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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND GRANISETRON

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ABSTRACT

During treatment of cancer by chemotherapeutic agents one of the major side effect is induction of emesis in patients. There is always need of simultaneous administration of antiemetic drugs while patients on chemotherapeutic treatment. So there is necessity to develop methods for simultaneous estimation of anticancer and antiemetic. In this work simple, accurate, precise method for the simultaneous estimation of dutasteride (anticancer) and granisetron (antiemetic) was developed and validated by UV-visible spectroscopy using solvent methanol: water. Maximum absorption shown by dutasteride is at 246 nm whereas for granisetron it is 306 nm. The validation of uv-visible spectroscopic methods was carried out in accordance with ICH guideline.

INTRODUCTION

Dutasteride (Fig. 1) is a 4-azasteroid compound, chemically it is 5a, 17b-N-{2,5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide. It is a selective inhibitor of both type1 and type 2 isoforms of steroid 5-alpha –reductase, an intracellular enzyme that converts testosterone to dihydrotestosterone. It is used in the treatment of benign prostate hyperplasia [1,2,3].

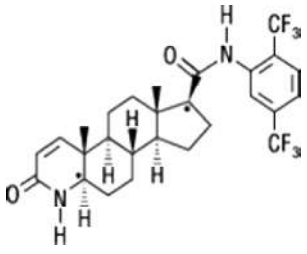

Fig 1 Structure of Dutasteride

Granisetron hydrochloride (Fig 2) is an effective and potent antiemetic drug which is used in the treatment of vomiting and nausea resulting from cancer chemotherapy and radiation in adults and children. Chemically it is endo-N-(9-methyl-9-azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride. Granisetron hydrochloride selectively blocks type3 serotonin (5-HT3) receptors [4, 5].

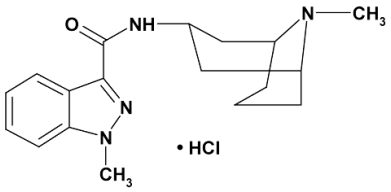

Fig 2 Structure of granisetron hydrochloride

Literature survey reveals that several analytical and Bioanalytical methods was reported for the analysis of Dutasteride and granisetron individually or in combination with other drugs but no method were reported for dutasteride and granisetron in combination. For Dutasteride, the methods reported were alone or in combination with other drugs. These include, HPLC[6,7,8] and HPTLC [9] methods in bulk and pharmaceutical dosage form, stability indicating LC methods [10,11], LC-MS [12,13] methods, spectrophotometric analysis of Dutasteride in tablets [14] were reported. For granisetron methods include UV/Vis spectrophotometric and HPLC methods for the determination of DEX [15,16,17] and GRA [18,19] individually.

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Objectives: 1) To develop a new spectrophotometric methods for Simultaneous estimation of Dutasteride and Granisetron.
2) To establish UV methods for the separation and simultaneous estimation of Dutasteride and Granisetron.

MATERIALS AND METHODS

Pharmaceutically pure samples of Dutasteride were obtained as gifts from IPCA Lab ltd. Mumbai & Granisetron was purchased from Research Lab. Methanol (Research Lab) and distilled water (1:1) was used as solvent in the study. Double beam UV spectrophotometer V-630 JASCO corporation, Japan was used to measure absorbance of the resulting solution.

Selection of solvent and wavelength

Solubility of dutasteride and granisetron was checked in solvents like ethanol, water, methanol and 0.1 N HCl. UV spectrums of the two drugs in these solutions were recorded. The absorbance of the two drugs was found maximum in methanol: water solvent compared to other solvents and two wavelengths 246 nm, and 306 nm were selected which were the $\lambda_{max}$of dutasteride and granisetron respectively.

Preparation of stock solution of Dutasteride and Granisetron

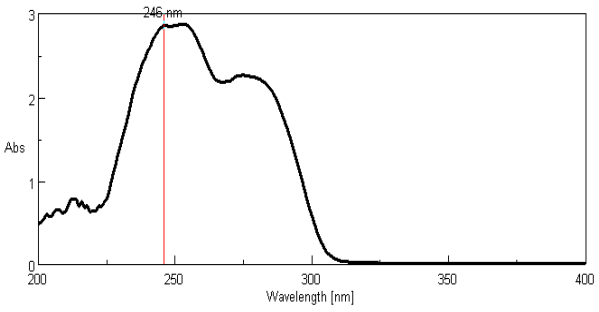
An accurately weighed quantity of 10 mg each dutasteride and granisetron was transferred to the 10 ml volumetric flask and dissolved in methanol: water (1:1).The volume was made up to the mark with the same to make concentration of 1000 µg/ml. The standard stock solutions (100µg/ml) were further diluted separately to obtain working standard of concentration 10µg/ml of dutasteride and granisetron each.

Study of spectra and selection of wavelengths

Each working standard solution was scanned between the range 200-400 nm in 1 cm cell against blank. Maximum absorbing wavelength of dutasteride and granisetron were selected from spectral data and isobestic wavelength selected from overlain spectra of zero order. The λ max for dutasteride and granisetron and isobestic point was 246nm, 306 nm and 295 nm respectively.

