ESTIMATION OF DUTASTERIDE AND TAMSULOSINE BY RP-HPLC

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Abstract

Pharmaceutical analysis plays a major role today and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principle from various branches of science like chemistry, physics, microbiology, etc., pharmaceutical analytical techniques are applied mainly in two areas viz., qualitative analysis and quantitative analysis, although there are several other applications. A simple, sensitive and precise RP-HPLC method was developed for the determination of dutasteride in tablet dosage form. The RP-HPLC separation was achieved on phenomenex C18 column (250 mm, id 4.6 mm, 5 µm) using mobile phase methanol:water (90:10 v/v) at a flow rate of 1 ml/min at an ambient temperature.

INTRODUCTION TO PHARMACEUTICAL ANALYSIS:

Drug and pharmaceuticals are chemicals or like substance, which are of organic, inorganic or other origin. Whether may be the origin, we use some property of the medicinal agents to measure them quantitatively and qualitatively.

Pharmaceutical analytical techniques, which are being used, can be categorized as following:

Spectral methods:

Where we use light absorption or emission characteristics of drugs (eg) UV Spectroscopy, IR spectroscopy, NMR spectroscopy, etc.,

Chromatographic methods:

Where we use affinity or partition coefficient differences between drugs (eg) thin layer chromatography (TLC), high performance liquid chromatography (HPLC), paper chromatography etc.,

Electro analytical technique:

Based on the electrochemical property of drugs (eg) potentiometry, conductometry, polarometry, amperometry etc.,

Biological and microbiological methods:

Where we use either animals or microorganisams for analysis (eg) boiological assay of antibiotics and vitamins

Radioactive methods:

Like radio immuno assay and related technique are used.

Physical methods:

Where we use measure some physical characteristics of drugs eg: differential thermal analysis (DTA), differential scanning colorimtry (DSC), thermo mechanical analysis (TMA) etc.,

CHROMATOGRAPHY:

It is an analytical technique that is used to separate the mixture in solution in toindividual components.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

In the modern pharmaceutical industry, HPLC is a major analytical tool applied at

all stages of drug discovery, development and production. Fast and effective development of rugged analytical HPLC methods is more efficiently undertaken with a thorough understanding of HPLC principles, theory and instrumentation.

Liquid Chromatography (LC), which is one of the forms of Chromatography, is an analytical technique that is used to separate a mixture in solution into its individual components. The separation relies on the use of two different "phases" or "immiscible layers," one of which is held stationary while the other moves over it. The separation occurs because, under an optimum set of conditions, each component in a mixture will interact with the two phases differently relative to the other components in the mixture. HPLC is the term used to describe Liquid Chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. An HPLC instrument, therefore, consists of an injector, a pump, a column, and a detector.

Table.1.1: Various Types and Applications of HPLC

TYPE	SAMPLE POLARITY	MOLECULAR WEIGHT RANGE	STATIONARY PHASE	MOBILE PHASE
Adsorption	non-polar to somewhat polar	10^0 - 10^4	silica or alumina	non-polar to polar
Partition (reversed-phase)	non-polar to somewhat polar	10^0 - 10^4	non-polar liquid adsorbed or chemically bonded to the packing material	relatively polar
Partition (normal-phase)	somewhat polar to highly polar	10^0 - 10^4	highly polar liquid adsorbed or chemically bonded to the packing material	relatively non-polar
Ion Exchange	highly polarto ionic	10^0 - 10^4	ion-exchange resins made of insoluble, high-molecular weight solids functionalized typically with sulfuri	aqueous buffers with added organic solvents to moderate solvent strength

			c acid (cationic exchange) or amine (anionic exchange) groups	
Size-Exclusion	non-polar to ionic	$10^3 - 10^6$	small, porous, silica or polymeric	polar to non-polar
			particles	

As soon as the instrumentation has been selected, based totally at the criteria advised above, it's far critical to decide analyte parameters of hobby. To increase a technique it's far necessary to don't forget few houses of the analytes, most beneficial ranges of analyte parameter values. Instance consist of capacity issue (a goal variety of k
=2 to ten is generally applicable), UV-VIS wave duration of detection, m/e ratio to be scanned and most fulfilling emission wave lengths.

