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"SIMULTANEOUS ESTIMATION OF MULTCOMPONENT FORMULATION BY UV VISIBLE SPECTROSCOPY & HPLC"

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ABSTRACT

The present study describes a simple, accurate, precise analytical method. The maximum absorbance was found to be at 210nm for Deflazacort and 232nm for Tamsulosin HCl. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 80:20 Methanol: Acetonitrile was fixed due to good symmetrical peaks and for good resolution.The percent recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 2.762 Deflazacort and 3.161 for Tamsulosin HCl. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests.

Keywords:

: RP- HPLC, UV- spectrophotometry, Deflazacort, Tamsulosine HCI

1. INTRODUCTION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The term „Chromatography" covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.

Chromatographic methods can be classified most practically according to the stationary and mobile phases, as shown in the

Table 1

Stationary phase	Mobile phase	Method
Solid	Liquid	Adsorption column, Thin-layer, Ion exchange chromatography, etc.
	Gas	Gas- Solid chromatography
Liquid	Liquid	Partition column, Thin-layer, HPLC, Paper chromatography, etc.
	Gas	Gas- Liquid Chromatography.

Table no. 1.1 : Classification of Chromatographic methods

TYPES OF HPLC TECHNIQUES:

Based on modes of chromatography:

- Normal phase chromatography
- Reverse phase chromatography Based on principle of separation:
- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography Based on elution technique:
- Isocratic separation
- Gradient separation Based on the scale of operation:
- Analytical HPLC
- Preparative HPLC

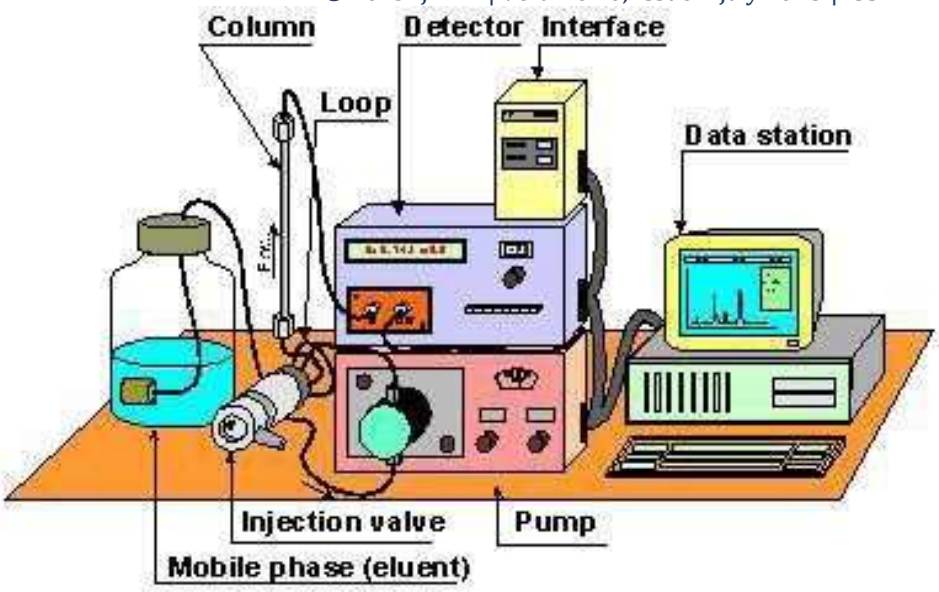

Figure 1.1 : Block diagram of High-Performance Liquid Chromatography System

2. AIMS AND OBJECTIVES

3. To estimate Deflazacort and Tamsulosin simultaneously in tablet dosage forms by uv visible spectroscopy method.
4. To validate the method according to ICH guidelines.
5. To develop selective, precise and sensitive method for the development and estimation of the combination
6. The analytical method so developed is to be validated.
7. The work involves use of analytical techniques such as UV-Visible Spectrophotometry and HPLC.
8. The method intends to provide suitable method for the quality control in pharmaceutical industry.
9. The analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceutical products formulated with single as well as multiple drugs.

3. DRUG PROFILE -

3.1 DEFLAZACORT

Synonym: AZACORT

Chemical Structure

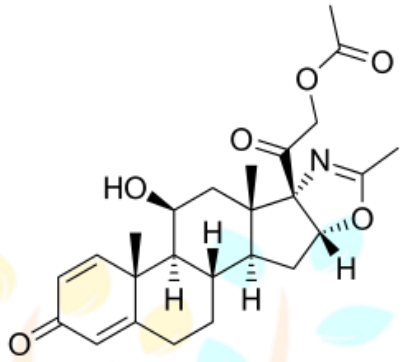

Fig. 3.1 Chemical Structure of Deflazacort

IUPAC:2-[(1S,2S,4R,8S,9S,11S,12S,13R)-11-hydroxy-6,9,13-trimethyl-16-oxo-5-oxa-7-azapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-6,14,17-trien-8-yl]-2-oxoethyl acetate

Molecular Framework: Aliphatic heteropolycyclic compounds

Molecular formula: $C_{25}H_{31}NO_6$ Molecular weight: 441.524 g/mol

Monoisotopic: 441.215137722

Physico-Chemical Properties

Appearance: White crystals or fine white powder.

Solubility: Soluble in water.

Melting Point: 255-258 °C

pKa: Strongest Acidic - 14.74 Strongest Basic- 0.48

logP: 2.56

Indication: it is a corticosteroid indicated for the treatment of Duchenne muscular dystrophy (DMD) in patients 5 years of age and older Pharmacology.

Mechanism of action: Deflazacort is a corticosteroid prodrug, whose active metabolite, 21-desDFZ, acts through the glucocorticoid receptor to exert anti- inflammatory and immunosuppressive effects. The precise mechanism by which deflazacort exerts its therapeutic effects in patients with DMD is unknown.

Pharmacodynamics: Clinical studies have indicated that the average potency ratio of deflazacort to prednisolone is 0.69–0.89 and 6 mg of deflazacort is equivalent to 5 mg of prednisolone. However, the therapeutic dosage ratio has been reported to range from 1:1.2 to 1:1.5 Due to the short pharmacokinetic half-life of its active metabolite, pharmacodynamic effects of deflazacort are of shorter duration than those of methylprednisolone and prednisolone.

Absorption: After oral administration in the fasted state, the median Tmax with deflazacort tablets or suspension is about 1 hour (range 0.25 to 2 hours). Food Effect: Co-administration of deflazacort tablets with a high-fat meal reduced Cmax by about 30% and delayed Tmax by one hour, relative to administration under fasting conditions, but there was no effect on the overall systemic absorption as measured by AUC. The administration of deflazacort with food or crushed in applesauce did not affect the absorption and bioavailability of deflazacort. Research findings: The pharmacokinetic parameters of 21-OH DFZ after the single oral administration of 6 mg, 12 mg and 24 mg DFZ tablets were as follows: (37.7 +/- 11.6), (61.5 +/- 17.7) and (123 +/- 23) ng x
mL(-1) for C(max); (1.90 +/- 0.32), (1.96 +/- 0.27) and (2.13 +/- 0.34) h for t1/2; (96.6
+/- 25.9), (190 +/- 44) and (422 +/- 107) ng x h x mL(-1) for AUC(0-14 h), respectively. After the multiple dose administration, the mean plasma concentration at steady-state C(av) was (7.00 +/- 1.66) ng x mL(-1) and the degree of plasma concentration fluctuation DF was 7.7 +/- 1.2.

Metabolism: Deflazacort is rapidly converted to the active metabolite 21-desDFZ by esterases after oral administration. 21-desDFZ is further metabolized by CYP3A4 to several other inactive metabolites.

Half Life: The plasma half-life is approximately 15 minutes; that for salicylate lengthens as the dose increases: doses of 300 to 650 mg have a half-life of 3.1 to 3.2 hours; with doses of 1 gram, the half-life is increased to 5 hours and with 2 grams it is increased to about 9 hours.

