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Spectroscopic Characterization of Charge-Transfer Complex of Granisteron Hydrochloride and σ-Acceptor Iodine in Dichloromethane

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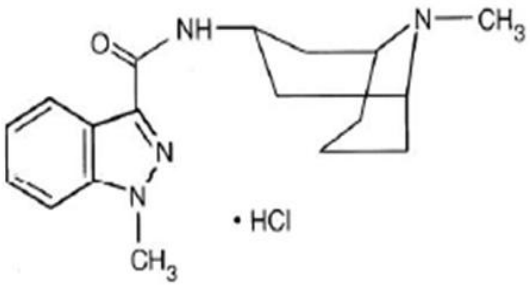
ABSTRACT

Simple, rapid and sensitive procedure was developed for the analysis of granisteron. HCl in pure form as well as in their pharmaceutical formulations using CT complex formed between the drug Granisteron hydrochloride and iodine (I_2) as σ- acceptor in dichloromethane. The complex was also investigated spectrophotometrically at equilibrium (λ= 365) nm for the iodine complex with different optimized experimental conditions. Job's method indicated the formation of 1:1 complex with iodine. The formation constant and molar absorbivity was determined by Benesi-Hildebrand equation. The validation of analytical procedures of granisteron HCl and its CT complex was estimated.

Introduction

Granisetron hydrochloride (GRAN) is a class of 5-HT_3 antagonists is used to prevent nausea and vomiting caused by cancer chemotherapy and radiation therapy. GRAN works by blocking serotonin; a natural substance in the body that causes nausea and vomiting due to the anesthetics [1,2]. Granisetron dosage forms are not yet official in USP and BP [3].



(Structure of Granisteron hydrochloride)

Capacio *et al.* [4] developed a high performance liquid chromatographic (HPLC) method for the determination of granisteron hydrochloride in guinea pig plasma. Separation was achieved on a spherical silica column and granisteron hydrochloride was detected at 305 nm. The correlation coefficients were reported to be 0.9978-0.9999 and the percent relative standard deviation (%RSD) was calculated to be about 10% for inter -day and within-day coefficients of variation (%CV) ranged from 4.9 to 9.5% and 3.6 to 7.6%, respectively. Percent errors for within-day test plasma samples were less than 8.2% of the expected concentration for all samples except for 1.125 ng/µL (14.6%). The limit of sensitivity was found to be 0.019 ng/µL.

Boppana [5] also reported HPLC methods with fluorescence detection for the determination of granisetron in human biological fluids. **Pinguet *et al.*** [6] described RP-HPLC method to measure the plasma concentration of granisetron with a mobile phase (a mixture of acetonitrile and sodium phosphate buffer (pH 4.5) in the ratio v/v (15:85). In addition, **Bin *et al.*** [7] established an RP-HPLC method to measure the plasma concentration of granisetron. On the other hand, **Nirogi *et al.*** [8] reported two LC-MS/MS methods for the determination of granisetron in human biological fluids. **Hewala *et al.*** [9] on the other hand developed a reversed phase HPLC method using photodiode array detection for the simultaneous determination of granisetron hydrochloride, benzyl alcohol, 1-methyl-1H-indazole-3-carboxylic acid (the main degradation product of granisetron) and benzaldehyde (the main degradation product of benzyl alcohol) in granisetron injections with Naphazoline hydrochloride as internal standard.

Zaheer and Imran [10] developed UV spectrophotometric methods for the estimation of Granisetron in bulk and tablet dosage form. Prabhakar et al [11] developed and validated UV- spectrophotometric method of Granisetron in bulk and pharmaceutical formulations. **Hewale *et al.*** [12] developed the first derivative spectrophotometric determination of Granisetron in presence of hydrolytic products and preservatives and applicable to pharmaceutical

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preparations. The literature survey indicates that there are very few spectrophotometric methods available and hence attempted to develop spectrophtometric method with better sensitivity.

Iodine form molecular complexes with a variety of drugs containing lone pair of electron (non bonding) like oxygen, nitrogen and sulphur which act as electron donors and iodine acts as σ- acceptor by forming intense color [13,14].

Experimental procedures

Reagents

Chemically, granisteron hydrochloride (GRAN) is endo-N-(9-methyl-9-azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride with an empirical formula of $C_{18}H_{24}N_4O.HCl$ and molecular weight of 348.9 (312.4 free base). It is a white to off-white solid that is readily soluble in water at 20°C. It is marketed as KYTRIL Tablet (oral administration) containing 1.12 mg of granisetron hydrochloride equivalent to granisetron 1.0 mg. The inactive ingredients include hydroxypropyl methylcellulose, lactose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide.

