulosin hydrochloride was transferred into different 100 ml volumetric flasks, dissolved and diluted up to mark with methanol to get 1000µg/ml solution for Tamsulosin hydrochloride. This 1000µg/ml stock solution was further diluted to obtained 100µg/ml solution for Tamsulosin Hydrochloride.

First Order Derivative Method

1. Working standard solution of Deflazacort

From 100µg/ml solution of Deflazacort 0.8, 1.6, 2.4, 3.2 and 4.0 ml was transferred into 10 ml volumetric flask and adjust with methanol up to mark to get the final concentration of 8, 16, 24, 32 and 40µg/ml.

2. Working standard solution of Tamsulosin hydrochloride

From 100µg/ml of Tamsulosin hydrochloride further dilute up to 10µg/ml. from this 10µg/ml transfer 1.6, 3.2, 4.8, 6.4 and 8.0 ml was transferred into 10ml volumetric flask and adjust with methanol up to mark to get the final concentration of 1.6, 3.2, 4.8, 6.4 and 8µg/ml.

Determination of Wavelength for Measurement

- Highest concentrations were selected for both the drugs 40µg/ml of Deflazacort and 8.0µg/ml of Tamsulosin hydrochloride were scanned under UV instrument. Each solution was scanned between 400-200 nm. Wavelengths were selected from the overlain spectra of Deflazacort and Tamsulosin hydrochloride.

Preparation of Calibration Curve

Calibration curve for Deflazacort

- Standard stock solution was further diluted to obtain range of 8, 16, 24, 32 and 40µg/ml for Deflazacort.
- This solutions were scanned against blank in 200-400nm.

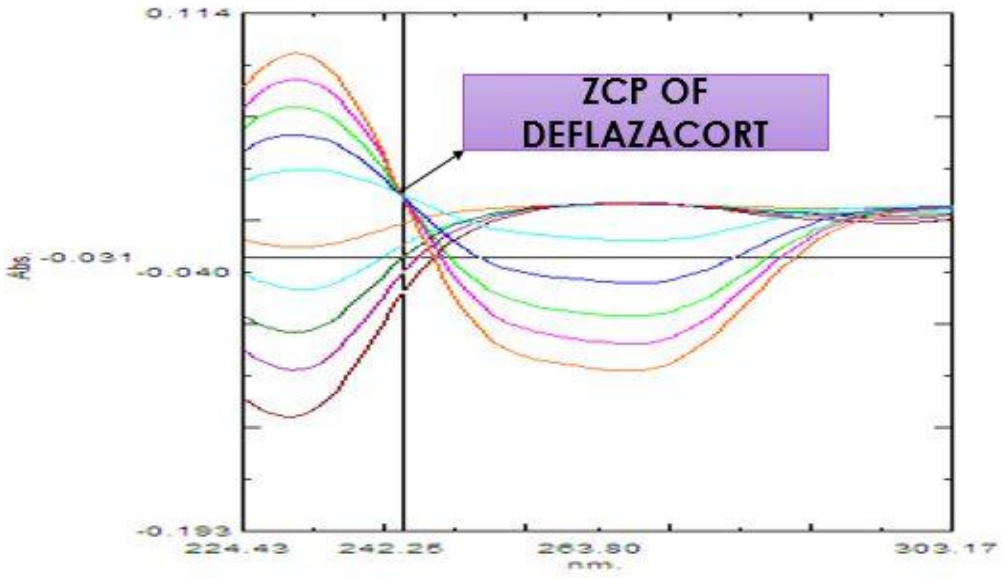


Figure 1: First-derivative spectra of Deflazacort (8-40 µg/ml) and Tamsulosin hydrochloride (1.6-8.0µg/ml) in methanol; ZCP of Deflazacort.

Calibration curve for Tamsulosin hydrochloride

- Standard stock solution was further diluted to obtain range of 1.6, 3.2, 4.8, 6.4 and 8.0µg/ml for Tamsulosin hydrochloride.
- This solutions were scanned against blank in 200-400nm.

