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A CRITICAL STUDY DESIGN AND VALIDATE AN ANALYTICAL METHOD FOR ESTIMATING APIXABAN DRUG

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ABSTRACT

A drug product's interaction with the excipients used in its formulation might result in a variety of contaminants. Additionally, a drug substance is put through a number of conditions during formulation that may cause it to degrade or undergo other negative reactions. For instance, heat can speed up the decomposition of medicinal compounds that are thermally susceptible when it is employed for drying or other purposes. Potentially susceptible to degradation brought on by hydrolysis are solutions and suspensions. When water or another solvent has been used for granulation, these processes can also take place in the dosage form in a solid state, such as in the case of capsules and tablets. In addition to adding its own contaminants, the water employed in the formulation may also create favourable conditions for hydrolysis and metal catalysis. Other possible solvents can undergo comparable reactions. If no measures are taken, easily oxidised materials may oxidise. Similar photochemical processes can occur with materials that are sensitive to light. Because to the breakdown of the API or other interactions during storage, a variety of contaminants may be formed. Stability studies must therefore be carried out in order to assess, predict, and guarantee the safety of pharmacological products. Pre-formulation studies to determine the compatibility of the active pharmaceutical ingredient (API) and the excipients are known as stability studies. Accelerated stability evaluations of the test or finished drug product are also known as stability evaluations via kinetic studies and projection of expiration date. Routine stability studies of drug products in marketed, sample, or dispensed packages under various conditions of temperature, light, and humidity are also known as stability studies. We can find out what potential contaminants can be created by degrading reactions by conducting stability experiments under a variety of exaggerated temperature, humidity, and light conditions. To assess impurities, it's critical to design a workable stability programme. A good stability programme effectively combines the need for regularity with scientific concerns.

KEY WORDS: *Design, Validate, Analytical Method, Estimating, Apixaban Drug.*

INTRODUCTION

Impurity profiles are not precisely defined. It details the contaminants that are present in it. The known and unidentified contaminants in a typical batch of API (Active Pharmaceutical Ingredient) produced by a certain controlled production process are described in the impurity profile. It contains information about each impurity's identity or some qualitative analytical designation (such as retention time), its range, and its type. The impurity profile of a substance under study lists all contaminants that could possibly be present. Additionally, it makes an estimate of the actual concentration of the various impurities. The impurity profile typically depends on how the API was built or where it came from.

SOURCE OF IMPURITIES IN DRUG PRODUCT

Drug impurities can come from a variety of sources and stages throughout the production of pharmaceutical dosage forms. It is never possible to distinguish clearly between degradation products and process-related contaminants. However, the majority of the impurities are peculiar to the manufacturing process's synthetic route. A medicine can be synthesised in a variety of ways, therefore it's feasible that the same product from several sources will include various impurities.

DRUG PROPFILE - APIXABAN

Chemically, ELIQUIS (apixaban) is 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl].-4,5,6,7-tetrahydro -1H-pyrazolo[3,4-c]pyridine-3-carboxamide. It has a molecular weight of 459.5 and the chemical formula C25H25N5O4. An off-white to pale-yellow powder is apixaban . Apixaban does not ionise at physiological pH (1.2–6.8); its average solubility in water throughout this pH range is 0.04 mg/mL. ELIQUIS tablets contain the inactive components anhydrous lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulphate, and magnesium stearate. They come in strengths of 2.5 mg and 5 mg of apixaban for oral administration. The film coating is made up of lactose monohydrate, hypromellose, titanium dioxide, triacetin, and tablets containing either red iron oxide (5 mg) or yellow iron oxide (2.5 mg). Since May 2012, apixaban has been accessible in Europe. Apixaban is used medically to reduce the risk of embolism and stroke in people with nonvalvular atrial fibrillation. The National Institute for Health and Clinical Excellence advises using apixaban to prevent stroke and systemic embolism in those with non-valvular atrial fibrillation and at least one of the risk factors listed below. In patients with atrial fibrillation, apixaban and other more recent anticoagulants (dabigatran and rivaroxaban) appear to be just as effective as warfarin in avoiding non-hemorrhagic stroke and are linked to a decreased risk of intracranial bleeding.For both free and clot-bound facto Xa, apixaban is a highly selective, orally

accessible, and reversible direct inhibitor. Apixaban can be estimated using several LC-MS techniques from human plasma, but there is no assay for the drug using HPLC and UV Spectrophotometry. Additionally, apixaban has not yet been formally reported in any of the pharmacopoeias (USP, EP, JP, and IP). According to ICH criteria, the current HPLC and UV Spectrophotometric procedures were created and validated. The procedures for determining apixaban in formulation with minimal background interferences using RP-HPLC and UV Spectrophotometry are straightforward, sensitive, and repeatable. The accuracy, precision, and other analytical method validation factors listed below have all been attempted to design and validate. the apixaban chemical structure.