- All solvents used were of analytical-reagent grade, CH_2Cl_2 from CHD, India. $1x10^{-2}$ M of Granisteron hydrochloride, GRAN (MW=348.9) stock solution in CH_2Cl_2 was prepared, and then diluted to obtain the required concentrations.
- For I_2 (MW 259): 0.259 gm in 100 ml CH_2Cl_2 to obtain 10^{-2} M, more diluted solutions from $2x10^{-5}$ to $1.6x10^{-3}$ M of drug and acceptor were prepared by dissolving an exact volumes of GRAN and I_2 in the same solvent.

Pharmaceutical Formulation Solutions

- EM-EX® Film-Coated tablet contains 1.12 mg Granisetron Hydrochloride equivalent to Granisetron 1 mg supplied from Amoun pharmaceutical company.
- In case of EM-EX® F.C.T (GRAN) each tablet (0.2 gm) contains 1 mg GRAN, so weigh 3.489 gm of powder in 25ml, sonicate for 10 min then filter to obtain $2x10^{-3}$ M of GRAN stock (A), from stock (A) dilute to obtain the required concentrations.

Instrumentation

UV–visible spectrophotometer, SHIMADZU 1700 (Japan) was used for absorption measurements. Transfer pipettes (5 to 50) ul (GERMANY), VORTEX neolab 7-20120, Digital balance, Sonicator Elma® Elmasonic S30H (Germany) was used in this study.

Statistical Analysis

Statistical analysis of the calibration curve parameters (slope, intercept, correlation coefficients and standard deviations) was done using a personal computer with the aid of Microsoft excel software.

Results and discussion

Spectroscopic determination of GRAN

Identification of band maxima

Standard solution of drug $1x10^{-2}$ M (1ml) was mixed with 1ml of $1x10^{-2}$ M of iodine into 10 ml volumetric flask and was completed the volume with dichloromethane, brown color was obtained indicated the formation of charge transfer complex (CT) between drug and iodine. The CT complex was measured in range 200 to 800 nm and displays a new band with maximum wavelength at 365 nm **Fig (1)**.

Effect of concentration of acceptor

Into a series of 10 ml volumetric flask different volume of $1x10^{-3}$ M of iodine solution ranged from 0.25 to 5 ml was transferred and then add 1 ml of $1x10^{-3}$ M of drug vortex for 30 sec, completed the volume with dichloromethane to 10 ml, with waiting for 30 minutes before measuring the absorbance, **Fig (2)**. The Fig. shows that a 1:1 complex is formed at equilibrium.

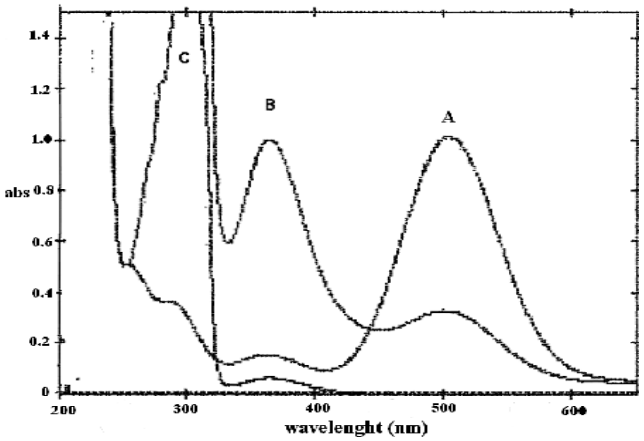

Fig 1: UV-vis spectra of **(A)** I_2, $1X10^{-3}$M solution in dichloromethane **(B)** complex (GRAN-I_2)and (C) GRAN.

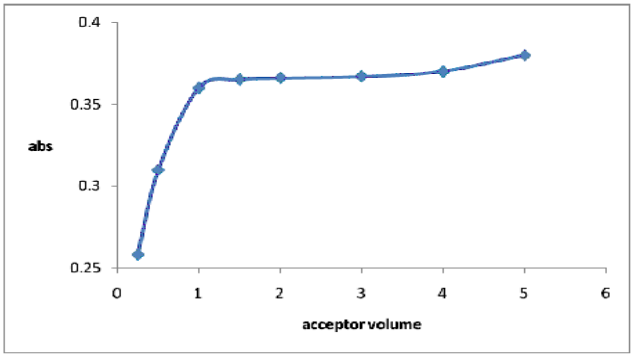

Fig 2: Plot of I_2 conc vs. absorbance of GRAN-I_2 complex in dichloromethane at room temperature.