Once the instrumentation has been assembled and analyte parameter had been considered, requirements have to be used for the continued improvement, optimization and initial assessment of the method.

initial analytical figures or benefit should be ascertained together with sensitivity, measured as reaction in step with quantity (attention or mass) injected limits of detection; limit of quantitiation; linearity of calibration plots; and multi-detector ratios.

It's miles essential that technique improvement be carried out the use of simplestanalytical standards those had been well recognized and characterised wherein purity is already known.

METHOD DEVELOPMENT:

The development of a way of evaluation is commonly based totally on earlier artwork of present literature, the usage of the equal or quite comparable instrumentation. Within the development level, selections regarding desire of column, mobile phase, detectors and technique of quantitiation need to be addressed. In this manner, improvement considers all the parameters touching on any technique.

Optimization:

for the duration of optimization level, the initial units of conditions that have evalued shape the first degree of improvement are progressed are maximized in terms of decision, peak form, plate counts, asymmetry, potential, elution time, detection limits, limit of quantitiation and overall potential to quantify the precise analyte of interest. while optimizing any method, an strive have to be made to provide analytical figures of merit which might be had to meet the assay necessities described at the initial tiers of approach development.

If the initial analytical records defined form the approach seems promising, it's miles essential to evaluate

its performance quantitatively. The scope of the methodassessment must be extensive sufficient to consist of technology of statistics that is without delay usable for affirmation of the analyte in any pattern for instance UVabsorbance or mass spectra.

Other capability optimization dreams consist of baseline decision of the analyte of interest from other pattern aspect, unique height identification, on-line demonstration of purity, and interfacing of automatic records for routine pattern analysis. Absolute quantitiation must use simplified methods that require minimal pattern handing and evaluation time. Optimization standards must be determined with awareness of the desires not unusual to any new approach, consisting of decreased evaluation time and fee and correct identity of the analyte.

To verify that the optimized technique satisfies the dreams of unequivocal analyte identification and quantitiation, stepped forward quantitative accuracy and precision, faster sample turnaround time, absence of interference, and automation, positive standard criteria can be considered:

- Chromatographic resolution is good enough.
- For maximum samples, limits of detection are decrease by at least one order of value than needed.
- Calibration plots are linear over numerous orders of value, starting with limits of quantitiation. Sample through put is elevated with minimal device equilibration.
- Pattern practise before evaluation is minimized.
- Interference is minimized and diagnosed and strategies are established to avoid such troubles.
- Information is acquired via laptop or reporting integrator and may be manipulated, translated, interpreted, published or stored in diverse bureaucracy. If applicable, laptop software program permit for rapid acquisition, storage and manipulation of facts in both a stand-by myself paintings station or customer-server environment.
- Reproducibility of analytical figures of benefit is confirmed, with suited accuracy and precision.
- Value in step with analysis is minimized.
- Machine optimization is one of the maximum time- and energy-ingesting elements of the general approach development method. It calls for an iterative procedure, constant replication, and the purchase of a huge amount of quantitative information. Too often, an optimization outcome in a method that meets the instantaneous necessities of the analyst however ignores viable destiny wishes. Preferably, the analyst optimize each new approach to the fullest realistic quantity, on the way to make certain a broad application of the approach and obviate the repetition of experiments for future techniqueimprovement.

Optimization of the method yield maximized sensitivity, height symmetry, minimized detection and quantitiation limits, a extensive linear dynamic range and a excessive diploma of accuracy and precision.

VALIDATION

Validation can be regarded as the establishment of an experimental facts base that certifies an analytical technique plays

in the way for which it changed into intended and is the obligation of the approach development laboratory. Approach transfer, alternatively, is the creation of a tested approach into a designated so that it can be used within the same potential for which it was at first developed. .