3.2 TAMSULOSIN

Synonym: (R)-5-(2-((2-(2-Ethoxyphenoxy)ethyl)amino)propyl)-2-ethoxybenzenesulfonamide

Chemical Structure:

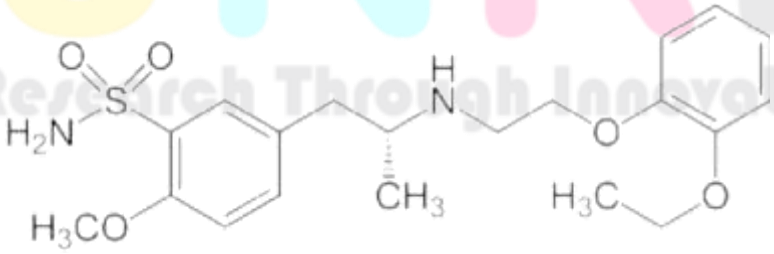

Fig. 3. 2 Chemical structure of Tamsulosin

IUPAC:5-[(2R)-2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl]-2-ethoxybenzene-1sulfonamide

Molecular Framework: Aromatic homo monocyclic compounds

Molecular formula: $C_{20}H_{28}N_2O_5S$ **Molecular weight:** 408.512 g/mol **Monoisotopic**: 408.171892706 **Physico**

Chemical Properties

Appearance: White crystals or fine white powder.

Solubility: Soluble in water.

Melting Point: 226-228°C

Boiling point: 595.5 ºC at 760mmHg

pKa: Strongest Acidic - 9.93

Strongest Basic - 9.28 **logP**: - 2.3

Indication: Used in the treatment of signs and symptoms of benign prostatic hyperplasia (reduction in urinary obstruction and relief of associated manifestations such as hesitancy, terminal dribbling of urine, interrupted or weak stream...etc.)

Pharmacology-

Mechanism of action: Tamsulosin is a selective antagonist at alpha-1A and alpha-1B- adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men.

Pharmacodynamics: Tamsulosin, a sulfamoyl phenethyl amine-derivative alpha- adrenoceptor blocker with enhanced specificity for the alpha-adrenoceptors of the prostate, is commonly used to treat benign prostatic hyperplasia (BPH). The drug is commercially available in a racemic mixture of 2 isomers, and is pharmacologically related to doxazocin, prazosin, and terazosin. However, unlike these drugs, tamsulosin has a higher affinity for the alpha-1A- adrenergic receptors, which are located in vascular smooth muscle. Studies show that tamsulosin has about 12 times greater affinity for alpha-1 adrenergic receptors in the prostate than those in the aorta, which may result in a reduced incidence of adverse cardiovascular effects.

Absorption:

Absorption of tamsulosin HCI from capsules 0.4 mg is essentially complete (>90%) following oral administration under fasting conditions.

Metabolism: Tamsulosin HCI is extensively metabolized by cytochrome P450 enzymes in the liver, however, the pharmacokinetic profile of the metabolites in humans has not been established.

Route of elimination: Tamsulosin hydrochloride is extensively metabolized by cytochrome P450 enzymes in the liver and

less than 10% of the dose is excreted in urine unchanged. The metabolites of tamsulosin hydrochloride undergo extensive conjugation to glucuronide or sulfate prior to renal excretion. On administration of the radiolabeled dose of tamsulosin hydrochloride to four healthy volunteers, 97% of the administered radioactivity was recovered, with urine (76%) representing the primary route of excretion compared to feces (21%) over 168 hours.

Half- life: 5-7 hour

4. PLAN OF WORK:

The experimental work has been planned as follows: Review of the literature for Deflazacort and Tamsulosin regarding their physical and chemical properties, various analytical methods that were conducted for Deflazacort and Tamsulosin forms the basis for development of new analytical UV visible spectroscopy method Deflazacort and Tamsulosin combination.

DEVELOPMENT OF THE METHOD BY RP- HPLC

1. Selection of the solvent to be used as diluents and mobile phase:

Choosing the suitable solvent in which the drug is soluble and stable. They must be easily available, economical and of the HPLC grade

2. Selection of Mobile phase:

For the mobile phase, the first variable to be decided is whether an organic or aqueous eluent should be used. With the RP-HPLC analysis, either an aqueous eluent or a very polar organic solvent such as methanol or acetonitrile should be fixed. If the K' values are too large with an aqueous solvent, organicsolvent should be tried. If the K' value are too low with organic solvent the separation should be attempted using a mixture of two solvents with various properties.

i. K'-capacity factor is a measurement of the degree where the peak of the interest is located with respect to void volume, i.e. Elution time of non-retained components. Generally the value of K' is > 2.

ii. If a buffer is used, the p^H as well as ionic strength of the buffer can be tried.

3. In order to select the wavelength to carry out the analysis, critical examination of the Ultraviolet absorbance spectra of the drug should be done.

4. A perfect study of the structure of drug and its physicochemical properties to select the chromatographic parameters.

5. Selection of method for quantitative chromatographic analysis. Determination of working concentration range.

6. Validation of the developed method by following ICH guidelines.

5. MATERIALS AND METHODS

5.1 Material

Sr. No.	Name Of Materials	Supplier
1	Deflazocart	Swapnroop Drug and Pharmaceutical, Aurangabad
2	Tamsulosin Hcl	Swapanroop Drug and Pharmaceutical, Aurangabad
3	Defcort tm	Macleods Pharmaceuticals Pvt Ltd.
4	Methanol (HPLC Grade)	Merck, Hyderabad
5	Acetonitrile (HPLC Grade)	Merck, Hyderabad

Table no. 5.1 List of Matarial

5.2 Equipment

Sr. No.	**Name Of Equipment**	**Make**
1	High Performance Liquid Chromatography	Waters 2695 (Compact system consisting Inertsil-C_{18} ODS Column and PDA detector)
2	UV-Visible Spectrophotometer	Systronics 119
3	FTIR Spectrophotometer	Agilent Technologies, Carry 630 FTIR
4	Sonicator	FAST CLEAN
5	Electronic Balance	SARTORIOUS
6	Melting Point	Esico International

Table no. 5.2 List of Equipment

6. RESULT AND DISCUSSION

6.1 SELECTION OF WAVE LENGTH:

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Dalfampridine shows λ max at 225nm.

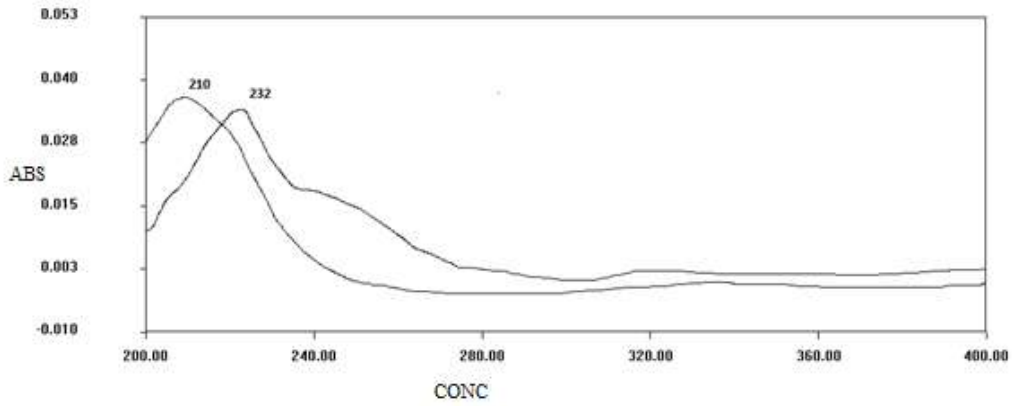

Fig. 6.1. UV Spectrum of Drug combination

6.2 Method Validation by UV Spectrophotometer: -

6.2.1 LINEARITY OF TEST METHOD:

A Series of solutions are prepared using Deflazacort and Tamsulosin HCL working standards at concentration levels from 4-24µg/ml.

ACCEPTANCE CRITERIA:

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

LINEARITY (Deflazacort):

From the standard stock solution, a series of solutions were prepared at Concentration range 4-24µg/ml.