Effect of solvent on the formation of charge transfer complex

The effect of solvent on the formation of charge transfer complex was studied using different solvents like acetonitrile, ethanol, methanol, benzene, acetone,

toluene, chloroform and dichloromethane. Benzene, acetone, methanol and ethanol were unsuitable due to formation of CT complexes with iodine and limit of solubility in acetonitrile. Dichloromethane afforded maximum sensitivity when compared with other solvents and was used in this study.

Effect of time on the stability of the formed charge transfer complex

The effect of time was studied by recording the absorbance at 365 nm at different time intervals from 5 to 45 min at room temperature as illustrated in **Fig. (3)**, 30 minutes were used as the best time which gives maximum absorbance at equilibrium.

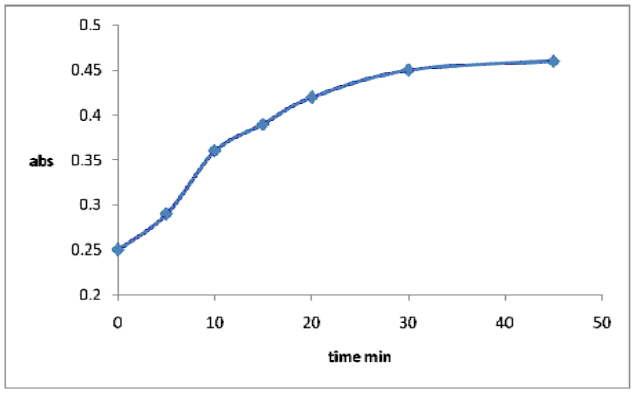

Fig 3: Plot of time vs. absorbance of GRAN–I_2 complex at room temperature.

Determination of the stoichiometry of reaction

Job's method of continuous variation [15] was applied in order to ascertain the stoichiometry of the reaction between drug and iodine. Solution of $1x10^{-3}$M of both drug and iodine were used. A series of solutions were prepared in which the total volume of the drug and iodine was kept at 10 ml. The reagents were mixed in various preparation allowed to stand at $25^{O}C$ for 30 minutes and then diluted to volume with 10 ml dichloromethane. The absorbance was measured at 365 nm. Results revealed 1:1 complexation ratio under optimum conditions, **Fig. (4)**.

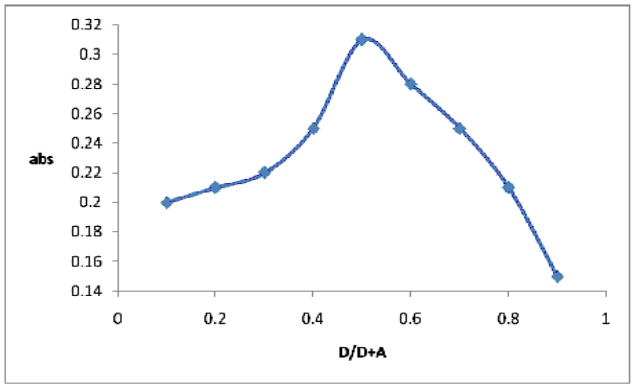

Fig 4: Plot of molar ratio (D/D+A) vs absorbance of GRAN–I_2 complex.

The Benesi-Hildebrand Method for Determination of K_f for DA association and ε Values for DA CT absorption

The Benesi-Hildebrand analysis involves the measurement of the CT complex absorbance as a function of varied concentration of acceptor [A] when [A]>>[D] [16] , where [D] is the concentration of donor.

$[D]_o/Abs = (1/[A])\ (1/K_f\ \varepsilon) + 1/\varepsilon$ (1)

A plot of $1/[A]_o$ vs $[D]_o/Abs$ gives (ε) from the intercept = $1/\varepsilon$ and formation constant (K_f) from the slope = $(1/K_f\ \varepsilon)$.

Which DA donor acceptor complex.

ε molar absorbivity.

K_f formation constant.

Association constant and free energy

To determine K_f, $1x10^{-2}$ M and $1x10^{-3}$ M solutions of GRAN and of iodine were prepared, respectively. 2 ml from the stock of GRAN was taken, and from iodine; different solution volumes from 0.05 to 0.5 ml, were taken and then completed to 10 ml with dichloromethane and the absorbance for the CT complex at 365 nm, was measured **Fig. (5)**.