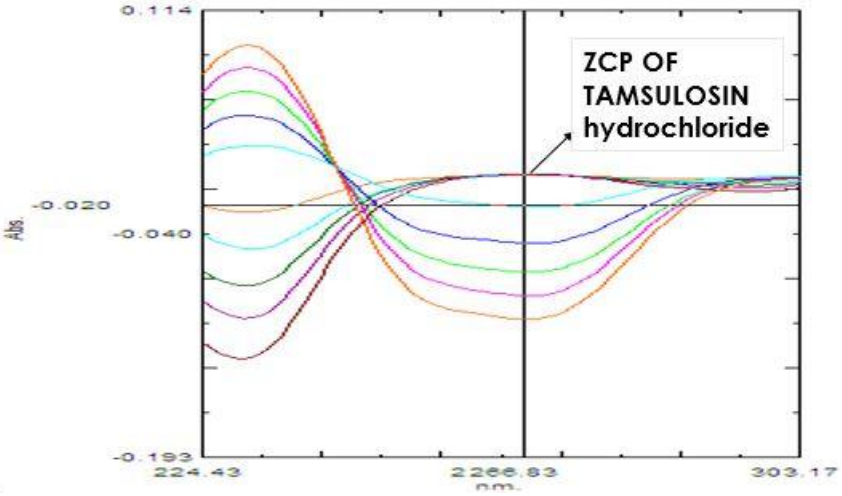


Figure 2: First-derivative spectra of Deflazacort (8-40 µg/ml) and Tamsulosin hydrochloride (1.6-8.0µg/ml) in methanol; ZCP of Tamsulosin hydrochloride.

Validation of Proposed Spectophotometric Method

1. **Linearity and range**

- The linearity response was determined by analyzing independent levels of calibration curve in the range of 8-40µg/ml for Deflazacort and 1.6-8.0µg/ml for Tamsulosin hydrochloride.
- Plot the calibration curve of absorbance vs. concentration and determine correlation coefficient and regression line equations for Deflazacort and Tamsulosin hydrochloride.

2. **Accuracy**

- The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to pre analyzed sample. Each determination was performed in triplicate.
- The accuracy of the method was checked by recovery experiment performed at three different levels of 80%, 100% and 120%. Percentage recovery for Deflazacort and Tamsulosin hydrochloride were calculated.
- Recovery between 98-102% justifies the accuracy of the method.

3. **Precision**

- The precision expressed as standard deviation or relative standard deviation.
- Deflazacort and Tamsulosin hydrochloride were taken in a ratio was analyzed at three level of concentration for three times.

- For Precision study intermediate concentrations were selected from linearity range for both drugs, i.e. 16, 24 and 32µg/ml and 3.2, 4.8, and 6.4µg/ml concentration range were taken in a ratio for Deflazacort and Tamsulosin hydrochloride respectively.

A. 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 16µg/ml Deflazacort and 3.2µg/ml Tamsulosin hydrochloride, 24µg/ml Deflazacort and 4.8µg/ml Tamsulosin hydrochloride, 32µg/ml Deflazacort and 6.4µg/ml Tamsulosin hydrochloride were analyzed three times on the same day at 1 hour interval. Absorbances of the solutions were measured and %R.S.D was calculated.
- % R.S.D. was calculated and it should be less than 2%.

B. Interday precision

- Deflazacort and Tamsulosin hydrochloride were taken in a ratio was analyzed at three levels of concentration for three consecutive days.
- Both the drugs Deflazacort and Tamsulosin hydrochloride content equivalent to 16µg/ml Deflazacort and 3.2µg/ml Tamsulosin hydrochloride, 24µg/ml Deflazacort and 4.8µg/ml Tamsulosin hydrochloride, 32µg/ml Deflazacort and 6.4µg/ml Tamsulosin hydrochloride were analyzed three times on the consecutive days. Absorbances of the solutions were measured and % R.S.D was calculated.
- % R.S.D. was calculated and it should be less than 2%.

4. 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.
- LOD was calculated out by using following formula:
- $DL = 3.3\ \sigma / S$
- **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.
- LOQ was calculated out by using following formula:
- $DL = 10\ \sigma / S$

Where,

- σ = the standard deviation of the response
- S = the slope of the calibration curve
- The slope S may be estimated from the calibration curve of the analyte.

Assay Analysis

- Applicability of the proposed method was tested by analyzing the commercially available tablet formulation (Defcort-TM) containing 30 mg of Deflazacort and 0.4 mg of Tamsulosin hydrochloride.

RESULT AND DISCUSSION

Linearity and Range

- The linearity range for Deflazacort was found to be 8-40 µg/ml and for Tamsulosin hydrochloride 1.6-8.0 µg/ml. Linearity data for Deflazacort and for Tamsulosin hydrochloride linearity data are shown below in table.