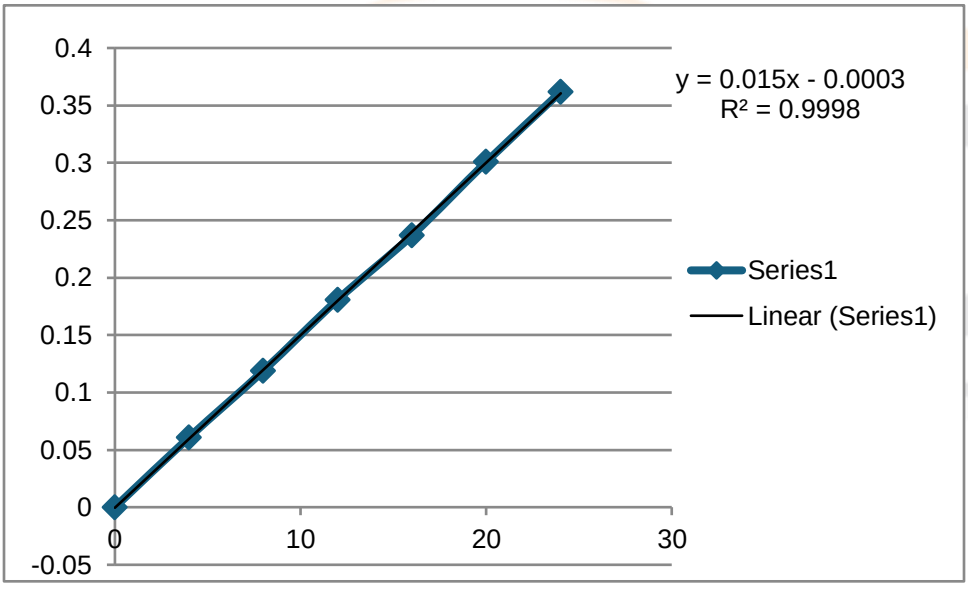

Fig. 6.2. Calibration curve for deflazacort

The graph is plotted concentration versus absorbance & the linear curve was obtained.

The Correlation coefficient was calculated from obtained results.

Concentration(µg/ml)	Absorbance	Correlation Coefficient
4	0.061	
8	0.119	
12	0.181	0.999
16	0.237	
20	0.301	
024	0.362	

Table no. 6.1 . -Data for linearity test (Deflazacort)

LINEARITY (Tamsulosin HCL):

From the standard stock solution, a series of solutions were prepared at Concentration range 4-24µg/ml.

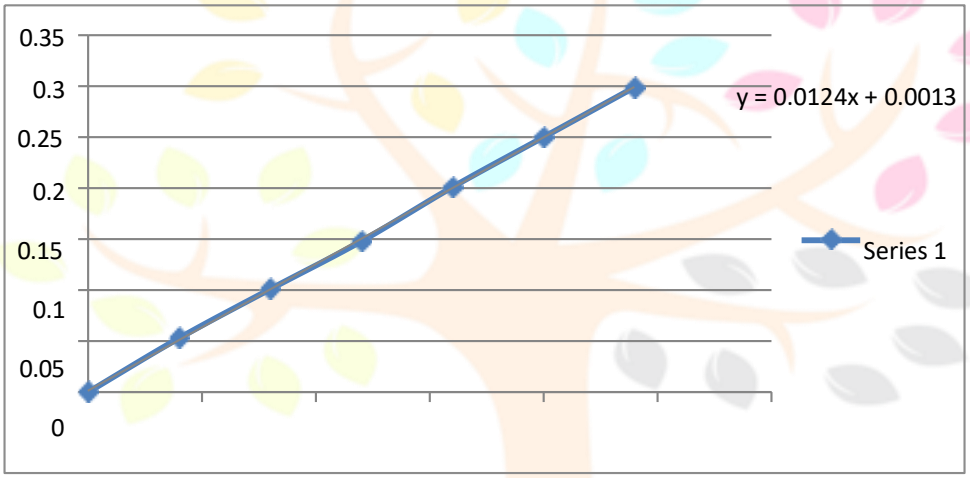

Fig.6. 3 Calibration curve for Tamsulosin HCL

The graph is plotted concentration versus absorbance & the linear curve was obtained.

TheCorrelation coefficient was calculated from obtained results.

CONCENTRATION(MG/ML)	ABSORBANCE	CORRELATION COEFFICIENT
4	0.053	
8	0.101	
12	0.148	0.999
16	0.201	
20	0.250	
24	0.299	

Table no. 6.2 -Data for linearity test (Tamsulosin HCL)

6.2.2 PRECISION:

1 REPEATABILITY:

a. System precision: Standard solution prepared as per test method and injected five times.

b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

ACCEPTANCE CRITERIA:

The % relative standard deviation of individual Deflazacort and Tamsulosin HCL, from the six units should be not more than 2.0%.

The individual assays of Deflazacort and Tamsulosin HCL should be not less than 98% and not more than 102.0%.

(a) System precision (Deflazacort):

	Sr.no	Abs	%Assay
Concentration 12ppm	1	0.182	101.11
	2	0.180	100
	3	0.181	100.55
	4	0.181	100.55
	5	0.181	100.55
Statistical Analysis	**Mean**	0.181	100.55
	SD	0.0007	0.39
	% RSD	0.39	0.39

Table no. 6.3. Data of Repeatability (System precision)

(B) System precision (Tamsulosin HCL):

	Sr.no	Absorbance	%Assay
Concentration 12ppm	1	0.146	100.67
	2	0.147	101.38
	3	0.146	100.69

	4	0.146	100.69
	5	0.146	100.69
Statistical Analysis	**Mean**	0.146	100.92
	SD	0.0005	0.358
	% RSD	0.352	0.355

Table no. 6.4. Data of Repeatability (System precision)

METHOD PRECISION (DEFLAZACORT):

	S.no	**Abs**	**%Assay**
Concentration 12ppm	1	0.181	100.55
	2	0.180	100
	3	0.180	100
	4	0.180	100
	5	0.180	100
Statistical Analysis	**Mean**	0.180	100
	SD	0.0004	0.248
	% RSD	0.248	0.0024

Table no. 6.5. Data of Repeatability (Method precision)

(D) METHOD PRECISION (TAMSULOSIN HCI):

	S.no	**Abs**	**%Assay**
Concentration 12 ppm	1	0.145	100.
	2	0.146	100.69

	3	0.146	100.69
	4	0.146	100.69
	5	0.146	100.69
Statistical Analysis	**Mean**	0.145	100.46
	SD	0.0005	0.358
	% RSD	0.354	0.356

Table no. 6.6. Data of Repeatability (Method precision)

INTERMEDIATE PRECISION (ANALYST TO ANALYST VARIABILITY):

A study was conducted by two analysts as per test method

ACCEPTENCE CRITERIA:

The individual assays of Deflazacort and Tamsulosin HCL should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Intermediate precision: For Analyst 1 Ref: Tables 5 & 6 (Method precision).

	S.no	**Abs**	**%Assay**
Concentration 12ppm	1	0.181	100.55
	2	0.181	100.55
	3	0.180	100
	4	0.181	100.55
	5	0.180	100
Statistical Analysis	**Mean**	0.180	100.33
	SD	0.0005	0.304
	% RSD	0.303	0.303

Table no. 6.7. Data of Intermediate precision (Analyst 2) for Deflazacort

	Sr.no	**Absorbance**	**%Assay**
Concentration 12ppm	1	0.145	100
	2	0.146	100.69
	3	0.145	100
	4	0.146	100.69
	5	0.145	100
Statistical	**Mean**	0.145	100.34

Analysis	**SD**	0.0005	0.380
	% RSD	0.376	0.379

Table no. 6.8. Data of Intermediate precision (Analyst 2) for Tamsulosin HCL

6.2.3 ACCURACY (RECOVERY):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Deflazacort and Tamsulosin HCL into each volumetric flask for each spike level to get the concentration of Deflazacort and Tamsulosin HCL equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Deflazacort and Tamsulosin HCL were calculated.