According to **equation (1)**, plot of $[D]_o$/absorbance against $(1/[A_o])$ gives a straight line fitted with equation: y= 0.000001x+0.000922. The slope equals to $(1/\varepsilon\ K_f) = 1x10^{-6}$, the intercept equals to $1/\varepsilon$ with $\varepsilon = 1.084x10^3$, So K_f = 1/slope x ε = $9.22\ x10^2$ and the free energy of the complex, ΔG_O = - RT ln K = -2.303 RT log K = -2.303 x 8.314 x 298 x log $(9.22x10^2)$ = -16.916 $kJ.mol^{-1}$.

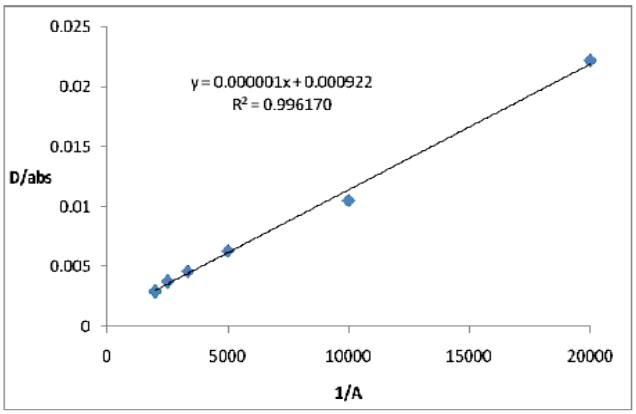

Fig 5: Plot of 1/A versus D/absorbance of GRAN–I_2 complex.

Analytical investigation

Calibration curve of GRAN-I_2

The calibration curve was constructed in the range $2x10^{-5}$-$1.6x10^{-3}$ M by preparing $2x10^{-3}$ M stock solution of GRAN and $1x10^{-2}$ M of iodine. The calibration points are given in **Table (1)**.

By adoption the optimum conditions, the calibration curve is constructed with iodine at 365 nm. The relationship between the absorbance of the CT complex and concentration was shown in **Fig (6)**. Linear relationship was obtained in the range $2x10^{-5}$-$1.6x10^{-3}$ M, **Table (1)**.

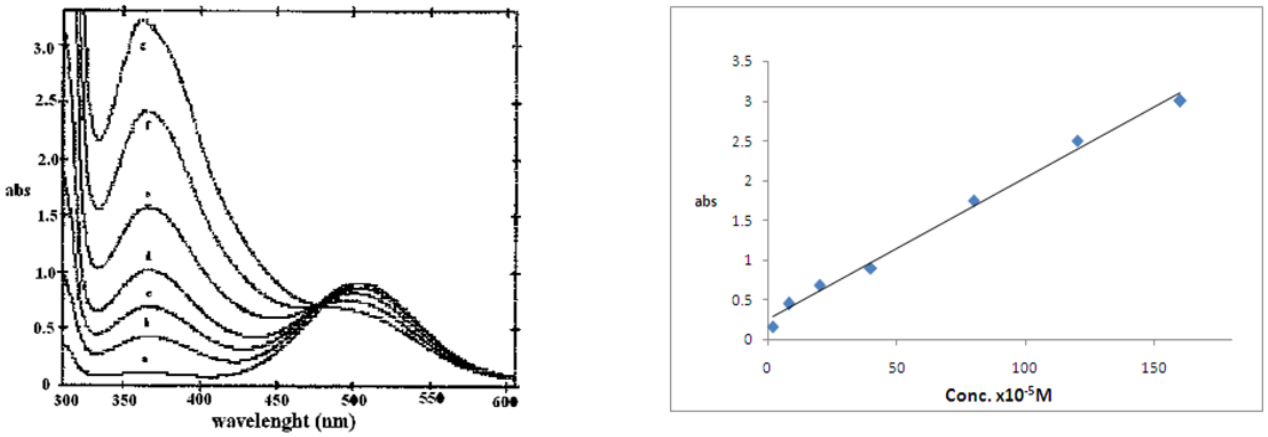

Fig 6: Calibration of GRAN-I_2 complex using solvent as blank. [GRAN] x 10^{-5}, [I_2] = 1x10^{-3} M.

Table 1: Concentration range of GRAN-I_2.

GRAN-I_2	
Conc. of drug X10^{-5} M	**abs**
2	0.16
8	0.45
20	0.68
40	0.90
80	1.75
120	2.50
160	3.00

Table 2: Assay of GRAN-I_2 using proposed spectroscopic technique.