Validation is described as follows by means of exclusive agencies:

European Committee (EC):

Motion of imparting in accordance with the concepts of desirable manufacturing practicethat any method, procedure, device, material, hobby or system truly ends in the predicted consequences. In short validation is a key procedure for effective first-class guarantee.

Food and Drug Administration (FDA):

Provides a high degree of assurance that specific process will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO):

Action of presenting that any technique, method, equipment, fabric, interest or systemvirtually results in the expected effects.

Analytical method validation:

Method validation is the procedure to affirm that the analytical method hired for a selected test is suitable for its supposed use. strategies need to be confirmed or revalidated.

- earlier than their advent into ordinary use
- Every time the situations change for which the approach has been proven, e.g.,devices with extraordinary characteristics.
- Every time the technique is changed, and the change is out of doors the authentic scope of the technique. The international convention on Harmonization (ICH) of Technical requirements for the Registration of Pharmaceutical for human use has advanced a consensus text on the validation of analytical tactics. The report includes definitions for eight validation traits.

Additives of approach validation:

Chromatographic methods validation is subdivided into four categories:

Category 1: validation of analytical strategies for assay.

Category 2: validation of analytical techniques for impurities and degrdents.

Category 3: validation of analytical techniques for dissolution.

Category four: validation of analytical techniques for identification

The parameters as defined via the ICH and by way of different companies are;

Precision:

"The precision of an analytical procedure expresses the closeness of agreement (diploma of scatter) among a sequence of measurements obtained from multiple sampling of the identical homogeneous pattern below the prescribed conditions. Precision may be taken into consideration at three stages; repeatability, intermediate precision and reproducibility."

Precision have to be acquired preferably the use of real samples. As parameters, the same old deviation (SD), the relative popular deviation (coefficient of variant) and theself assurance c language ought to be calculated for each degree of precision.

Repeatability expresses the analytical variability underneath the equal

working conditions over a short c programming language of time (within-assay, intra-assay). at the least nine determinations masking the required variety or six determinations at a hundred % test attention have to be accomplished. Intermediate precision includes the affect of extra random effects within laboratories, in step with the meant use of the procedure, for example, exclusive days, analysts or system, etc.

Reproducibility, i.e., the precision among laboratories (collaborative or interlaboratory studies), isn't required for submission, but can be taken into account for standardization of analytical procedures.

Specificity:

"Specificity is the capacity to evaluate unequivocally the analyte within the presence of additives which can be predicted to be present. usually those would possibly include

impurities, degradants, matrix, and so on. loss of specificity of an man or woman technique can be compensated with the aid of other assisting analytical method(s)". With recognize to identification, discrimination between carefully associated compounds in all likelihood to be gift ought to be tested through nice and poor samples. inside the case of chromatographic assay and impurity exams, available impurities / degradants can be spiked at suitable ranges to the corresponding matrix or else degraded samples can be used. For assay, it could be confirmed that the result is unaffected with the aid of the spiked fabric. Impurities need to be separated in my opinion and/or from different matrix additives. Specificity can also be demonstrated through verification of the end result with an impartial inside the case of chromatographic separation, decision factors need to be received for important separation. checks for height homogeneity, as an instance, by way of diode array detection (DAD) or mass spectrometry (MS) are encouraged.

Linearity:

"The linearity of an analytical manner is its potential (within a given variety) to

attain take a look at consequences that are immediately proportional to the attention (quantity) of analyte in the pattern".

It could be tested without delay at the analyte, or on spiked samples using at the least five concentrations over the complete operating variety. besides a visual evaluation of the analyte sign as a characteristic of the concentration, suitable statistical calculations are encouraged, including a linear regression. The parameters slope and intercept, residual sum of squares and the coefficient of correlation need to said. A graphical presentation of the statistics and the residuals is suggested.

Range:

"The variety of an analytical method is the interval between the higher and lower concentration (amounts) of analyte within the pattern (together with those concentrations) for which it has been demonstrated that the analytical process has a suitable level of precision, accuracy and linearity."