ACCEPTANCE CRITERIA:

The mean % recovery of the Deflazacort and Tamsulosin HCL at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

Concentration	Abs	Amount added(ppm)	Amount found(ppm)	% Recovery	Statistical Analysis of % Recovery	
50%	0.118	8	7.8	98.33	**MEAN**	98.88
50%	0.119	8	7.9	99.16		
50%	0.119	8	7.9	99.16	**%RSD**	0.486
100 %	0.180	12	12	100	**MEAN**	100.18
100 %	0.181	12	12.06	100.55		
100%	0.180	12	12	100	**%RSD**	0.320
150%	0.238	16	15.86	99.16	**MEAN**	99.30
150%	0.239	16	15.93	99.58		
150%	0.238	16	15.86	99.16	**%RSD**	0.242

Table no. 6.9. Data of Accuracy (Deflazacort)

Concentration	Abs	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50%	0.098	8	8.08	101.04	**MEAN**	100.69
50%	0.097	8	8	100		
50%	0.098	8	8.08	101.04	**%RSD**	0.597
100 %	0.146	12	12.08	100.69	**MEAN**	100.92
100 %	0.146	12	12.08	100.69		
100%	0.147	12	12.16	101.38	**%RSD**	0.397
150%	0.196	16	16.25	101.56	**MEAN**	101.38
150%	0.195	16	16.16	101.04		
150%	0.196	16	16.25	101.56	**%RSD**	0.296

Table no. 6.10. Data of Accuracy (Tamsulosin HCL)

6.2.4 RUGGEDNESS OF TEST METHOD:

System to system variability:

System to system variability study was conducted on different UV-VISIBLE systems, under similar conditions at different times. Five samples were prepared and each was analyzed as per test method.

Comparison of both the results obtained on two different UV-VISIBLE systems, shows that the assay test method are rugged for System to system variability.

ACCEPTANCE CRITERIA:

The % relative standard deviation of Deflazacort and Tamsulosin HCL from the six sample preparations should be not more than 2.0%

The % assay of Deflazacort and Tamsulosin HCL should be between 98.0%-102.0%.

Ruggedness: For system 1 Refer: (Method precision).

Sr .NO:	Absorbance DEF	Assay % of DEF	Assay % of TAM	Absorbance of TAM
1	0.180	100	101.38	0.147
2	0.179	99.44	100.69	0.146
3	0.180	100	101.38	0.147
4	0.180	100	101.38	0.147
5	0.180	100	101.38	0.147
Mean	0.180	100	100.15	0.146
%RSD	0.351	0.351	0.354	0.352

Table no. 6.11. Data of system to system variability (Deflazacort & Tamsulosin HCL) SYSTEM 2

6.2.5 LIMIT OF DETECTION AND QUANTITATION (LOD and LOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

LOD= $\frac{3.3\ \sigma}{S}$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

LOQ = $\frac{10\ \sigma}{S}$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Detection limit was determined based on the standard deviation of the response & slope of Calibration curve.

$$\text{LOD (DEF)} = \frac{3.3\times SD}{\text{Slope}}$$

$$= \frac{3.3\times 0.0011}{0.015}$$

$$= 0.242$$

$$\text{LOQ (DEF)} = \frac{10\times SD}{\text{Slope}}$$

$$= \frac{10\times 0.0011}{0.015}$$

$$= 0.7333$$

$$\text{LOD (TAM HCL)} = \frac{3.3\times SD}{\text{Slope}}$$

$$= \frac{3.3\times 0.0007}{0.012}$$

$$= 0.192$$

$$\text{LOQ (TAM HCL)} = \frac{10\times SD}{\text{Slope}}$$

$$= \frac{10\times 0.0007}{0.012}$$

$$= 0.583$$

6.3 HPLC METHOD DEVELOPMENT AND VALIDATION

6.3.1 METHOD DEVELOPMENT

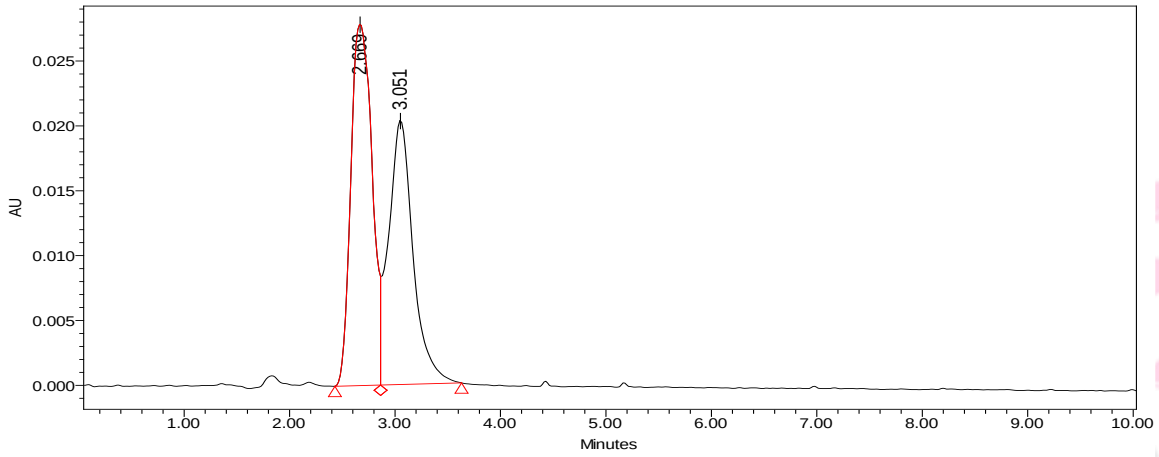

Fig.6.1. Chromatogram of Trial 1

Inference : Two peaks are not seperated, completely merged and RT of DEF (2.689) & TAM HCL (3.061)

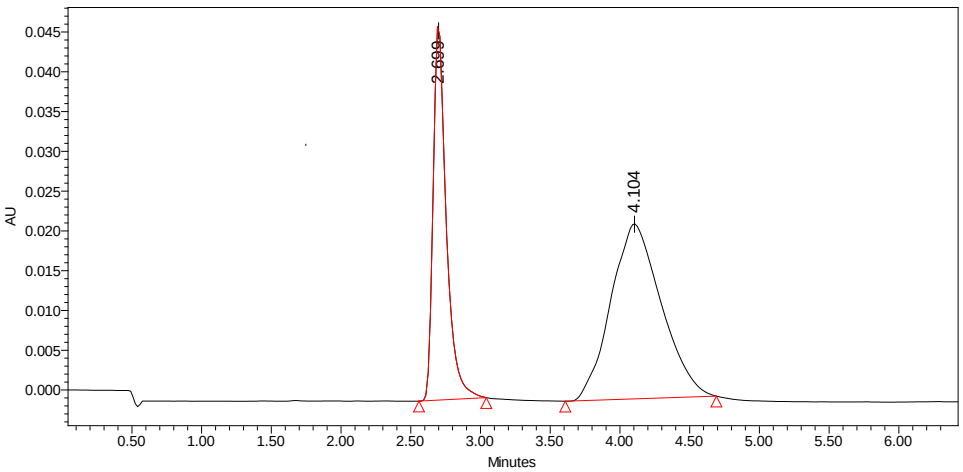

Fig 6.2. Chromatogram of Trial 2

Inference: Peaks shapes are not good And RT of DEF (2.699) &TAM HCL (4.104)

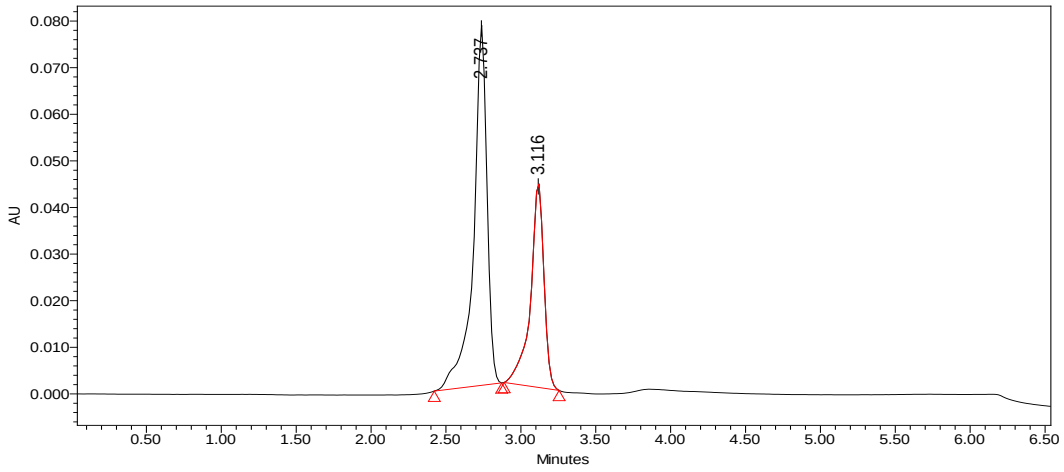

Fig.6.3. Chromatogram of Trial 3

Inference : peaks are not seperated completely and RT of DEF (2.707) & TAM HCL(3.116)