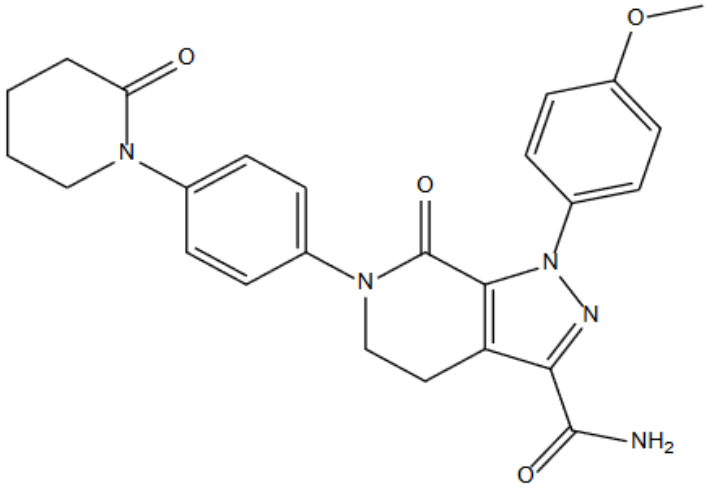

FIGURE - 1: CHEMICAL STRUCTURE OF APIXABAN

IUPAC Name : 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4, 5, 6,7- tetrahydro-1H-pyrazolo[3,4-c] pyridine-3-carboxamide

Empirical Formula: $C_{25}H_{25}N_5O_4$

Molecular Weight: 459.497 g/mol

Mechanism of action:

In order to prevent coagulation, apixaban directly inhibits both free and clot-bound factor Xa in a reversible manner.

PHARMACOKINETICS:

Apixaban works by preventing coagulation, which stops the formation of blood clots. FXa inhibition causes Apixaban to prolong clotting tests like the prothrombic time (PT), international normalised ratio (INR), and activated partial thromboplastin time (aPTT). However, the changes seen in these clotting tests at the anticipated therapeutic dose are small, highly variable, and not helpful in tracking the anticoagulation effect of apixaban. The anticoagulant effect of apixaban should not be evaluated using the Rotachrom® Heparin chromogenic assay.

ABSORPTION:

The stomach and small intestine are where apixaban is absorbed. The absolute bioavailability is roughly 50% for dosages up to 10 mg. When taken orally, meals do not impact absorption, and it typically takes 3–4 hours for maximal plasma concentrations to be reached. Large oral dosages equal to or greater than 25 mg result in poor absorption and reduced bioavailability. The ascending colon and distal small intestine are where the majority of absorption takes place.

ELIMINATION AND METABOLISM:

Most commonly, apixaban is converted to metabolites through o-demethylation and hydroxylation. The 3-oxopiperidinyl moiety is the main site of biotransformation. CYP3A4/5 is the primary metabolic enzyme, whereas CYP1A2, 2C8, 2C9, 2C19, and 2J2 are secondary metabolic enzymes. There are no active metabolites, and the main substance in circulation is Apixaban as is 5% of the dosage that is provided is excreted in the urine and faeces. It is expelled through the gut and bile into the faeces for disposal.

INDICATION:

Apixaban is prescribed to people with nonvalvular atrial fibrillation to lower their risk of stroke and systemic embolism. Additionally, it has been used to reduce the possibility of venous thrombosis after orthopaedic surgery.

RESEARCH METHODOLOGY

CHEMICALS AND REAGENTS:

TABLE -1: LIST OF CHEMICALS AND REAGENTS

S. No.	Name	Make	Grade
1.	Sodium acetate	Merck, India ltd, Mumbai	AR
2.	Acetonitrile	Merck, India ltd, Mumbai	HPLC
3.	Methanol	Merck, India ltd, Mumbai	HPLC
4.	Water	Milli-Q water purificationsystem, (Milford, USA)	Millipore
5.	0.45 µm Nylon filter	Rankem	NA

INSTRUMENT DETAILS:

