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Research Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DEXAMETHASONE AND GRANISETRON IN COMBINED DOSAGE FORMS

B. Raja[1] and A. Lakshmana Rao[2]*

[1]Anurag Pharmacy College, Ananthagiri- 508 206, Andhra Pradesh, India.
[2]V. V. Institute of Pharmaceutical Sciences, Gudlavalleru- 521 356, Andhra Pradesh, India.

ABSTRACT

A rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for simultaneous estimation of Dexamethasone and Granisetron hydrochloride. Chromatographic separation was achieved on reverse phase Hypersil BDS C_{18} column (150 X 4.6 mm, 5 µm) using the mobile phase consisting of phosphate buffer pH 6.0 and acetonitrile in the ratio of 70:30, v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min and detection was done by UV detector at 263 nm. The proposed method was found to be simple, fast, accurate, precise and reproducible and could be applied for routine quality control analysis for simultaneous determination of Dexamethasone and Granisetron hydrochloride in pharmaceutical dosage forms.

Keywords: Dexamethasone, Granisetron, RP-HPLC, Validation.

INTRODUCTION

Dexamethasone (Figure 1) is a synthetic adrenocortical steroid. It acts as an anti-inflammatory and immunosuppressant[1]. Chemically it is 9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1, 4-diene, 3, 20-dione. Dexamethasone is commonly used for allergic rhinitis, prevention of nausea and vomiting induced by cancer chemotherapy, cerebral edema and ophthalmic disorders. Dexamethasone is a glucocorticoid agonist. Unbound Dexamethasone crosses cell membranes and binds with high affinity to specific cytoplasmic glucocorticoid receptors. This complex binds to DNA elements which results in a modification of transcription and hence, protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses and reduction in edema or scar tissue. The antiinflammatory actions of Dexamethasone are thought to involve phospholipase A_2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

Granisetron hydrochloride (Figure 2) is an effective and potent antiemetic drug which is used in the treatment of vomiting and nausea. It is also effective in the management of postoperative nausea and vomiting due to the anesthetics[2]. Chemically it is *endo*-N-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1*H*-indazole-3-carboxamide hydrochloride. Granisetron is a selective type 3 serotonin (5-HT_3) receptor antagonist. Its main effect is to reduce the activity of the vagus nerve, which is a nerve that activates the vomiting center in the medulla oblongata.

Literature survey has revealed that co-administration of steroids increases the

antiemetic efficacy of 5-HT_3 receptor antagonist. Granisetron when combined with Dexamethasone has found to be the most effective regimen for prevention of post operative nausea and vomiting and during chemotherapy of cancer[3-5]. Hence, a combined dosage form of Dexamethasone and Granisetron and can be considered as a novel avenue for research.

Literature survey reveals that few HPLC methods are reported for the determination of Dexamethasone and Granisetron and individually and only one HPLC method have been reported for the simultaneous estimation of Dexamethasone and Granisetron[6]. Hence, the purpose of presented work is to develop and validate a simple, rapid, accurate and precise RP-HPLC method for simultaneous estimation of Dexamethasone and Granisetron in a combined dosage form.

EXPERIMENTAL

Chromatographic Conditions

The chromatographic separation was achieved on Waters HPLC 2695 series consisting of isocratic binary pump, auto sampler, 2487 dual absorbance detector and thermostat column compartment connected to Waters Empower software. The analysis of the drug was carried out on Hypersil BDS C_{18} column (150 x 4.6 mm; 5 µm), flow rate 1.0 mL/min, wave length 263 nm, column temperature 30°C, injection volume 20 µL and run time 10 minutes.

Chemicals and Solvents

The working standards of Dexamethasone and Granisetron hydrochloride were provided as gift samples from Chandra Labs, Hyderabad, India. Dexamethasone and Granisetron hydrochloride tablets were purchased from local market. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai, India. HPLC grade water obtained from Milli Q water purification system was used throughout the study.

