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A New Validated RP-HPLC Method for Determination of Antiemetic Drug (Granisetron) In Bulk and Pharmaceutical Dosage Form

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ABSRACT

RP-HPLC technique has been created and approved for the examination of Granisetron API. Promote the proposed RP-HPLC technique has magnificent affectability, exactness and reproducibility to develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Granisetron, different chromatographic conditions were applied Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results.. RP-HPLC various columns are Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm column was preferred because using this column peak shape, resolution and absorbance were good. Mobile Phase &diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be Soluble in DMSO (100 mM), Acetonitrile and methanol. Sparingly Soluble in ethanol and Water. Using these solvents with appropriate composition newer methods can be developed and validated. Recognition wavelength was chosen in the wake of checking the standard arrangement of medication more than 200 to 400nm. From the U.V range of Granisetron it is apparent that a large portion of the HPLC works can be refined in the wavelength scope of 206 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best examination. The outcome demonstrates the created strategy is amazingly, one more appropriate technique for test and steadiness related contamination thinks about which can help in the examination of Granisetron in various definitions.

Keywords: RP-HPLC, Granisetron, Determination, Validation parameters

INTRODUCTION [1, 2]

Analytical instrumentation plays an important role in the products and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits require to assure safe food, drugs, water and air. An instrumental method of Chemical analysis has now become the backbone of experimental chemistry.

Points to be considered in the selection of a procedure include:

- Stability of the absorbance with respect to time, minor variations in pH, ionic strength and temperature.
- Degree of selectivity of a complexing agent including the effect of other species likely to be present and the effect of an excess reagent.
- Conformity of the Beer–Lamberts law and plot calibration data for the range of concentration measured.

The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substance is required in samples known of containing other absorbing substances which potentially interferes in the assay, if the recipe of the sample formulation is available to the analyst, the identity and

concentration of the interference are known and the extent of interference in the assay may be determined.

Chromatography [2]

"Chromatography is a scientific system where in an example blend under test is isolated into various parts." This is both a subjective and quantitative technique [1]. The example gets isolated affected by a versatile stage (moving stage) over a stationary stage [2]. These isolated parts are later distinguished and furthermore measured [3].

High-Performance Liquid Chromatography (HPLC)

HPLC is an essential explanatory technique normally used to independent and evaluate segments of fluid examples. In this system, an answer (first stage) is pumped through a section that contains a pressing of little permeable particles with a second stage bound to the surface [4]. The distinctive solubility of the example segments in the two stages cause the segments to travel through the segment with various normal speeds, in this way making a partition of these segments. The pumped arrangement is known as the versatile stage, while the stage in the segment is known as the stationary stage[5].

Principle

Partition depends on the analyte's relative dissolvability between two fluid stages. HPLC utilizes assorted sorts of stationary stage (consistently, hydrophobic drenched carbon chains), a pump that moves the convenient stage (s) and analyte through the section, and a marker that gives a trademark upkeep time to the analyte. Analyte maintenance time shifts relying upon the temperature of the segment, the proportion/structure of solvent (s) utilized, and the stream rate of the portable stage. With HPLC, a pump (instead of gravity) gives the higher weight required to drive the portable stage and analyte through the thickly pressed segment (Figure 2)[6].

Instrumentation of HPLC [3]