[Taken], (M)	[Found], (M)	mean	R%	SD	% RSD
4.0x10^{-5}	4.008x10^{-5} 4.10x10^{-5} 3.99x10^{-5} 3.90x10^{-5}	3.99x10^{-5}	99.74	0.082	0.081
8.0x10^{-5}	8.02x10^{-5} 8.05x10^{-5} 8.10x10^{-5} 7.99x10^{-5}	8.04x10^{-4}	100.5	0.046	0.046
8.0x 10^{-4}	8.01x10^{-4} 8.02x10^{-4} 7.9x10^{-4} 7.95x10^{-4}	7.9710^{-4}	99.62	0.055	0.054

Validation of analytical procedure

Validation of the present proposed procedures for assay of GRAN-I_2 was examined via repeatability, recovery, selectivity, within day and between day calibrations.

The obtained results were confirmed the reliability of the proposed procedure for the trace quantitation of bulk GRAN. Repeatability of results of the procedure was examined by performing four replicates measurements for $4x10^{-5}$, $8x10^{-5}$ and $8.0x10^{-4}$ M of GRAN-I_2 and it gives mean percentage recoveries 99.74, 100.5 and 99.62 %, respectively, **Table 2**.

The selectivity of the described procedures was tested by analysis of $4x10^{-5}$, $8x10^{-5}$ and $8x10^{-4}$M standard granisteron hydrochloride and a standard formulation (EM-EX®) which gives recoveries 97.5, 100.3 and 99.3, respectively, **Table (3)**. No significant differences in the recoveries or the relative standard deviations were obtained in the absence and presence of excipients. Thus, the proposed procedure can be considered selective. LOD and LOQ [17] were calculated according to equations (2), (3) as given in **Table (4)**.

LOD = 3.3 S.D / b (2)

LOQ = 10 S.D / b (3)

Where S.D is the standard deviation and b is the slope of the calibration curve.

The robustness of the proposed procedure was examined by studying the effect of variation of some of the neck procedural conditions such as time (15 – 30) min, volume of acceptor (0.8, 1.2 ml). The mean percentage recoveries based on four replicate measurements were not significantly affected and consequently the optimized procedure was reliable for assay of GRAN and could be considered robust **Table (5)**.

Table 3: Assay of bulk and formulation EM-EX® film-coated tablet.

[standard] (M)	[Found], Formulation (M)	Conc.	Mean	S.D	RSD
$4x10^{-5}$	$4.01x10^{-5}$ $4.02x10^{-5}$ $3.98x10^{-5}$ $3.6x10^{-5}$	$3.9x10^{-5}$	97.5	0.2	0.19
$8x10^{-5}$	$8.03x10^{-5}$ $8.01x10^{-5}$ $8.10x10^{-5}$ $7.98x10^{-5}$	$8.03x10^{-4}$	100.3	0.05	0.05
$8x\ 10^{-4}$	$8.01X10^{-4}$ $8.12X10^{-4}$ $7.9X10^{-4}$ $7.8X10^{-4}$	$7.95x10^{-4}$	99.3	0.13	0.12

Table 4: Statistical Data of GRAN-I_2 complex.

Statistical Parameters	GRAN-I_2
Correlation Coefficient	0.992
Regression equation Y=ax+b	y = 0.017x + 0.252
Concentration Range (M)	$2x10^{-5} – 1.6x10^{-3}$
S.D of blank	0.017
LOD (M)	$3.3x10^{-5}$
LOQ (M)	$1.0x10^{-4}$

Table 5: Results of robustness of GRAN-I_2 complex.

Robustness conditions	Recovery
Iodine volume 0.8 ml	99%
Iodine volume 1.2 ml	98%
Equilibrium time 15 min	100.2
Equilibrium time 15 min	100.5

Conclusion

Simple, sensitive, extraction-free, rapid and cost-effective spectrophotometric method based on charge transfer complex formation reaction was developed and validated for the determination of GRAN drug. The reagents used in the proposed method are cheap, available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The method is more selective than many of the reported spectrophotometric methods which gives higher ranges 5-25 ug/ml **Venkata *et al.*** [18] and employs a higher wavelength to measure absorbance readings where the errors due to inactive ingredients are minimized to a large extent. The method is free from interferences from the common excipients and other basic substances present, which don't form CT complex.

The statistical parameters and the recovery data reveal good accuracy and precision of the method which can be used as general method for the determination of these types of drugs in pure powder and dosage forms.

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