TABLE -2: LIST OF INSTRUMENTS

S. No.	Instrument Name	Make and Model	Manufacturer/supplier
1.	HPLC	Shimadzu LC10ATVP	Shimadzu corporation,Kyoto, Japan
2.	UV	Shimadzu 1800	Shimadzu corporation,Kyoto, Japan
3.	Analytical Balance	Shimadzu AUX220	Shimadzu corporation,Kyoto, Japan
4.	Sonicator	Ultrasonic CleanerPower sonic 420	Hwashin technology co,Korea
5.	Centrifuge	pendorf Centrifuge5810	Chennai, India

PREPARATION OF SOLUTIONS:

- **PREPARATION OF STOCK AND WORKING STANDARD SOLUTION FOR HPLC AND UV SPECTRO PHOTOMETRY**

Accurately weighing 10 mg of apixaban into a 100 ml volumetric flask, adding 10 ml of methanol, sonicating it to dissolve it, and adding the remaining methanol (0.1 mg/ml) to fill the remaining space. To achieve a concentration

of 20 g/ml, which was used as the working standard and 100% of the goal concentration, 4 ml of this stock solution were pipetted out and increased to 20 ml.

- **PREPARATION OF STOCK AND WORKING SAMPLE SOLUTION FOR HPLC AND UV SPECTRO PHOTOMETRY**

Ten tablets were measured, placed in a mortar, crushed, and then evenly combined. Test stock solutions of apixaban at a concentration of 100 g/ml were made by dissolving 10 mg of the drug, which was weighed in a 100 ml volumetric flask, adding 10 ml of methanol, sonicating it for five minutes, and filling the remaining space. The solution was then filtered through a 0.45 syringe filter.To create a working sample solution with a concentration of 20 g/ml, 4 ml of the aforementioned stock solution were pipette out and added to a 20 ml volumetric flask.

SELECTION OF MOBILE PHASE: High UV transparent with good baseline

SELECTION OF STATIONARY PHASE:

Since it is a neutral moiety, the compound needs a hydrophobic surface to be retained. For general purpose method development at low pH, SunFire C18 columns are used. The C18 ligand is very retentive, particularly for basic chemicals, and thus is perfect for impurity profile tests and purification.

RESULTS AND DISCUSSION

In order to create a reverse phase HPLC process, the theoretical plates (N), run time, and asymmetry of the system were taken into consideration. The apixaban was eluted by the developed optimised technique in around 4 minute's shows, respectively, the chromatograms of the standard solution and the blank solution. The 10-minute runtime is the total. System suitability tests are done to make sure the chromatographic system is performing properly and are a crucial component of method development. Six replicate injections of the standards at working concentration were examined for retention time (Rt), the number of theoretical plates (N), and peak asymmetry (A).

SELECTION OF WAVELENGTH FOR UV:

By observing the UV spectrum of the drug solution in the 200–400 nm region, it was possible to establish the ideal wavelength for HPLC and UV analysis. The 280nm wavelength was chosen for simultaneous estimation.

CHROMATOGRAPHIC CONDITIONS:

TABLE -3: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Chromatographic mode	**HPLC**
Detector	**PDA-detector**
Stationary Phase	Sun fire C18,(150×4.6 mm:5 µ)
Buffer	1.50 g of sodium acetate in 1000 buffer bottleadded 100 ml water sonicate to dissolve and makeup to the volume with water. The bufferwas filtered through 0.45 µ filters to remove all fine Particles and gases.
Mobile phase	The mobile phase was prepared by mixing buffer and acetonitrile 60:40 v/v and later itwas sonicated for 10 min for the removal of air bubbles**.**
Elution mode	Isocratic
Detection wavelength (nm)	280
Flow rate (mL/min)	1.0
Injection volume (µL)	10.0
Column temperature	Ambient