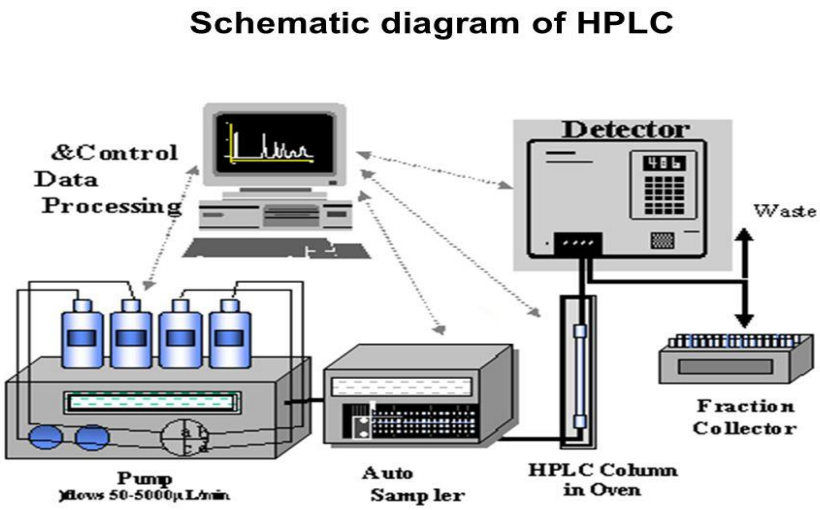


Fig 1: Schematic Diagram of HPLC

HPLC instrumentation joins a pump, injector, segment, identifier and integrator or obtainment and show system. The center of the system is the place parcel occurs.

Analytical method development [5]

Expository science manages strategies for recognizable proof, division, and evaluation of the concoction parts of normal and counterfeit materials. The decision of investigative system depends on numerous contemplations, for example, compound properties of the analyte and its fixation test lattice, the speed and cost of the examination, sort of estimations i.e., quantitative or subjective and the quantity of tests. A subjective strategy yields data of the synthetic personality of the species in the example. A quantitative strategy gives numerical data with respect to the relative measures of at least one of the analytes in the example [13]. The means of technique improvement and strategy approval the means of technique advancement and strategy approval

Sample preparation

The motivation behind example readiness is to make a handled example that prompts better explanatory outcomes contrasted and the underlying example. The readied test ought to be an aliquot moderately free of impedances that is good with the HPLC strategy and that won't harm the section. The principle test planning systems are fluid extraction (LLE) and strong stage extraction (SPE). In these strategies the analyte of intrigue was isolated from test framework, so that as few conceivably meddling species as conceivable are brought through to the diagnostic detachment arrange [14].

Detection

After the chromatographic partition, the analyte of intrigue is identified by utilizing appropriate indicators. Some business indicators utilized in LC are: bright (UV) finders, fluorescence locators, electrochemical identifiers, refractive list (RI) identifiers and mass spectrometry (MS) identifiers. The decision of identifier relies upon the example and the motivation behind the examination. The UV locators are the most widely recognized HPLC identifiers since they are powerful, shoddy, and simple to deal with and since numerous solutes retain light in this recurrence go. The standard UV identifier estimates the absorbance at one single wavelength at the time. A diode-cluster finder (DAD) can quantify a few wavelengths in the meantime, and since no parts are moved to change wavelength or to examine, there are no mechanical blunders or float with time. HPLC with a mass spectrometer locator (LC-MS) demonstrated better affectability and selectivity thought about than HPLC-UV techniques [15].

Analytical Method Validation [6]

The approval parameters including scientific improvement, QC administrative undertakings and the people requiring the logical information [16]. The working strategy or the Validation Master Plan (VMP) ought to plainly characterize the jobs and duties of every division engaged with the approval of systematic strategies [17].

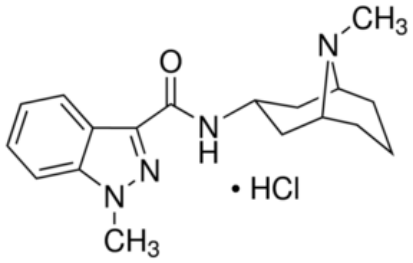
Parameters for method validation

The parameters for technique approvals have been characterized in various working gatherings of National and global boards and are portrayed in the writing. Shockingly, a portion of the definitions shift between the diverse associations [18]. An endeavor at harmonization was made for pharmaceutical applications through the ICH where delegates from the Industry and administrative offices from the United States, Europe and Japan characterized Parameters, prerequisites and, to some degree, system for logical strategies approval [19]. The different parameters are: Selectivity/Specificity, Precision and Reproducibility, Accuracy and Recovery, StabilityRange, Limit of Detection, Limit of Quantization, Repeatability, Reproducibility, Measurement Uncertainty, Sensitivity, Ruggedness.