Preparation of mobile phase and diluents

700 mL of phosphate buffer pH 6.0 (1.6 g of potassium dihydrogen phosphate and 0.3g of dipotassium hydrogen phosphate was dissolved in 1000 mL water, adjusted pH 6.0±0.1 with orthophosphoric acid) was mixed with 300 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard stock solution

Accurately weighed and transferred 10 mg of Dexamethasone and 1 mg of Granisetron working standards into 100 mL volumetric flask, about 60 mL of diluent was added, sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Preparation of standard solution

Pipetted 10 mL of the standard stock solution into 100 mL volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution

20 tablets of Dexamethasone and Granisetron were weighed and calculated the average weight. Accurately weighed and transferred the sample equivalent to 10 mg of Dexamethasone and 1 mg of Granisetron into 100 mL volumetric flask. About 60 mL of diluent was added, sonicated to dissolve it completely and made volume up to the mark with diluent. Mixed well and filtered through 0.45 µm filter. Further pipetted 10 mL of the above stock solution into a 100 mL volumetric flask and dilute up to the mark with diluent.

UV spectra's of Granisetron and Dexamethasone

Absorbance maxima of Dexamethasone and Granisetron were detected at 238.60 nm and 301.20 nm, respectively. Both the spectra's were overlapped at 263.40 nm. The overlaid UV spectrum of Dexamethasone and Granisetron were shown in Figure 3.

Method validation

The developed analytical method was validated as per ICH guidelines[7] for the parameters like linearity, accuracy, precision, ruggedness, specificity and system suitability.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of drug in the samples within a given range. Linearity was performed by preparing mixed standard solutions of Dexamethasone and Granisetron and at six concentration levels. The linearity of detector response for Dexamethasone and Granisetron was demonstrated by prepared solutions of over

the concentration range of 25 to 150 µg/mL. The plot of peak area of each sample against respective concentration of Dexamethasone and Granisetron was found to be linear. Beer's law was found to be obeyed over this concentration range. The correlation coefficient shall not be less than 0.998. Linearity results were presented in Table 1 and 2.

Accuracy

Accuracy indicates the deviation between the mean value found and the true value. The accuracy of the method was determined by standard addition method by means of recovery experiments. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD at each level was calculated and results were presented in Table 3 and 4. Satisfactory recoveries ranging from 99.73 to 99.94 for Dexamethasone and 99.03 to 99.88 for Granisetron respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Preparation of precision solution

10 mL of standard stock solution was tranferred to 100 mL volumetric flask and diluted up to the volume with diluent. Same procedure was repeated for remaining six preparations. The %RSD of the result of six preparations for precision study was 0.07 and 0.11 for Dexamethasone and Granisetron respectively, which were well within the acceptance criteria of not more than 2.0. The results of precision study were reported in Table 5 and 6.

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts. The results of the study were tabulated in Table 7 and 8.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 2.9971 µg/mL and 9.0821 µg/mL for Dexamethasone and 0.0115 µg/mL and 0.0349 µg/mL for Granisetron. The LOD and LOQ showed that the method is sensitive for Dexamethasone and Granisetron.

System suitability test

The specificity of this method was determined by complete separation of Dexamethasone and Granisetron. The typical chromatogram of Dexamethasone and Granisetron was shown in Fig. 4 with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of Dexamethasone and Granisetron was less than 2% and resolution was satisfactory. The average retention time for Dexamethasone and Granisetron were found to be 1.988 and 4.746 respectively, for five replicates. The peaks obtained for Dexamethasone and Granisetron were sharp and have clear baseline separation. Analysis was also performed for active Dexamethasone and Granisetron, as well as placebo sample at different conditions. After analysis it was found that there is no interference of peak in the Dexamethasone and Granisetron region for the placebo & active sample. Hence the developed method was specific for the analysis of this product**.** The system suitability parameters are given in Table 9.

Estimation of Dexamethasone and Granisetron in tablet dosage forms

Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Dexamethasone and Granisetron in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of the tablet powder equivalent to 10 mg of Dexamethasone and 1 mg of Granisetron was transferred into a 100 mL volumetric flask and dissolved in 60 mL of diluent. The contents of the flask were sonicated for 15 min and the volume was made up with the diluent. The solution was filtered through a 0.45 µm membrane filter. Further pipetted 10 mL of the above stock solution into a 100 mL volumetric flask and dilute up to the mark with diluent.

The solution was injected into the column six times. The average peak area of the drug was

computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The assay results of tablet dosage formulation by the proposed method are presented in Table 10.