OPTIMIZED METHOD:

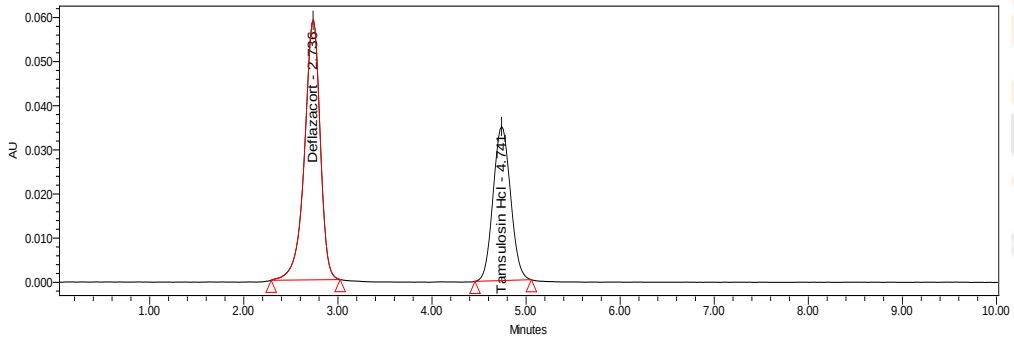

Fig. 6.4 . Chromatogram of standard

Inference: Got chromatogram at RT's of 2.736min to Deflazacort and 4.741min to Tamsulosin HCL

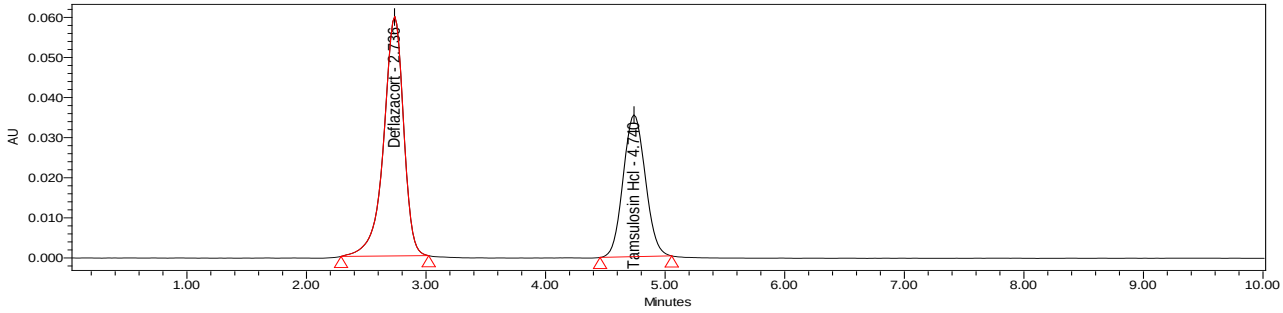

Fig.6.5. Chromatogram of sample

Inference: Got same chromatogram with same RT values as of standard.

6.4 VALIDATION DATA

SYSTEM SUITABILITY:

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.736	4037216	5368.357111	1.496148
2	2.736	4063397	5441.422408	1.471228
3	2.738	4115511	5413.470928	1.484832
4	2.736	4126557	5357.317249	1.466473
5	2.738	4195611	5398.478881	1.506952
Mean	2.7368	4107658	5395.809	1.485127
SD	0.001095	61391.54	-------	-------
% RSD	0.040026	1.494563	-------	-------

Table no. 6.12. Data of System Suitability for Deflazacort

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.740	2164732	6648.722084	1.119216
2	4.742	2161848	6673.911816	1.142210
3	4.743	2198427	6630.743655	1.167058
4	4.741	2231236	6778.292084	1.140170
5	4.741	2254490	6687.924039	1.132946
Mean	4.7414	2202147	6683.919	1.14032
SD	0.00114	40693.03	-------	-------
% RSD	0.024047	1.84788	-------	-------

Table no. 6.13. Data of System Suitability for Tamsulosin HCl

SPECIFICITY:

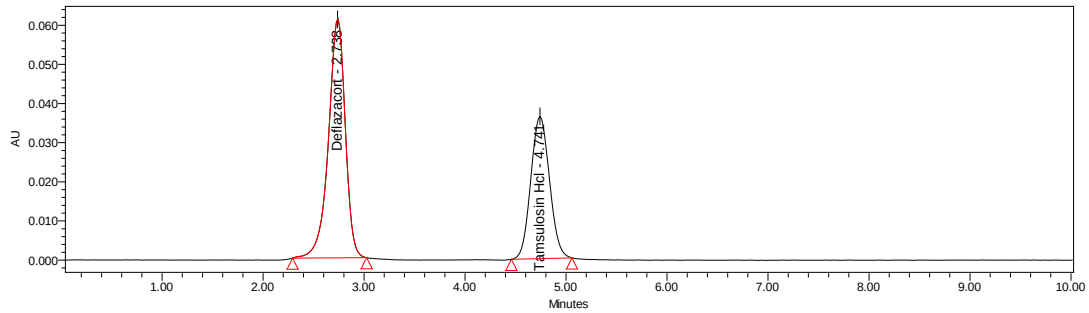

Fig. 6.6. Chromatogram of standard

Inference: Got a peak for standard at an Rt of 2.738min for Deflazacort and 4.741min for Tamsulosin HCL

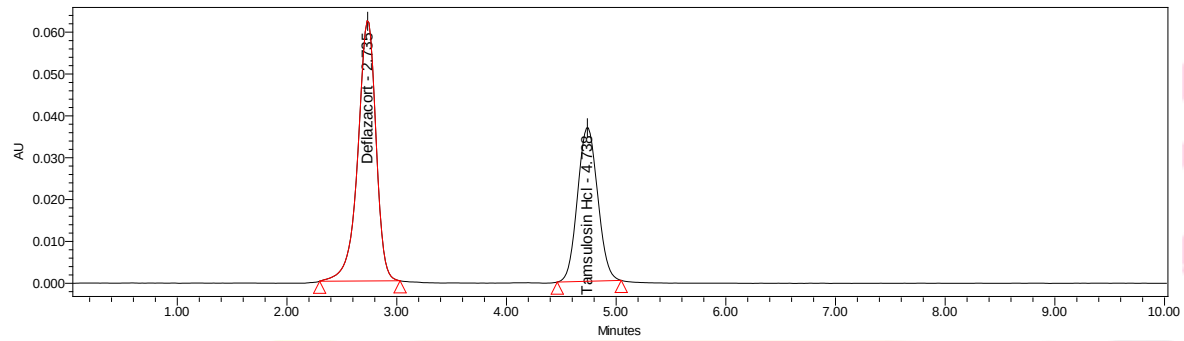

Fig. 6.7. Chromatogram of sample

Inference: Got a peak for sample at an Rt of 2.735min for Deflazacort and 4.738min for Tamsulosin HCL

PRECISION:

Repeatability:

(a) System precision:

	Injection	**Peak Areas of Deflazacort**	**%Assay**

Concentration 40ppm	1	4037216	98.86
	2	4063397	99.86
	3	4115511	100.56
	4	4126557	99.36
	5	4195611	99.54
Statistical Analysis	**Mean**	4107658	99.636
	SD	61391.54	0.630777
	% RSD	1.494563	0.633082