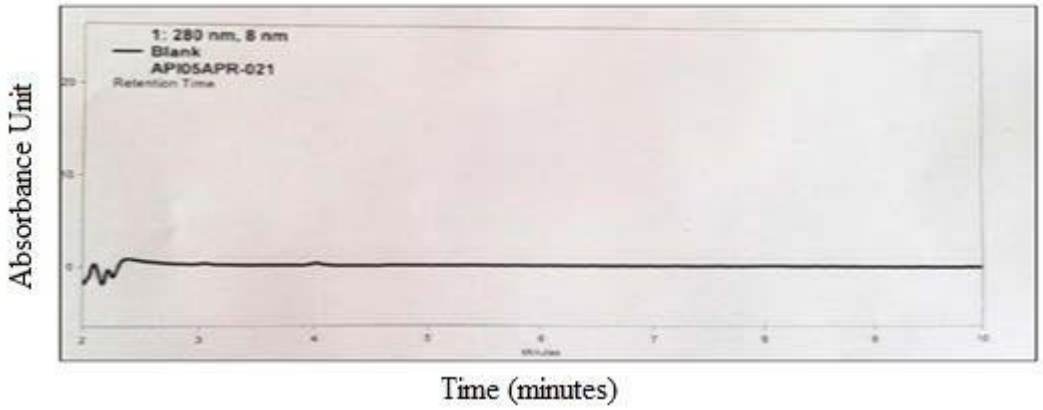

FIG.-2: CHROMATOGRAM OF BLANK

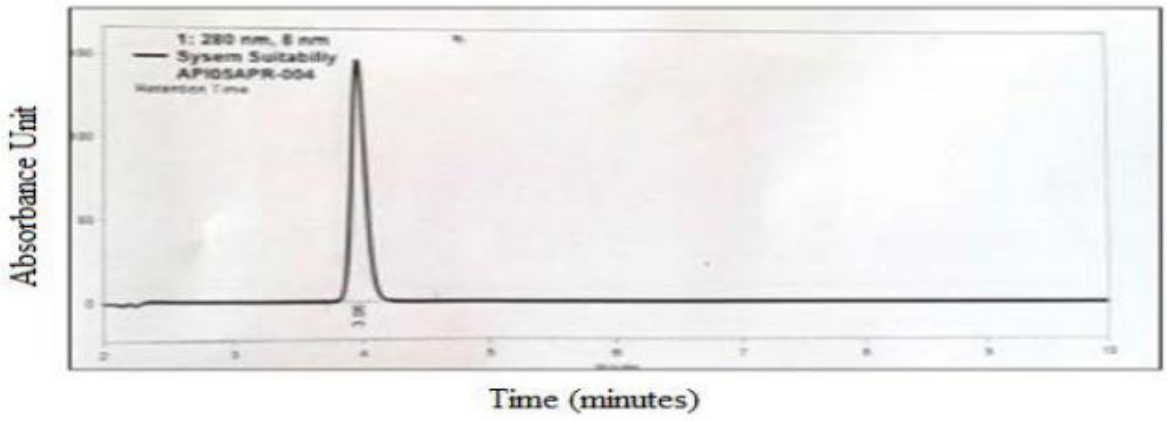

FIG. -3: CHROMATOGRAM OF STANDARD

ANALYTICAL METHOD VALIDATION:

The process of proving that analytical techniques are appropriate for their intended purpose is known as method validation. In order to establish documented proof that an operation or facility will consistently produce products that meet a set of specifications, analytical method validation is more particularly the process of doing so. System Suitability, Specificity, Precision, Linearity, LOD & LOQ, Accuracy (Recovery), and Robustness were taken into consideration.

METHOD VALIDATION

The process of determining through laboratory tests if the method's performance characteristics satisfy the criteria for the intended analytical application is known as validation of the analytical method. The International Conference on Harmonisation (ICH) recommendations for the validation of analytical procedures were followed in the development and validation of HPLC and UV Spectrophotometric methods. The methods' linearity, accuracy, system precision, day-to-day precision, limit of detection (LOD), and limit of quantitation (LOQ) parameters were all validated.

SYSTEM SUITABILITY TEST FOR HPLC:

Asymmetry (A), theoretical plates, and other system suitability parameters were calculated and compared to a reference value.

TABLE -4.: SYSTEM SUITABILITY STUDIES RESULTS (HPLC METHOD).

S.No.	Parameters	HPLC METHOD
1	Retention time(Rt)	**3.96**
2	Number of theoretical plates(N)	**5674**
3	Peak asymmetry (A)	**1.21**

SPECIFICITY

The peaks found in the standard solution and sample solution at working concentration for blank are exclusively caused by the medicines since blank has no peak during the retention time of apixaban, according to a mixing of standards drug solution and sample chromatogram. Consequently, it can be said that the developed method is said to be specific.

PRECISION

SYSTEM PRECISION - The absorbance of the reference solution (UV Method) at working concentration demonstrated % RSD (Relative reference Deviation) less than 1 in six replicate recordings, indicating acceptable repeatability and subsequently the accuracy of the system. Results for system precision are presented in Table.

TABLE -5: SYSTEM PRECISION RESULTS OF APIXABAN.