RESULTS AND DISCUSSION

The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drugs Dexamethasone and Granisetron in a combined dosage form. Hypersil BDS C_{18} column in isocratic mode, with mobile phase phosphate buffer pH 6.0 and acetonitrile (70:30 v/v) (pH was adjusted to 6.0 with orthophosphoric acid) resulted in peak with good shape and resolution. The flow rate was 1 mL/min and Dexamethasone and Granisetron were measured with UV detector at 263 nm. Linearity was assessed by plotting concentration vs area within the range of 25-150 µg/mL for both Dexamethasone and Granisetron with correlation coefficient of 0.999 with good linearity response greater than 0.995. The % recovery was found to be within limits of the acceptance criteria with recovery range 99.73 to 99.94% for Dexamethasone and 99.03 to 99.88% for Granisetron. The high percentage of recovery indicates that the proposed method is highly accurate. The %RSD for intra-day and inter-day precision is less than 2% for Dexamethasone and Granisetron. The detection limit of the proposed method was 2.9971 and 0.0115 µg/mL and the quantification limit was 9.0821 and 0.0349 µg/mL for Dexamethasone and Granisetron respectively, which indicate the sensitivity of the method. The assay procedures were repeated for six times and the results were found to give 100.87% of Dexamethasone and 100.22% of Granisetron. The number of theoretical plates calculated was 3501 and 4398 for Dexamethasone and Granisetron respectively, which indicates efficient performance of the column. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the simultaneous estimation of the drugs Dexamethasone and Granisetron by the proposed HPLC method.