Table no. 6.14. Data of Repeatability (System precision) for Deflazacort

	Injection	**Peak Areas of Tam HCL**	**%Assay**
Concentration 40ppm	1	2164732	98.66
	2	2161848	99.30
	3	2198427	101.53
	4	2231236	100.53
	5	2254490	99.98
Statistical Analysis	**Mean**	2202147	100.00
	SD	40693.03	1.107678
	% RSD	1.84788	1.10

Table no. 6.15. Data of Repeatability (System precision) for Tamsulosin HCL

(b)Method precision:

Method precision:

	Injection	Peak Areas of Deflazacort	%Assay
Concentration 40ppm	1	4196762	98.54
	2	4237539	99.58
	3	4219201	98.86
	4	4278401	99.56
	5	4235847	99.86
	6	4219201	99.06
Statistical Analysis	**Mean**	4231159	99.24333
	SD	27435.85	0.503812
	% RSD	0.648424	0.507653

Table no. 6.16. Data of Repeatability (Method precision) for Deflazacort

	Injection	Peak Areas of Tamsulosin HCL	%Assay
Concentration 40ppm	1	2245703	99.55
	2	2291408	99.88
	3	2278639	99.40
	4	2239286	100.30
	5	2267407	100.53
	6	2278639	99.28
Statistical Analysis	**Mean**	2266847	99.82333
	SD	20436.91	0.505754
	% RSD	0.901557	0.506649

Table no.6.17. Data of Repeatability (Method precision) for Tamsulosin HCL

Intermediate precision:

	Injection	Peak Areas of Deflazacort	%Assay
	1	4219201	99.78
	2	4237216	99.95

Concentration 40ppm	3	4235847	100.00
	4	4195611	98.55
	5	4226557	101.50
	6	4237216	101.37
Statistica l Analysis	**Mean**	4225275	100.19
	SD	16219.94	1.100898
	% RSD	0.383879	1.09

Table no. 6.18. (i) Data of Intermediate precision (Analyst 2) for Deflazacort

	Injection	**Peak Areas of Tamsulosin HCL**	**%Assay**
Concentrat ion 40ppm	1	2278639	99.99
	2	224732	99.66
	3	2267407	101.53
	4	2254490	99.98
	5	2231236	99.97
	6	2267407	101.10
Statistical Analysis	**Mean**	2260652	100.37
	SD	16338.36	0.753536
	% RSD	0.722728	0.75

Table no.6.19. (ii)Data of Intermediate precision (Analyst 2) for Tamsulosin HCL

ACCURACY (RECOVERY)

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection 1	20	20.15	100.75	**MEAN**	99.69333
50% Injection 2	20	19.86	99.31		
50% Injection 3	20	19.80	99.02	**%RSD**	0.92

100 % Injection 1	40	39.88	99.70	**MEAN**	99.83333
100 % Injection 2	40	40.12	100.30		
100% Injection 3	40	39.80	99.50	**%RSD**	0.41
150% Injection 1	60	60.12	100.21	**MEAN**	99.97333
150% Injection 2	60	59.76	99.61		
150% Injection 3	60	60.06	100.10	**%RSD**	0.31

Table no.6.20. (i) Data of Accuracy for Deflazacort

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Injection 1	20	20.04	100.22	**MEAN**	100.06
50% Injection 2	20	19.97	99.85		
50% Injection 3	20	20.02	100.11	**%RSD**	0.18
100 % Injection 1	40	40.01	100.02	**MEAN**	100.04
100 % Injection 2	40	40.05	100.14		
100% Injection 3	40	39.98	99.96	**%RSD**	0.091
150% Injection 1	60	60.08	100.14	**MEAN**	100.02
150% Injection 2	60	59.97	99.96		
150% Injection 3	60	59.98	99.98	**%RSD**	0.09

Table no.6.21. (ii)Data of Accuracy for Tamsulosin HCL

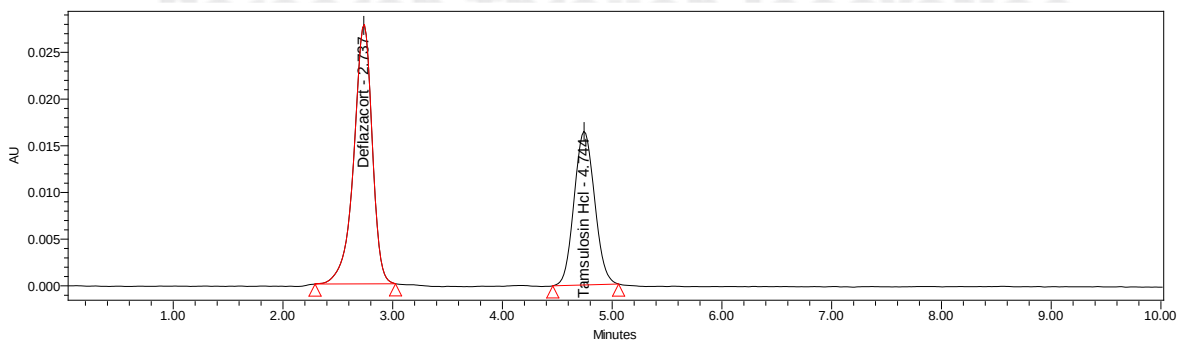

Fig no :6.8 Chromatograms for accuracy (50%)

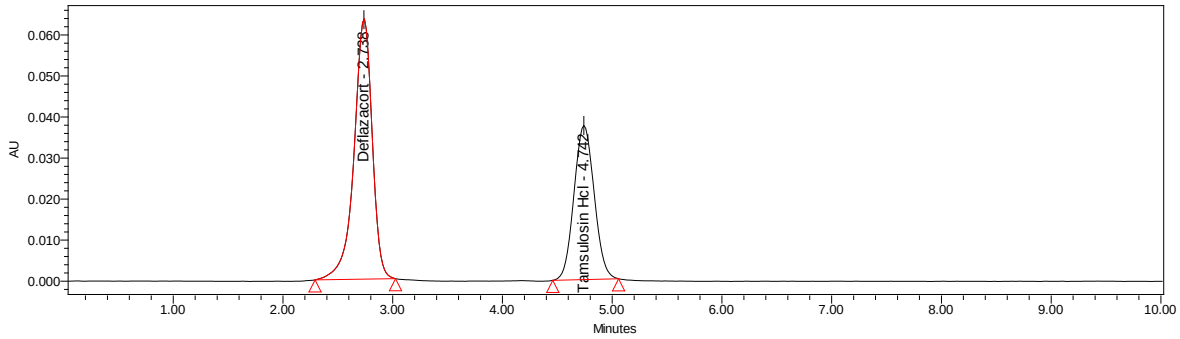

Fig no 6.9 : Chromatograms for accuracy (100%)

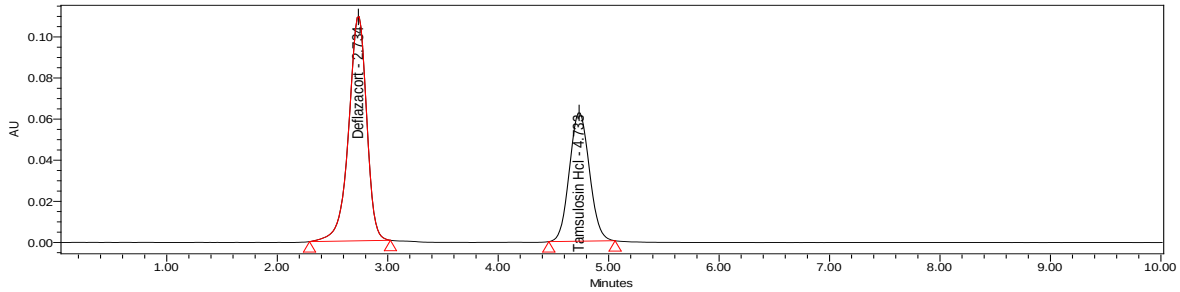

Fig no:6.10 chromatograms For Accuracy (150%)