DRUG PROFILE

Drug name: Granisetron

Chemical Structure:



Description: A serotonin receptor (5HT-3 particular) foe that has been utilized as an antiemetic and antinauseant for malignancy chemotherapy patients[11].

Chemical Formula: $C_{18}H_{24}N_4O$

Solubility: Soluble in DMSO (100 mM), water (70 mg/ml at 25° C), ethanol (1 mg/ml at 25° C), and methanol. Promptly dissolvable in Water, Sparingly Soluble in Methylene Chloride.

Medication Category: Antiemetic

Mechanism of action: Granisetron is a strong, particular for of 5-HT3 receptors. The antiemetic action of the medication is achieved through the restraint of 5-HT3 receptors present both halfway (medullary chemoreceptor zone) and incidentally (GI tract) and chemoreceptor trigger zone[13].

Medical Uses: A particular serotonin (5HT3) receptor adversary. Utilized as an antiemetic. Its fundamental impact is to lessen the action of the vagus nerve. It doesn't have much impact on regurgitating because of movement infection [15].

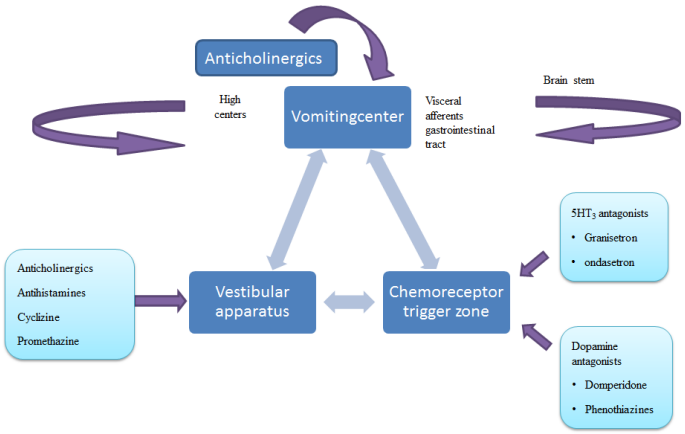


Fig2: Mechanism of Action of Granisetron

Materials and Methods:

Instruments Used:HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters, T60-LAB INDIA UV–Vis spectrophotometer, Electronic Balance (SHIMADZU ATY224) Ultra Sonicator(Wensar wuc-2L) Thermal Oven Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d. P^H Analyzer (ELICO) Vacuum filtration kit (BOROSIL)

Chemicals / Reagents Used:Doubled distilled water 99.9% HPLC grade- Sd fine-Chem ltd; Mumbai, HPLC Grade Water-HPLC grade- Sd fine-Chem ltd; Mumbai, Hydrochloric Acid-Sd fine-Chem ltd; Mumbai, Acetonitrile-LobaChem; Mumbai, Sodium Hydroxide-Sd fine-Chem ltd; Mumbai Ethanol-Sd fine-Chem ltd; Mumbai, Octano-Sd fine-Chem ltd; Mumbai.

Solubility Study:Methanol: Soluble, Ethanol:slightly soluble, Acetonitrile-Soluble, DMSO-Freely soluble, Water-Sparingly soluble**.**

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Granisetron, so that the same wave number can be utilized in HPLC UV detector for estimating the Granisetron. The scanned UV spectrum is attached in the following page,

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Granisetron standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