S.No	HPLC METHOD	UV METHOD
n	Area	Absorbance
1	931308	0.80
2	937062	0.798
3	936473	0.797
4	938276	0.799
5	926865	0.796
6	944462	0.802
Mean	935741	0.799
% RSD	0.65	0.25

METHOD PRECISION

By creating an assay of a sample and testing its repeatability at working concentration for both procedures, method precision was obtained.

TABLE -6: METHOD PRECISION RESULTS OF APIXABAN DRUG SUBSTANCE.

S.No	HPLC METHOD	UV METHOD
n	**Assay**	**Assay**
1	99.90	100.00
2	100.97	100.13
3	99.61	100.63
4	99.61	100.63
5	100.99	99.87
6	99.88	100.13
Mean	**100.16**	**100.23**
%RSD	**0.65**	**0.32**

LINEARITY - For both procedures, a methanolic solution of the drug with a concentration range of 5–50 g/ml was prepared in order to create the calibration curve. Concentration v/s peak area (HPLC technique) and concentration v/s absorbance (UV method) were used to plot the linearity graph, and linear regression analysis was utilised to evaluate the linearity.

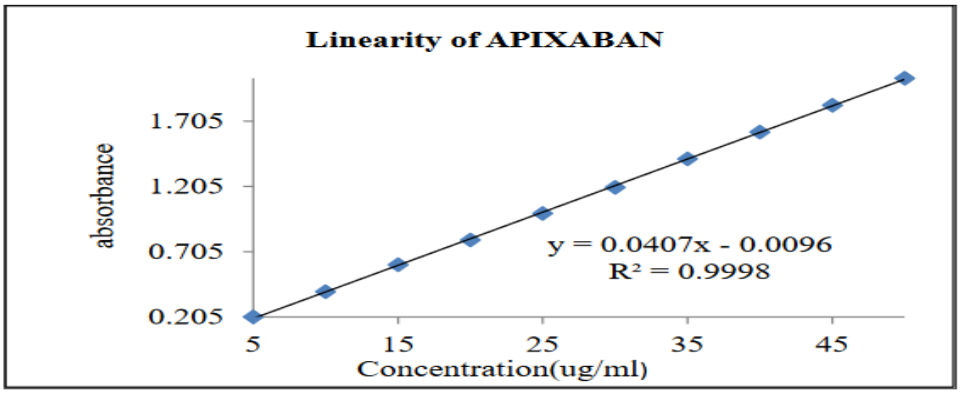

FIG. -4: LINEARITY GRAPH OF APIXABAN (UV METHOD)

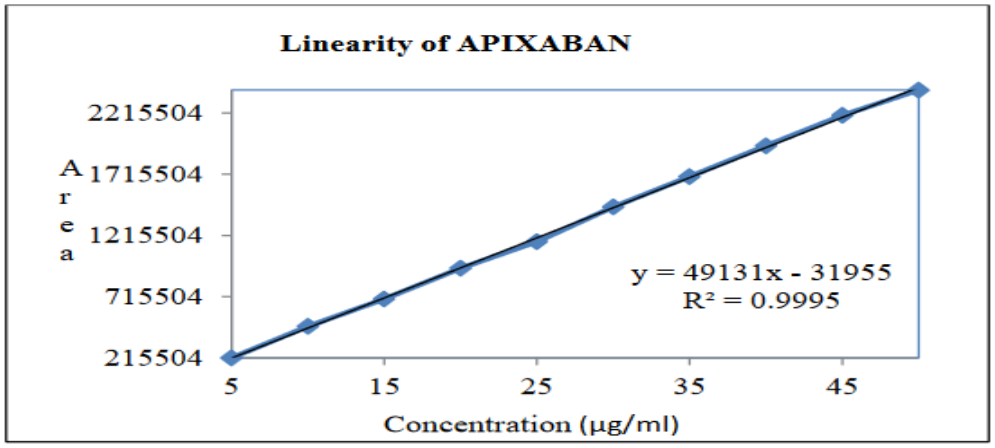

FIG-5: LINEARITY GRAPH OF APIXABAN (HPLC METHOD)

TABLE- 7: CALBRATION DATA FOR APIXABAN.

S.No	Concentration(µg/ml)	HPLC Area	UV Absorbance
1	5	215504	0.205
2	10	473058	0.399
3	15	697488	0.606
4	20	947961	0.795
5	25	1164331	0.999
6	30	1449075	1.198
7	35	1696996	1.416
8	40	1948152	1.621
9	45	2195960	1.827
10	50	2403046	2.033

TABLE -8 : OPTICAL CHARACTERISTICS OF APIXABAN DRUG SUBSTANCE.