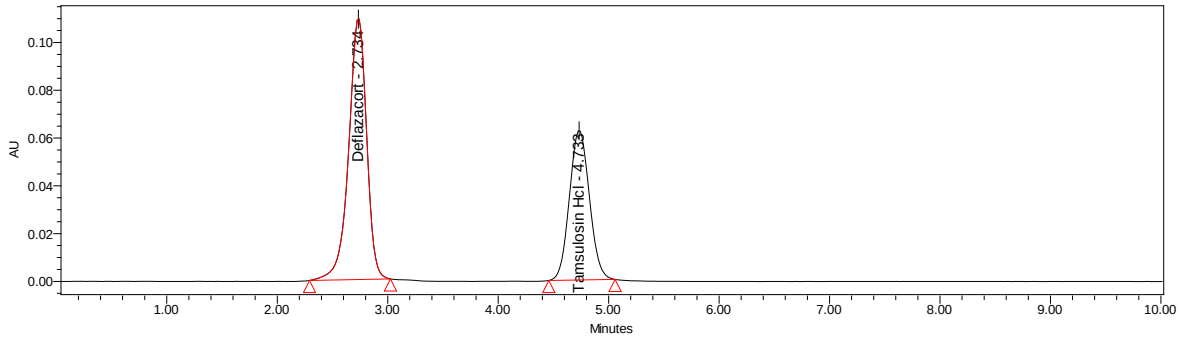

Fig no : 6.11 Chromatogram for standard 2 (150% accuracy)

LINEARITY:

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	10893
20	2110652	y-Intercept	69769
30	3250149	Correlation Coefficient	0.999
40	4307216		
50	5320468		
60	6427385		

70	7452108		
80	8769527		

Table no. 6.22. (i) Data of Linearity (Deflazacort)

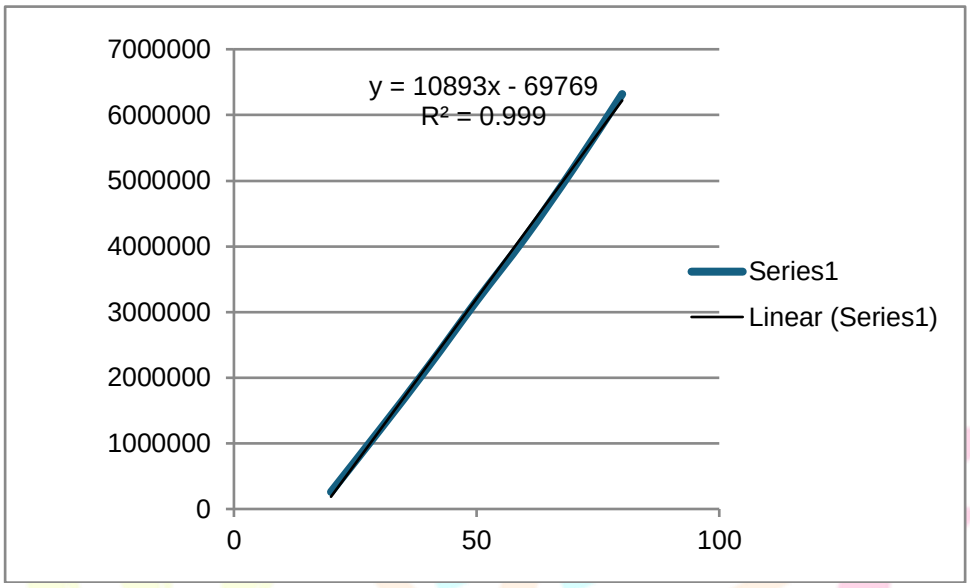

Fig: 6.12 (a) Linearity Plot (Concentration Vs Response) of Deflazacort

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	10310
20	560960	y-Intercept	540.63
30	1602034	Correlation Coefficient	0.999
40	2164732		
50	3171457		
60	4138838		
70	5276830		
80	6523097		

Table no.6.23. (ii) Data of Linearity (Tamsulosin HCL)

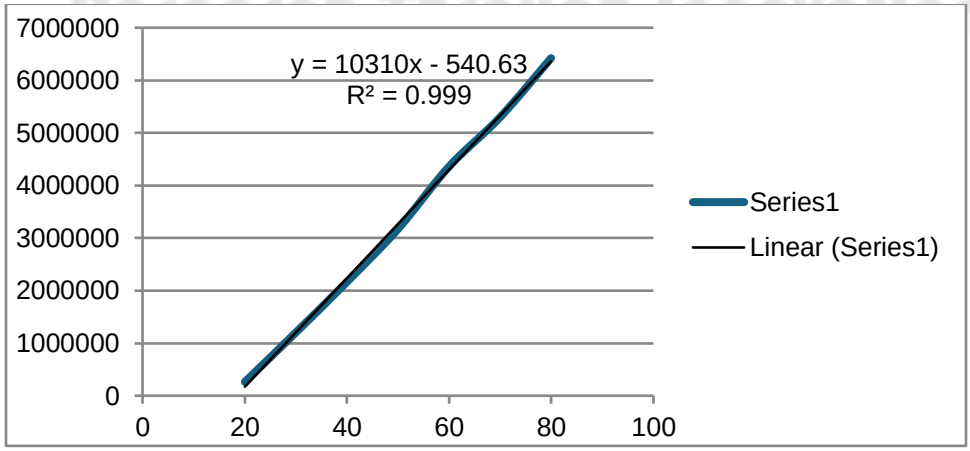

Fig: 6.13 (b) Linearity Plot (Concentration Vs Response) of Tamsulosin HCL

Ruggedness:

a) System to System variability:

S.NO:	Peak area	Assay % of Deflazacort	Peak area	Assay % of Tamsulosin Hcl
1	4063397	99.98	2161848	98.65
2	4115511	99.30	2198427	98.63
3	4126557	98.60	2231236	98.86
4	4195611	99.30	2254490	98.52
5	4196762	98.55	2245703	98.63
6	4195541	98.73	2238426	98.55
Mean	4148897	99.07667	2221693	98.64
%RSD	1.345138	0.56	1.578192	0.12

Table no. 6.24. (i)Data of system to system variability (Deflazacort) &(Tamsulosin HCl) System-2

Robustness:

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	4037216	1.496148		4196762	1.47228		4219201	1.485372

	4063397	1.471228		4237539	1.496148		4237216	1.499385
	4115511	1.506952		4219201	1.486521		4235847	1.498063
	4126557	1.484832		4278401	1.476089		4195611	1.494662
	4195611	1.466473		4235847	1.425063		4126557	1.497630
Avg	4107658	1.482517	**Avg**	4231159	1.47122	**Avg**	4225275	1.495022
SD	61391.54	0.013609	**SD**	27435.85	0.027435	**SD**	16219.94	0.005664
%RSD	1.494563	0.99	**%RSD**	0.648424	1.86	**%RSD**	0.383879	0.378877

Table no.6.25. Data for Effect of variation in flow rate (Deflazacort):

Flow 0.8 ml	**Std Area**	**Tailing factor**	**Flow 1.0 ml**	**Std Area**	**Tailing factor**	**Flow 1.2 ml**	**Std Area**	**Tailing factor**
	2245703	1.119216		2278639	1.114878		2161848	1.115372
	2291408	1.142210		2164732	1.114354		2198427	1.119385
	2278639	1.167058		2367407	1.113805		2231236	1.112055
	2239286	1.140170		2254490	1.118590		2254490	1.114561
	2267407	1.132946		2231236	1.119986		2245703	1.114621
Avg	4231159	1.14032	**Avg**	2266847	1.116323	**Avg**	2221693	1.115199
SD	27435.85	0.017452	**SD**	20436.91	0.002778	**SD**	55808.37	0.002654
%RSD	0.648424	1.530435	**%RSD**	0.901557	0.248825	**%RSD**	1.345138	0.237999

Table no. 6.26. Data for Effect of variation in flow rate (Tamsulosin HCL)

Chromatograms of robustness

a) Effect of variation of flow rate (for 0.8 ml/min flow)