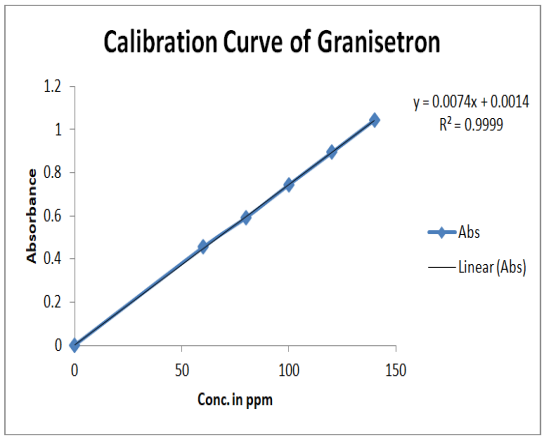


Fig 4: UV Calibration Curve for Granisetron

While scanning the Granisetron solution we observed the maxima at 206nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

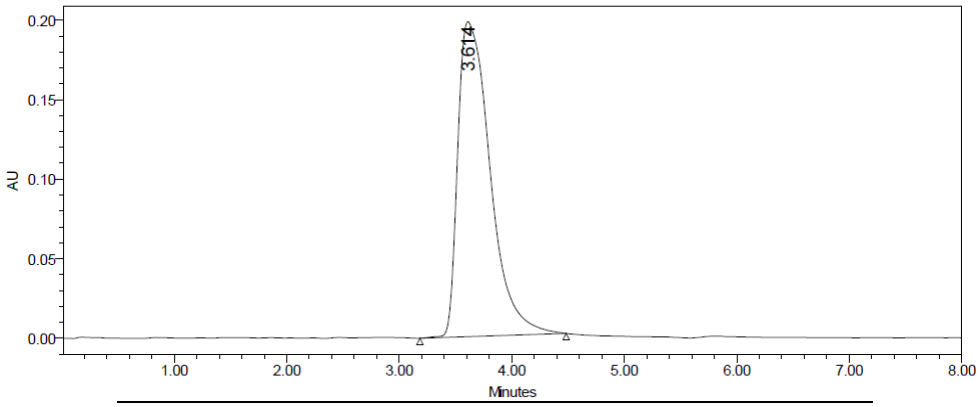
Table 1:Optimised Chromatographic conditions

Mobile phase	Phosphate Buffer : Methanol = 46:54 (pH-3.2)
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	206 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	3.622 minutes

METHOD VALIDATION

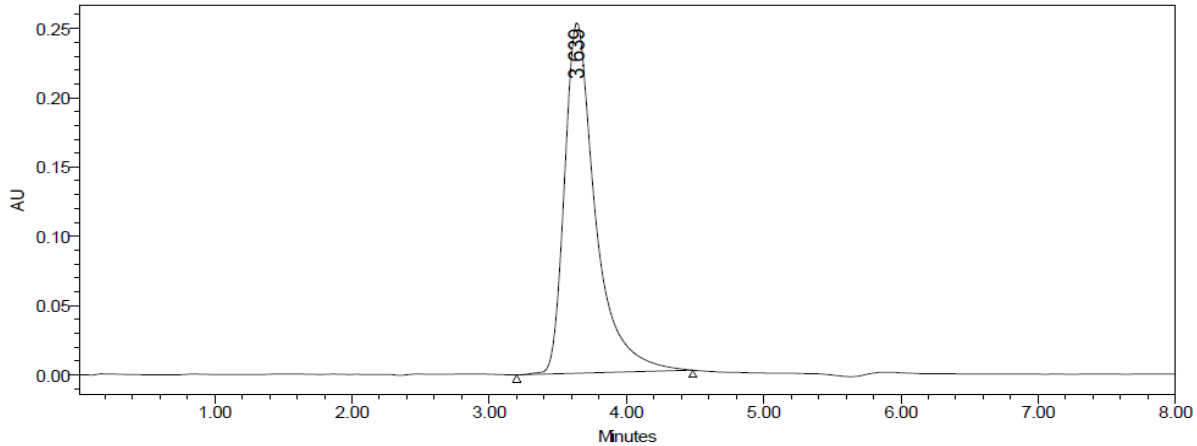
Accuracy

*Recovery study:*To decide the exactness of the proposed strategy, recuperation contemplates were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of GRANISETRON were taken and added to the pre-examined plan of fixation 100µg/ml. From that rate recuperation esteems were figured.