RESULTS AND DISCUSSION

Procedure for Calibration Curve of dutasteride and granisetron*:* The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution, pipette out 10 mg dutasteride and 10 mg granisetron in 10 ml of volumetric flask and diluted with mobile phase.



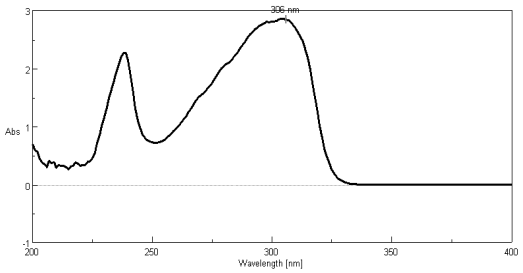


Fig 3 Overlain spectra of dutasteride Fig No -4 Overlain spectra of dutasteride

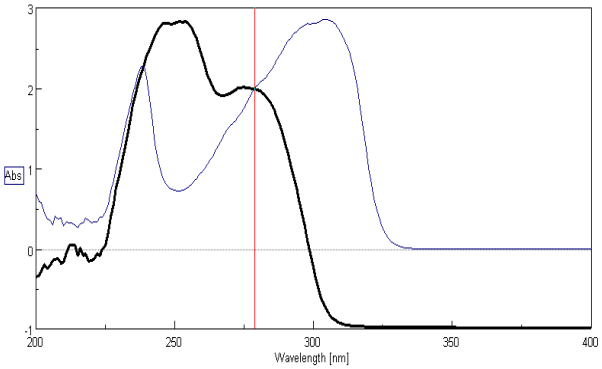


Fig 5 Iso-absorptive point of dutasteride and granisetron

Linearity and Range

The study of linearity and range was performed as per the ICH recommendation. Linearity study for the proposed method was established by least square linear regression analysis. dutasteride and granisetron standard was found to be linear in the range of 5-40µg/ml,5-40µg/ml respectively with r^2>0.995, r^2> 0.996 found at selected wavelengths. The relation between concentration and absorbance for individual drug was studied and shown in Table 1 and 2.

Table 1 Linearity data of dutasteride

Sr. No.	Concentration (µg/mL)	Absorbance at 246 nm
1	0	0.00
2	5	0.2413
3	10	0.4657
4	15	0.6347
5	20	0.9672
6	25	1.1623
7	30	1.3584
8	35	1.5482
9	40	1.8654
10	Correlation coefficients (r^2)	0.9954
11	Slope	0.0456
12	Intercept	0.0046

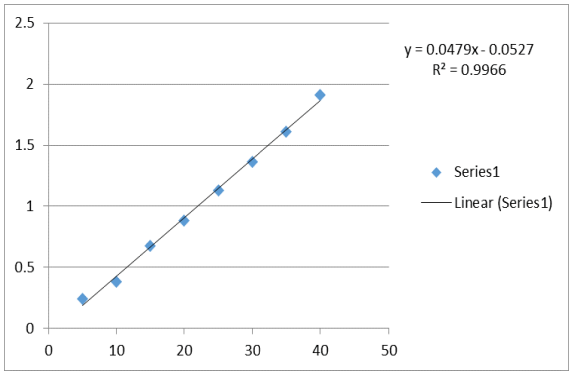


Fig 6 Calibration graph of dutatseride

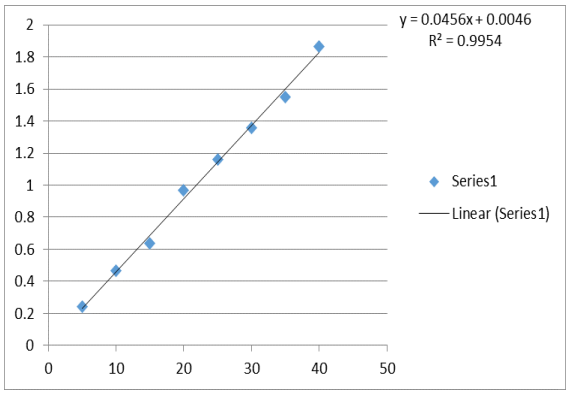


Fig 7 Calibration graph of granisetron

Table 2 Linearity data of granisetron

Sr. No.	Concentration (µg/mL)	Absorbance at 306 nm
1	5	0.2415
2	10	0.3852
3	15	0.6748
4	20	0.8854
5	25	1.1297
6	30	1.3649
7	35	1.6108
8	40	1.9102
9	Correlation coefficients (r^2)	0.996
10	Slope	0.047
11	Intercept	0.052

Recovery study

Accuracy (recovery) of the method is ascertained by recovery studies performed at different levels of concentrations (50, 100 and 150%). The % recovery was found to be, 99.4 to 100.6 for dutasteride, 97.6 to 102.3 for granisetron the value of standard deviation and % RSD were found to be > 2.0 %; show in the Table 3 and 4.