Parameters	Results	
	HPLC Method	UV Method
Detection wavelength(nm)	280	280
Beer's law limits (µg/ml)	5 – 50	5 – 50

Regression equation (y = mx+c)	y=19131x-31955	y=0.0407x-0.0096
Correlation coefficient (r^2)	0.999	0.999
LOD (µg/ml)	1.16	0.73
LOQ (µg/ml)	3.52	2.21

LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)SENSITIVITY:

The limits of quantitation (LOQ) and detection (LOD) used to determine the sensitivity of measuring apixaban using the described methodologies.The equations LOD=3.3/S and LOQ=10/S, where is the standard deviation of response of calibration plots and s is the slope of the corresponding calibration plot, were used to determine LOQ and LOD. LOQ and LOD were calculated for both approaches using data from the linearity curve. The limit of quantitation (LOQ) and limit of detection (LOD) were used to determine the sensitivity of measurement of the assay using the proposed approach. According to the HPLC method and the UV method, the limit of detection (LOD) and limit of quantitation (LOQ) were found to be 1.16 g/ml and 3.52 g/ml, respectively. Results for optical properties are compiled.

ACCURACY- The recovery experiments, sometimes referred to as the standard addition method, are carried out by adding a known quantity of the reference medications to a solution of a known concentration of a commercial pharmaceutical product that has already been examined. The recovery investigations were carried out by adding 10, 20, or 30 g/ml of solution of standard medication to solution of tablet that had previously been examined.

TABLE -9: RESULTS OF ACCURACY STUDIES FOR APIXABAN BY UV SPECTROPHOTOMETRIC METHOD.

Accuracy (Average of triplicates)	Level-I	Level-II	Level-III
Added (µg/ml)	10.0	20.0	30.0
Found (µg/ml)	9.78	19.96	29.82
Recovery (%)	97.8	99.8	99.4
RSD (%)	0.36	0.40	0.6

TABLE -10: RESULTS OF ACCURACY STUDIES FOR APIXABAN BY HPLC METHOD

Accuracy (Average of triplicates)	Level-I	Level-II	Level-III
Added (µg/ml)	10.0	20.0	30.0
Found (µg/ml)	9.95	19.96	30.28
Recovery (%)	99.5	99.7	100.9
RSD (%)	0.31	0.38	0

ROBUSTNESS:

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

By comparing system appropriateness characteristics found by altering the HPLC pump flow rate (5%), mobile phase composition (5%), and column temperature (2°c), the robustness of the approach was examined.

ULTRA VIOLET SPECTROPHOTOMETER:

An analytical method's robustness is a measure of its ability to be unaffected by subtle but intentional changes to method parameters and offers a clue as to its dependability under typical conditions. The robustness of the method is concluded.

CONCLUSION

For the estimation of Granisetron in bulk medications and formulations, a straightforward, precise, and repeatable reverse phase UPLC method was created. The optimised procedure uses Acetonitrile in gradient elution mode with a mobile phase pH 6.5 ammonium acetate buffer, a run time of 4 min, and a flow rate of 0.4 ml/min. UV detection was done using a 2 L injection volume and a 305 nm wavelength. Acquity BEHC18 column, 1.7 mm, 100 mm, and 2.1 mm. Granisetron's retention time was discovered to be 1.75 minutes. The created technique was approved in accordance with ICH Q2A (R1) guidelines. Over the range of 10.22-30.50 g/ml, the suggested UPLC method was linear; a correlation coefficient of 0.999 was discovered. Relative standard deviation was determined to be 0.32 for procedure precision and 0.34 for intermediate precision. Limits of Quantification and Detection were found to be 0.77 g/ml and 0.25 g/ml, respectively. In order to determine Granisetron pharmaceutical dosage forms, a new UPLC method was created and validated. This method ensured adequate precision and accuracy as well as the ability to determine lower drug concentrations in solid dosage forms using the RP-UPLC method. The procedure was discovered to be easy, precise, affordable, and quick; it can be used for normal laboratory analysis,

is appropriate for quality control of raw materials, formulations, and dissolution investigations, and it may be used for bioequivalence studies for the same formulation. The method that is intended to determine the enantiomer content of 2, 6-trans dimethyl morpholine by HPLC for 2, 6-trans dimethyl morpholine determined to be exact, linear, accurate, and robust, according to the data of the method validation parameters mentioned above.

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