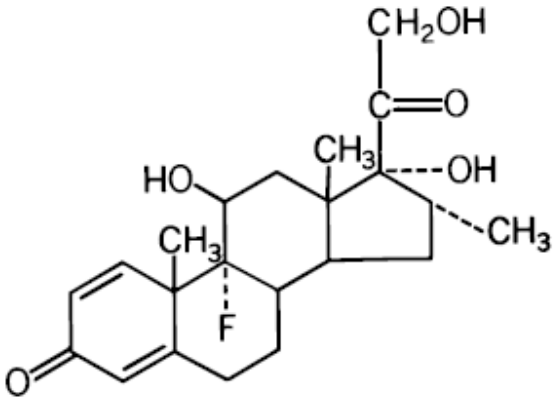

Fig. 1: Chemical structure of Dexamethasone

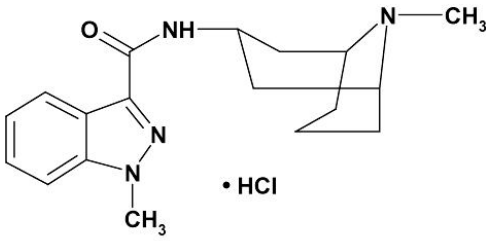

Fig. 2: Chemical structure of Granisetron hydrochloride

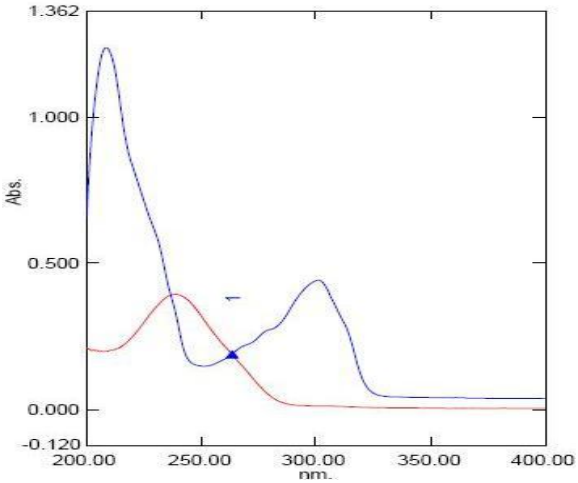

Fig. 3: Overlaid UV spectrum of Dexamethasone and Granisetron

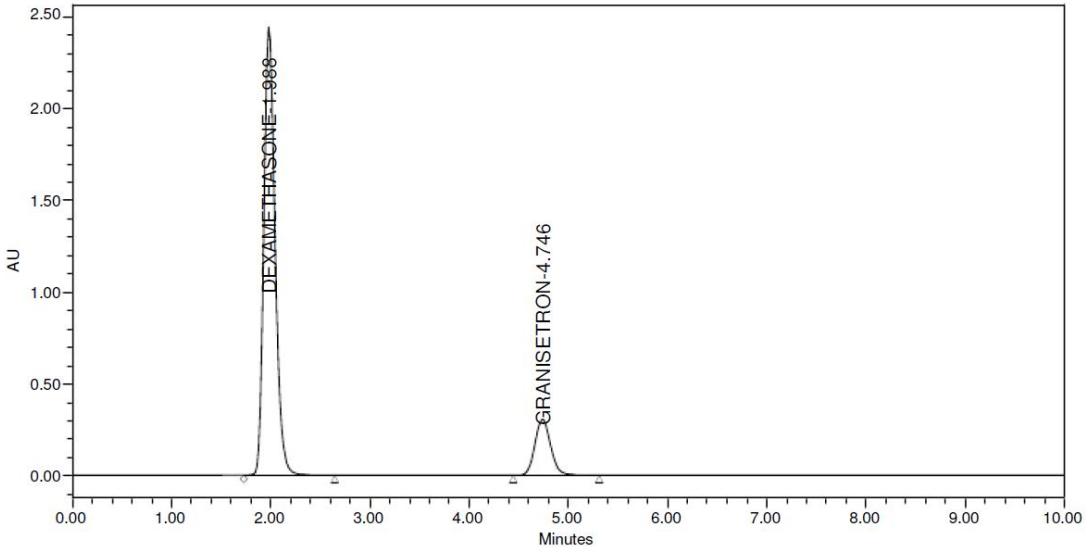

Fig. 4: Typical chromatogram of Dexamethasone and Granisetron

Table 1: Calibration data of Dexamethasone

Concentration (µg/mL)	Peak area (n=6)
25	5208946
50	10079255
75	14788178
100	19111841
125	23653569
150	28765777

Table 2: Calibration data of Granisetron

Concentration (µg/mL)	Peak area (n=6)
25	860106
50	1686183
75	2521137
100	3339383
125	4158299
150	4969276

Table 3: Recovery study for Dexamethasone

Level	Peak area	Amount recovered (µg)	%Recovery
50%	9567984	49.96	99.92
	9598456		
	9548758		
100%	19112654	99.94	99.94
	19165348		
	19158965		
150%	28678464	149.60	99.73
	28637690		
	28665764		

Table 4: Recovery study for Granisetron

Level	Peak area	Amount recovered (µg)	%Recovery
50%	1655476	49.53	99.06
	1658956		
	1638705		
100%	3330127	99.88	99.88
	3329856		
	3327869		
150%	4967689	148.54	99.03
	4943797		
	4942378		

Table 5: Precision study of Dexamethasone

Injection number	Peak area
1	18987563
2	18976875
3	18998560
4	18987590
5	18984580
6	18958946
Mean	18982352
SD	13417.099
%RSD	0.07

Table 6: Precision study of Granisetron

Injection number	Peak area
1	3321654
2	3318753
3	3326746
4	3321789
5	3327645
6	3319864
Mean	3322742
SD	3643.031
%RSD	0.11

Table 7: Ruggedness study for Dexamethasone

S. No.	Retention time	Peak area
1	1.984	18934675
2	1.984	18548795
3	1.985	18587458
4	1.983	18867996
5	1.983	18812764
6	1.983	18801985
Average	1.983667	18758946
SD	0.000787	155623.2
RSD	0.0396	0.8295

Table 8: Ruggedness study for Granisetron

S. No.	Retention time	Peak area
1	4.743	3343265
2	4.741	3328954
3	4.741	3343276
4	4.744	3328953
5	4.746	3319856
6	4.748	3315467
Average	4.743833	3329962
SD	0.002787	11562.9
RSD	0.0587	0.3472

Table 9: Analytical validation parameters

Parameter	Dexamethasone	Granisetron
Linearity (µg/mL)	25-150	25-150
Slope	186092.29	32891.93
Intercept	651518.33	44352.93
Correlation coefficient	0.999	0.999
LOD (µg/ml)	2.9971	0.0115
LOQ (µg/ml)	9.0821	0.0349
Theoretical Plates	3501	4398
Tailing Factor	1.22	1.11
Retention Time (min)	1.988	4.746

Table 10: Assay studies

Drug	Label claim (mg)	Amount found (mg)	%Assay
Dexamethasone	10	10.087	100.87
Granisetron	1	1.002	100.22

CONCLUSION

A simple, specific, sensitive, rapid, accurate and precise RP-HPLC method has been developed for simultaneous estimation of Dexamethasone and Granisetron hydrochloride. The result of the study follows the protocol of ICH guidelines and it can be successfully applied for the simultaneous estimation of the marketed products of Dexamethasone and Granisetron hydrochloride and in combined tablet formulations.

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