Table 3 Recovery data of dutasteride

Sr.No	Level %	Amount taken (ug/ml)	Amount added (ug/ml)	Amount recovered (ug/ml)	%Recovery ± SD*	RSD*
	50	5	7	12.01	100.6±0.035	0.3873
1.	50	5	7	12.02	100.2±0.041	0.0401
	50	5	7	11.99	100.4±0.010	0.0206
	100	10	14	23.98	99.92±0.831	0.0835
2.	100	10	14	23.97	99.93±0.567	0.0570
	100	10	14	23.98	99.95±0.813	0.0816
	150	15	21	36.01	99.4±0.459	0.0465
3.	150	15	21	36	99.5±0.010	0.0336
	150	15	21	36.02	99.6±0.813	0.0815

Table 4 Recovery data of granisetron

Sr.No	Level %	Amount taken (ug/mL)	Amount added (ug/ml)	Amount recovered (ug/ml)	%Recovery ± SD*	RSD*
	50	5	5	9.76	97.6±0.035	0.3873
1.	50	5	5	9.765	97.65±0.041	0.0401
	50	5	5	9.763	97.63±0.010	0.0206
	100	10	10	20.46	102.3 ±0.831	0.0835
2.	100	10	10	20.46	102.3 ±0.567	0.0570
	100	10	10	20.45	102.2±0.813	0.0816
	150	15	15	29.76	99.02±0.459	0.0465
3.	150	15	15	29.85	99.5±0.010	0.0336
	150	15	15	29.87	99.56±0.813	0.0815

Precision

Precision studies were carried out using parameter like intra-day and inter-day variability, the results for precision was obtained within acceptance limit. The % RSD > 2.0 indicating high reproducibility of the proposed method. The Relative Standard Deviation (RSD) for intra-day analysis of dutasteride and granisetron was found in the range of 0.0167-0.0221 and 0.0190- 0.0239 respectively. The RSD for Inter-day analysis of dutasteride and granisetron was found to be 0.00076-0.00069 and 0.0015 - 0.003 respectively as indicated in Table 5 and 6.

Table 5 Precision data for dutasteride

Conc. (µg/mL)	Intraday Mean ± S.D. (n=3)	RSD*	Interday Mean ± S.D. (n=3)	RSD*
4	0.0087 ± 0.0502	0.0167	0.038 ± 0.0023	0.00076
8	0.0081 ± 0.0597	0.0199	0.010 ± 0.0015	0.00050
12	0.0092 ± 0.0664	0.0221	0.0061 ± 0.0020	0.00069

* indicates determination of three replicates

Table 6 Precision data for granisetron

Conc. (µg/mL)	Intraday Mean ± S.D. (n=3)	RSD*	Interday Mean ± S.D. (n=3)	RSD*
5	0.012 ± 0.0572	0.0190	0.011 ± 0.0045	0.0015
10	0.011 ± 0.0558	0.0186	0.012 ± 0.0794	0.0264
15	0.0087 ± 0.0719	0.0239	0.0082 ± 0.0095	0.0031

* indicates determination of three replicates

Limit of Detection (LOD)

Limit of detection (LOD) for dutasteride and granisetron was found to be 0.2481µg/ml and 0.2053 µg/ml respectively showed in table 7 and 8.

Limit of Quantitation (LOQ)

Limit of quantitation (LOQ) for dutasteride and granisetron was found to be, and 0.7297µg/ml ,0.6222 µg/ml respectively table 7 and 8.

Table 6 Limit of Detection and Limit of Quantification of dutasteride

Sr no.	5 µg/mL	10 µg/mL	15 µg/mL	20 go/mol	25 go/mol
1	0.188	0.314	0.478	0.682	0.786
2	0.189	0.315	0.477	0.684	0.789
3	0.190	0.317	0.479	0.685	0.787
4	0.192	0.318	0.481	0.686	0.788
5	0.194	0.319	0.482	0.687	0.791
Mean	0.191	0.316	0.479	0.686	0.790
SD*	0.00286	0.00207	0.00277	0.00270	0.00319
LOD			0.2481 go/ml		
LOQ			0.7297 go/ml		

Table 7 Limit of Detection and Limit of Quantification of granisetron

Sr no.	5 go/ml	10 go/ml	15 go/ml	20 go/ml	25 go/ml
1	0.023	0.0328	0.0683	0.0853	0.103
2	0.0233	0.0332	0.0684	0.0856	0.104
3	0.0237	0.0327	0.0686	0.0854	0.105
4	0.0234	0.0332	0.0687	0.0857	0.107
5	0.0236	0.0342	0.0682	0.0858	0.108
Mean	0.0235	0.0340	0.0688	0.0857	0.107
SD*	0.00254	0.0329	0.00277	0.00258	0.00364
LOD			0.2053 go/ml		
LOQ			0.6222 go/ml		

CONCLUSION

The developed UV spectro-photometric method in that linearity, precision, accuracy, recovery were found to be more accurate, precise and reproducible. The method were found to

be simple and time saving .All proposed methods could be applied for routine analysis in quality control laboratories.

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