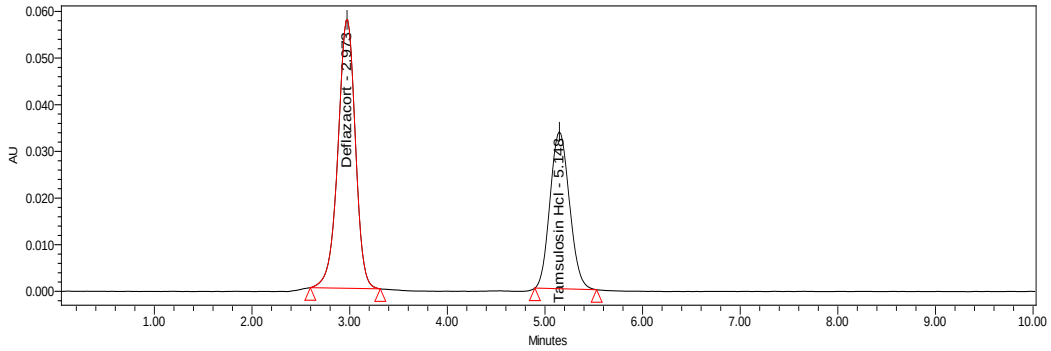

Fig. 6.14. Chromatogram for robustness standard - 1

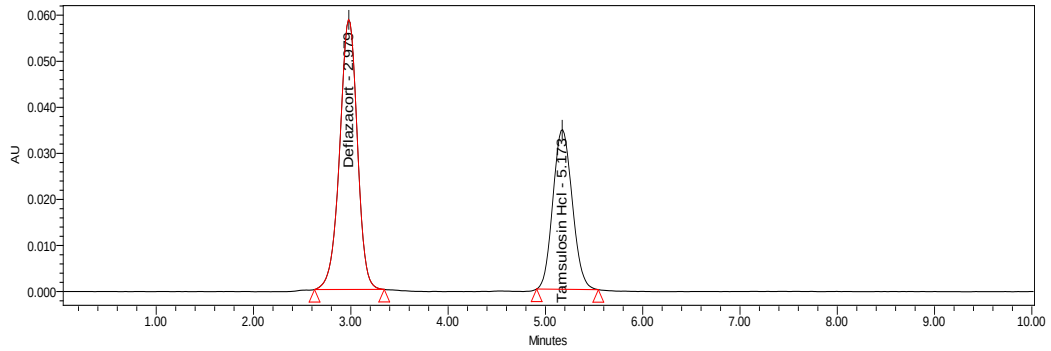

Fig. 6.15. Chromatogram for robustness standard - 2 Chromatograms for 1ml/min

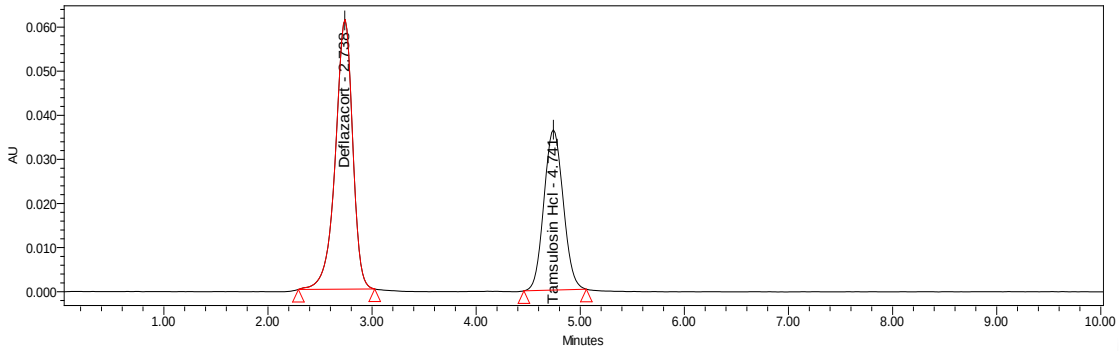

Fig. 6.16. Chromatogram for robustness standard – 1

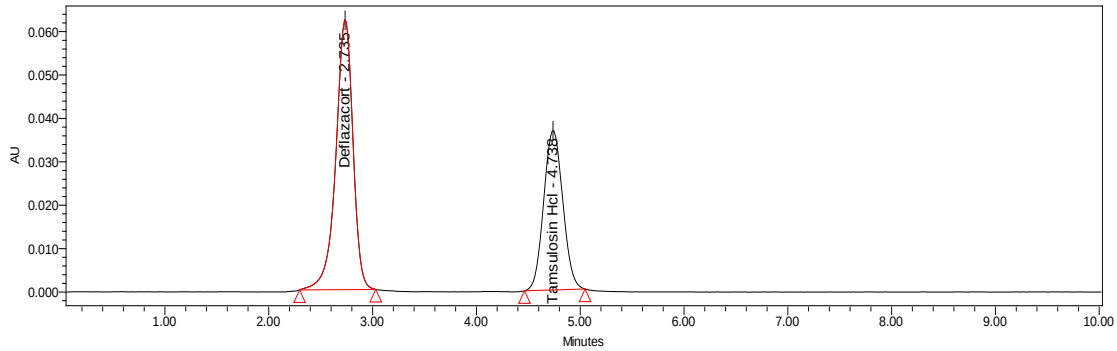

Fig. 6.17. Chromatogram for robustness standard – 2 Chromatograms for 1.2ml/min

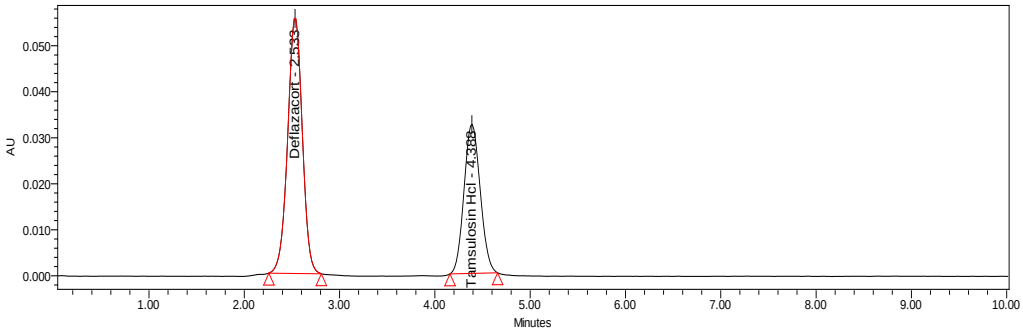

Fig. 6.18. Chromatogram for robustness standard – 1

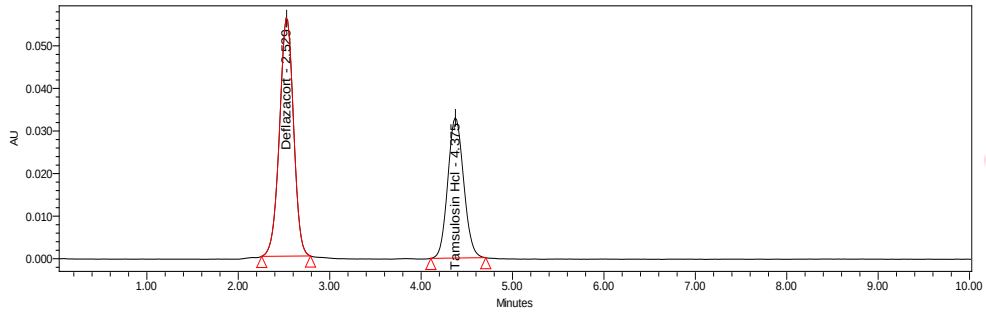

Fig. 6.19. Chromatogram for robustness standard – 2

LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ):
(i) Deflazacort:

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$\frac{3.3 \times 1621.94}{10893} = 0.491$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$10 \times 1621.94$$

= = 1.48 ----------

10893

(ii) Tamsulosin HCL;

LOD = 3.3 σ

S

3.3×1633.36

= = 0.522 ----------

10310

LOQ = 10 σ

S

10×1633.36

= = 1.58 ----------

10310

7.CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 210nm for Deflazacort and 232nm for Tamsulosin HCl. Common wavelength will be 225nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18}, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of 80:20 Methanol: Acetonitrile was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 2.762 Deflazacort and 3.161 for Tamsulosin HCl. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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