Table 1. Data of calibration curve for Deflazacort at ZCP of Tamsulosin hydrochloride 266.83 nm.		Data of calibration curve for Tamsulosin hydrochloride at ZCP of Deflazacort 242.25nm.	
CONC. (µg/ml)	**Amplitude of Deflazacort at 266.83nm**	**Conc. (µg/ml)**	**Amplitude of Tamsulosin HCl at 242.25nm**
8	-0.020	1.6	-0.012
16	-0.040	3.2	-0.022
24	-0.066	4.8	-0.031
32	-0.083	6.4	-0.040
40	-0.099	8.0	-0.052

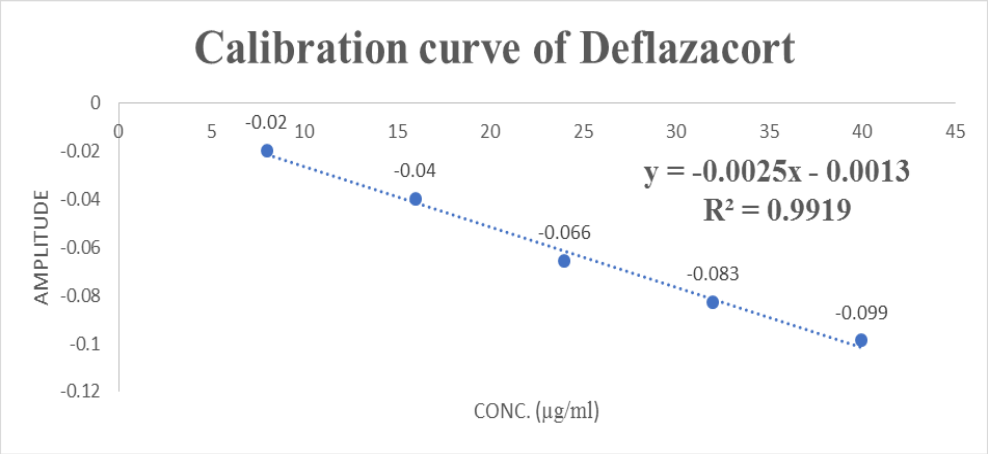


Figure 3: Calibration curve for Deflazacort amplitudes at ZCP of Tamsulosin hydrochloride (266.83nm).

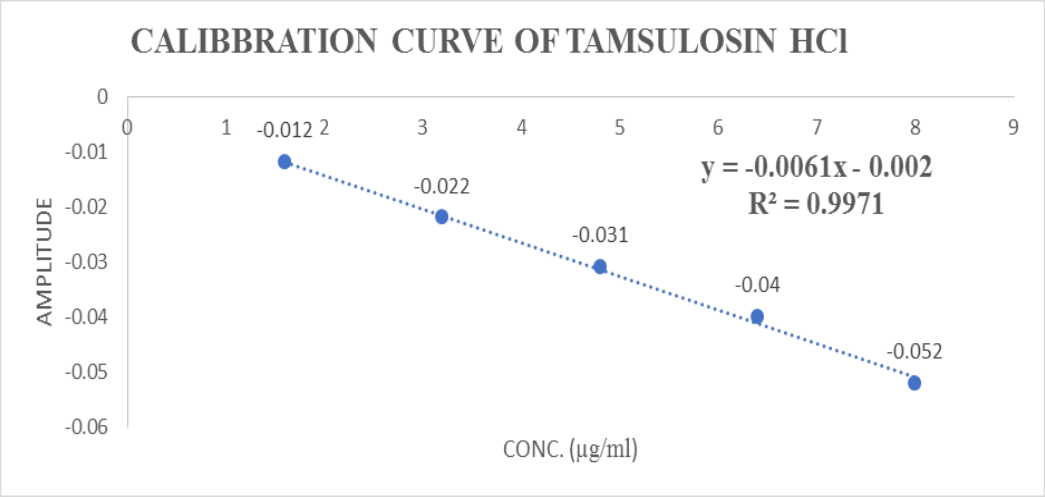


Figure 4: Calibration curve for Tamsulosin hydrochloride amplitudes at ZCP of Deflazacort (242.25nm).

Precision

A. Intraday Precision

- % RSD was found to 0.87 to 1.46% for Deflazacort and 1.33 to 1.84% for Tamsulosin hydrochloride.

Table 2: Intraday precision data for Deflazacort and Tamsulosin hydrochloride.

Conc. (µg/ml)		Amplitude		Mean ± SD (n=3)		% RSD	
Defla	Tam	Defla	Tam	Defla	Tam	Defla	Tam
16	3.2	0.04	0.0223	0.0397 ± 0.00058	0.0226 ± 0.00030	1.46	1.33
16	3.2	0.04	0.0229				
16	3.2	0.039	0.0226				
24	4.8	0.066	0.031	0.0663 ± 0.00058	0.0313 ± 0.00058	0.87	1.84
24	4.8	0.067	0.032				
24	4.8	0.066	0.031				
32	6.4	0.083	0.04	0.0830 ± 0.00100	0.0397 ± 0.00058	1.20	1.46
32	6.4	0.082	0.039				
32	6.4	0.084	0.04				

B. Interday Precision

- % RSD was found to 0.86 to 1.86% for Deflazacort and 1.42 to 1.71% for Tamsulosin hydrochloride.