Drug Name	Rt	Peak Area	Tailing Factor	Plate Count
Granisetron	3.614	3959294	0.98	2968

Fig 6: Chromatogram of 80% Accuracy-1



Drug Name	Rt	Peak Area	Tailing Factor	Plate Count
Granisetron	3.639	4948323	1.22	3025

Fig 7: Chromatogram of 100% Accuracy-1

Precision

Repeatability

The accuracy of every technique was found out independently from the pinnacle regions and maintenance times gotten by real assurance of six recreates of a fixed amount of drug Granisetron (API). The percent relative standard deviation was calculated for Granisetron are presented in the table.

Intermediate Precision:Intra-assay & inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Granisetron revealed that the proposed method is precise.

Linearity & Range

The calibration curve showed good linearity in the range of 0 – 140 µg/ml, for Granisetron (API) with correlation coefficient (r^2) of 0.999 (Fig-27). A typical calibration curve has the regression equation of y = 48313x + 71968 for Granisetron.

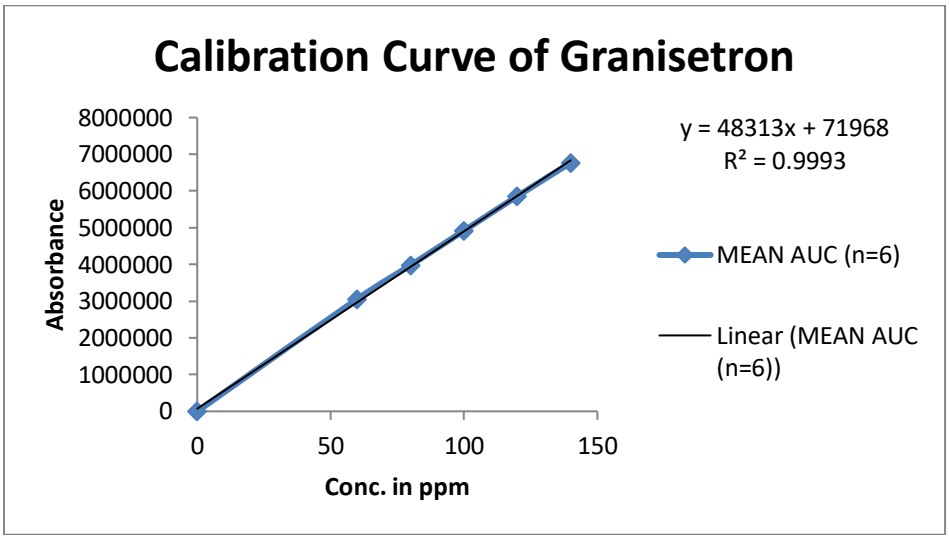
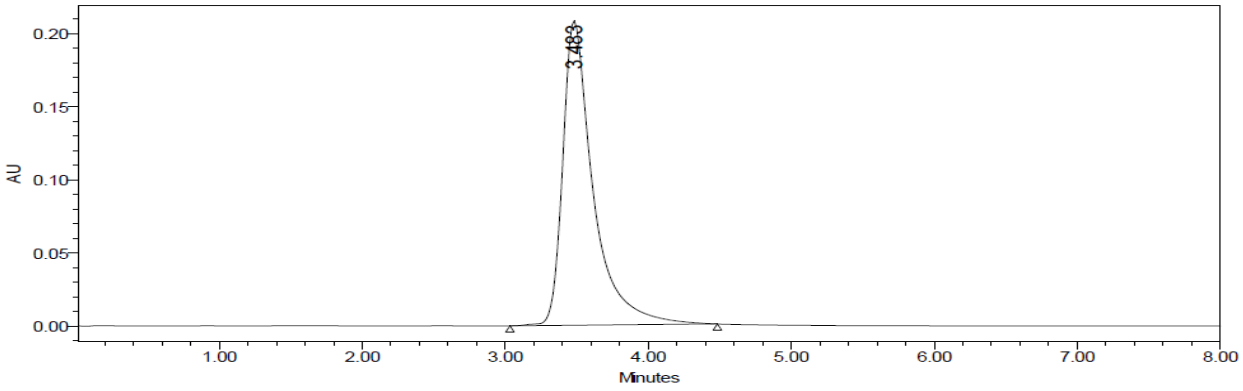