Limit of detection (LOD):

"The detection restrict of an individual analytical process is the bottom amount of analyte in a sample which can be detected

but now not always quantitated as an specific cost. The quantitation restriction of an person analytical process is the bottom awareness of analyze in a pattern which can be quantitatively determined with appropriate precisionand accuracy."
Various approaches can be applied:

Visual definition

Calculation from the signal-to-noise ratio (LOD and LOQ correspond to 3 or 2 and 10times the noise level, respectively)
Calculation from the standard deviation of the blank Calculation from the calibration line at low concentrationsLOD; LOQ ¼ F_SD b (2.6-1)
F: factor of 3.3 and 10 for LOD and LOQ, respectively

SD: standard deviation of the blank, standard deviation of the ordinate intercept, or residual standard deviation of the linear regression
b: slope of the regression line

The estimated limits should be verified by analyzing a suitable number of samples containing the analyte at the corresponding concentrations. The LOD or LOQ and the procedure used for determination, as well as relevant chromatograms, should be reported.**Limit of Quantitation (LOQ):**
The quantitation restrict is the bottom degree of analyte that can be accurately and precisely measured. This limit is needed most effective for impurity strategies and is decided via lowering the analyte attention until a level is reached where the precision of the method is unacceptable. If not determined experimentally, the quantitation restriction is frequently calculated because the analyte concentration that offers S / N = 10. An instance of quantitation restriction criteria is that the limit will be described as the bottomattention level for which an RSD 20 % is obtained whilst an intra-assay precision study isachieved.

Robustness:

According to ICH Q2A [1a] "the robustness of an analytical method is a measure of its capacity to remain unaffected by means of small, but planned variations in techniqueparameters and affords an indication of its reliability in the course of normal utilization". moreover, it's miles said in ICH Q2B [1b], "The assessment of robustness need to be taken into consideration during the development phase and depends on the sort of system below observe. It must show the reliability of an analysis with respect to deliberate versions in approach parameters. If measurements are susceptible to versions in analytical conditions, the analytical situations need to be suitably controlled or a precautionary announcement ought to be blanketed within the process. One consequence of the evaluation of robustness must be that a chain of machine suitability parameters (e.g., resolution test) is established to make sure that the validity of the analytical manner is maintained whenever used".

Ruggedness:

"The ruggedness of an analytical approach is the diploma of reproducibility of test effects obtained by using the analysis of the identical samples under a spread of situations, which include one of a kind laboratories, special analysts, distinct units, exceptional days, and so forth. Ruggedness is typically expressed as the lack of impact on take a look at consequences of operational and environmental variables of

the analytical method. Ruggedness is a measure of reproducibility of test effects beneath the version in conditions normally expected from laboratory to laboratory and from analyst to analyst". The degree of reproducibility is then evaluated by using assessment of the results obtained beneath varied situations with the ones under preferred conditions.

STABILITY-INDICATING METHOD (SIM):

According to FDA guidance, a balance-indicating technique is "a confirmed quantitative analytical technique which can hit upon the adjustments with time in the pertinent properties of the drug substance and drug product. A balance-indicating method as it should be measures the active elements, without interference from degradation products, technique impurities, excipients, or different potential impurities."

High performance liquid chromatography (HPLC) is an quintessential analytical device in assessing drug product stability. HPLC methods must be able to separate,detect, and quantify the various drug-associated degradants that could shape on storage or production, plus discover and quantify any drug-associated impurities that can be introduced for the duration of synthesis.

Pressured degradation research (chemical and physical strain testing) of latest chemical entities and drug products are essential to assist broaden and reveal the specificity of such balance-indicating techniques. further to demonstrating specificity, forced degradation research can be used to decide the degradation pathways and degradation products of the APIs that might form at some stage in storage, and facilitate components development, manufacturing, and packaging. Tactics for the guidance of precise degradation products needed for approach validation regularly emerge from these studies.

Purpose of forced degradation studies

- Understanding degradation mechanism
- Identification of potential degradents
- Development of stability indicating method
- Selection of compounds and excipients
- Optimization of manufacturing condition

Stress testing:

In order to establish whether the analytical method and assay was stability indicating, API and its formulation are stressed under various conditions to conduct forced degradation studies.