Table 3: Interday precision data for Deflazacort and Tamsulosin hydrochloride.							
Conc. (µg/ml)		Amplitude		Mean ± SD (n=3)		% RSD	
Defla	Tam	Defla	Tam	Defla	Tam	Defla	Tam
16	3.2	0.038	0.0229	0.0383 ± 0.00058	0.0227 ± 0.00032	1.51	1.42
16	3.2	0.039	0.0223				
16	3.2	0.038	0.0228				
24	4.8	0.068	0.034	0.0673 ± 0.00058	0.0337 ± 0.00058	0.86	1.71
24	4.8	0.067	0.034				
24	4.8	0.067	0.033				
32	6.4	0.081	0.039	0.0823 ± 0.00153	0.0393 ± 0.00058	1.86	1.47
32	6.4	0.082	0.039				
32	6.4	0.084	0.040				

Accuracy

- The % recoveries were found within 101.01 to 101.11% and 99.24 and 100.93% for Deflazacort and Tamsulosin hydrochloride respectively.
- The results are shown in table.
- Recovery 98 to 102% justifies the accuracy of the method.

Table 4: % Recoveries for Deflazacort and Tamsulosin hydrochloride.								
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	30	0.4	-	-	30	0.41	-	-
	30	0.4	-	-	30.09	0.40	-	-
	30	0.4	-	-	31.92	0.41	-	-
			Mean ± SD		**30.67±1.08**	**0.41±0.045**		
80%	30	0.4	24	0.32	54.6	0.71	101.11	98.61
	30	0.4	24	0.32	54.7	0.73	101.30	101.39
	30	0.4	24	0.32	54.5	0.74	100.93	102.78
			Mean ± SD		**54.60±0.10**	**0.73±0.015**	**101.11**	**100.93**
100%	30	0.4	30	0.4	60.1	0.79	100.17	98.75
	30	0.4	30	0.4	60.9	0.82	101.50	102.50
	30	0.4	30	0.4	60.8	0.81	101.33	101.25
			Mean ± SD		**60.60±0.44**	**0.81±0.015**	**101.00**	**100.83**
120%	30	0.4	36	0.48	66.5	0.89	100.76	101.14
	30	0.4	36	0.48	67.8	0.86	102.73	97.73
	30	0.4	36	0.48	65.7	0.87	99.55	98.86
			Mean ± SD		**66.67± 1.05**	**0.87±0.015**	**101.01**	**99.24**

LOD and LOQ

- LOD found for Deflazacort and Tamsulosin hydrochloride was 0.117 and 0.189 respectively. LOQ found for Deflazacort and Tamsulosin hydrochloride was 0.354 and 0.572 respectively.

Table 5: Summary of Validation Parameters.			
Sr no.	**Parameter**	**Deflazacort**	**Tamsulosin hydrochloride**
1.	Linearity Range	8 to 40 µg/ml	1.6 to 8.0 µg/ml
2.	Correlation coefficient	0.9919	0.9971
3.	PRESICION		
	1. Intraday precision (% RSD)	0.87 to 1.46%	1.33 to 1.87%
	2. Interday precision (%RSD)	0.86 to 1.86%	1.42 to 1.71%
4.	Accuracy	99.95 to 100.42%	99.31 to 100.31%
5.	LOD	0.117 µg/ml	0.189 µg/ml
6.	LOQ	0.354 µg/ml	0.572 µg/ml

Assay Analysis

- The percentage purity of drugs in combined dosage form was found to be 99.66±0.44% for Deflazacort and 102.5±2.88 % for Tamsulosin hydrochloride.

Table 6: Analysis of pharmaceutical tablet dosage form.					
Tablet content taken eq. to (mg)		**Amount found (mg)**		**Assay (% Estimated) (Mean±SD (n=3))**	
Defla	**Tam**	**Defla**	**Tam**	**Defla**	**Tam**
30	0.40	29.9	0.41	99.66±0.44	102.5±2.88

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. Limit of detection and limit of quantification was found to be 0.117 µg/ml and 0.354 µg/ml, respectively for Deflazacort and Limit of detection and limit of quantification was found to be 0.189 µg/ml and 0.572 µg/ml, respectively for Tamsulosin hydrochloride, 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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