Fig 8:Calibration Curve of Granisetron (API)



Drug Name	Rt	Peak Area	Tailing Factor	Plate Count
Granisetron	3.483	3979280	1.02	2986

Fig9:Chromatogram for Linearity (80 ppm)

Method Robustness

Impact of little changes in chromatographic conditions, for example, change in Flow rate (± 0.1ml/min), Wavelength of location (±2nm) and organic phase content in mobile phase (±5%) concentrated to decide the Robustness of the technique are additionally for (Table-6.26, % RSD < 2%) the created RP-HPLC strategy for the examination of Granisetron (API).

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

System Suitability Parameter

Framework appropriateness testing is a necessary piece of numerous explanatory methodologies. The tests depend on the

idea that the gear, hardware, logical tasks and tests to be examined comprise a necessary framework that can be assessed thusly. Following framework reasonableness test parameters were built up. The information is appeared in Table 2.

Table 2: Dataof System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.34
2	Asymmetry	T ≤ 2	Granisetron=0.16
3	Theoretical plate	N > 2000	Granisetron=3065
4	Tailing Factor	T<2	Granisetron=1.55

Estimation of Granisetron in Pharmaceutical Dosage Form

Label claim:Each tablet contains: 2 mg :Twenty pharmaceutical dosage forms were taken and the I.P. technique was taken after to decide the normal weight. Above measured tablets were at long last powdered and triturated well. An amount of powder comparable to 25 mg of medications were exchanged to 25 ml volumetric jar, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with mobile phase. The arrangement was separated through a film channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five repeats into the HPLC framework and the perceptions were recorded. A copy infusion of the standard arrangement was likewise infused into the HPLC framework and the pinnacle zones were recorded. The information is appeared in Table 3. The amount of drugs in Granicipwas found to be 1.96 (± 0.546) mg/tab for Granisetron& % assay was 99.82 %.

Table 3: Recovery Data for estimation Granisetron in Granicip

Brand name of Granisetron	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Granicip (2 mg) (Cipla Pharmaceuticals Limited)	2mg	1.96 (± 0.546)	99.82 (±0.287)

STABILITY STUDIES: Following convention was entirely clung to for constrained corruption of Granisetron Active Pharmaceutical Ingredient (API).

Results of Stability Studies: The results of the stress studies indicated the specificity of the method that has been developed. Granisetron was stable in acidic and photolytic stress conditions.

Table 4:Results of Forced Degradation Studies of Granisetron API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	97.32	2.68	100.0
Basic Hydrolysis (0.I MNaOH)	24Hrs.	87.64	12.36	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	88.65	11.35	100.0
UV (254nm)	24Hrs.	93.42	6.58	100.0
3 % Hydrogen Peroxide	24Hrs.	91.04	8.96	100.0

RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Granisetron, different chromatographic conditions were applied & the results observed are presented in previous chapters.Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be Soluble in DMSO (100 mM), Acetonitrile and methanol. Sparingly Soluble in ethanol and Water. Using these solvents with appropriate composition newer methods can be developed and validated. Recognition wavelength was chosen in the wake of checking the standard arrangement of medication more than 200 to 400nm. From the U.V range of Granisetron it is apparent that a large portion of the HPLC works can be refined in the wavelength scope of 206 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best examination. The

outcome demonstrates the created strategy is amazingly, one more appropriate technique for test and steadiness related contamination thinks about which can help in the examination of Granisetron in various definitions.

CONCLUSION

A sensitive and particular RP-HPLC technique has been created and approved for the examination of Granisetron API. Promote the proposed RP-HPLC technique has magnificent affectability, exactness and reproducibility.

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