Types of Stress Testing:

Acid based testing:

Acid-Base stress checking out can be performed on both the drug substance and on intermediates. When the favored drug product is a solution dosage shape, acid-base studies are used greater regularly. Diverse pH range combos may be used to carry out acid-base studies at the drug substance. Most of the time use of low temperature levels (ambient-70^{0}C) is used for acid-base studies at the drug substance but extreme conditions also are used if the drug substance doses now not degrade

Oxidation:

Oxidation strain checking out can also be accomplished on both the drug substance and on intermediates. Especially

hydrogen peroxide is used as an oxidative agent with diverse awareness changes

Photo-stability Studies:

Photo-Stability testing can be performed on the drug substance and the drug product. kindof 16% of the agencies perform photo-balance pressure testing on intermediates. One organization carry out strain testing most effective on the drug substance and every other organisation carry out pressure trying out best on the drug product. most people of groups (sixty three%) use ICH wellknown for his or her standard visible-light dose range. 37% use a selection greater than the ICH wellknown.

Thermal-humidity studies:

All organizations carry out thermal-humidity strain trying out at the drug substance. Ninety% of agencies carry out pressure checking out at the drug product and 20% perform pressure checking out on intermediates. Maximum agencies perform thermal- humidity stress checking out studies in each open and closed bin.

Conditions generally employed for forced degradation

egradation type	Experimental conditions	Storage conditions	Sampling time
Hydrolysis	Control API (no acid no base)	40°C, 60^{0}C	1,3,5 days
	0.1N HCL	40°C, 60^{0}C	1,3,5 days
	0.1NNAOH	40°C, 60^{0}C	1,3,5 days
	Acid control (no API)	40°C, 60^{0}C	1,3,5 days
	Base control (no API)	40°C, 60^{0}C	1,3,5 days
	pH 2,4,6,8	40°C, 60^{0}C	1,3,5 days
Oxidative	3% H2O2	25°C, 40^{0}C	1,3,5 days
	Peroxide control	25°C, 40^{0}C	1,3,5 days
	Azobisisobutyronitrile(AIBN)	40°C, 60^{0}C	1,3,5 days
	AIBN control	40°C, 60^{0}C	1,3,5 days
Photolytic	Light 1 x ICH	NA	1,3,5 days
	Light 3 x ICH	NA	1,3,5 days
	Light control	NA	1,3,5 days
Thermal	Heat chamber	60^{0}C	1,3,5 days
	Heat chamber	60^{0}C/ 75%RH	1,3,5 days
	Heat chamber	80^{0}C	1,3,5 days
	Heat chamber	80^{0}C/ 75%RH	1,3,5 days
	Heat control	Room temp	1,3,5 days

Conclusion

The estimation of Dutasteride and tamsulosin hydrochloride changed into achieved by way of RP-HPLC. The Phosphate buffer was pH 2.5 and the cell segment changed into optimized which includes Acetonitrile: Phosphate buffer combined within the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, five µm,

Make X Terra) column used as desk bound segment. The detection turned into performed the usage of UV detector at 274 nm. The answers were chromatographed at a consistent go with the flow price of 0.8 ml/min. the linearity variety of Dutasteride and tamsulosin hydrochloride have been discovered to be from 25-one hundred twenty five µg/ml. Linear regression coefficient was now not extra than zero.999. The values of % RSD are much less than 2% indicating accuracy and precision of the technique. The proportion restoration varies from 97-102% of dutasteride and tamsulosin hydrochloride LOD and LOQ changed into discovered to be inside limit. There was a sizable degradation in the presence of zero.1N HCl, 0.1N NaOH, 3% H2O2 and additionally on heat. C18 column guarantees better peak form, higher decision and lower stress at some stage in operation. So the approach is stability indicating. The proposed technique is specific, simple and correct to determine the quantity of dutasteride and tamsulosin in components.

Excessive percentage of recovery indicates that the technique is unfastened from the interference of excipients used in the formulation. So the method may be beneficial within the routine high-quality manage of those drugs.

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