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Stability Indicating RP-UPLC Method-Development and Validation

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ABSTRACT:
Analytical method development and validation play an important role in the discovery, development, and manufacture of API's or pharmaceuticals. Method development is the process of proving that an analytical method is acceptable for use to measure the concentration of an Active Pharmacuetical Ingredient in a specific compounded dosage form which allows simplified procedures to be employed to verify that an analysis procedure, accurately and consistently will deliver a reliable measurement of an active ingredient in a compounded preparation. The analytical method validation is essential for analytical method development and tested extensively for specificity, linearity, accuracy, precision, range, detection limit, quantitation limit, and robustness. In summary, analytical method development and validation allow confirming that an accurate and reliable potency measurement of a pharmaceutical preparation can be performed. Most of the drugs in multi-component dosage forms can be analyzed by the UPLC method because of the several advantages like rapidity, specificity, accuracy, precision, and ease of automation in this method. UPLC methods development and validation play important roles in new discovery, development, manufacture of pharmaceutical drugs, and various other studies related to humans and animals. An analytical procedure is developed to test a defining characteristic of the drug substance or drug product against established acceptance criteria for that characteristic. This review gives information regarding various stages involved in the development and validation of the UPLC method.

KEYWORDS: Ultra performance liquid chromatography (UPLC), RP-UPLC, Validation, HPLC, LOD.

I. INTRODUCTION:

Ultra performance liquid chromatography (UPLC) system is an innovative product that revolutionized high performance chromatography by outperforming conventional high performance liquid chromatography (HPLC UPLC reduces sample run times by a factor of 10, using the above 95% less solvent and significantly improves productivity in the laboratory. UPLC achieves speed by using novel sub two-micron particles that reduce chromatographic run time and resolution. UPLC was designed as a total system to take advantage of both ultra-high pressure and small Particle separation characteristics resulting in exceptionally superior performance Significant improvement in resolution, sensitivity and speed. [1]

UPLC system will eliminates significant time and cost per sample from analytical process while improving the quality of results, the system allows chromatographers to work at higher efficiency, flow rate, and backpressure. UPLC photodiode array detector (pda) detects and quantifies the lower concentration of sample substance, detects the impurities with the maximum sensitivity and compares spectra across wavelengths and broad concentration range. [1]

Ultra-Performance Liquid Chromatography (UPLC) uses sub-2 micron particles to effect dramatic increases in higher linear solvent velocity resolution, sensitivity and analysis speed. Particle size reduction to less than 2 micrometers is required instrumentation that can operate under pressure in the 6000-15,000 psi range. typical the peak width generated by the uplc system is 10-min. occurs on the order of 1-2 s for a 8-10 min separation. In the present work this technique is applied to in vivo studies metabolism, in particular the analysis of drug metabolites in bile. A decrease in the peak width increases the analytical sensitivity by up to three five-fold, and a decrease in peak width, and a concomitant increase in peak capacity, significantly reduces the spectral overlap resulting in MS. Both have better spectral quality and MS/MS mode. Application of UPLC/MS resulted in detection of excess drug metabolites, better separation and better spectral quality. [2]

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II. CHEMISTRY OF SMALL SIZE PARTICLES :

The chemistry of the particles used in this course of the method contributes the increased efficiency and potential to work at amplified linear velocity, thereby, providing both the speed and the resolution. Efficiency is one of the important separation parameters that play a vital role in UPLC as it depends on selectivity and retention just like HPLC. This may be understood with the help of the following basic resolution (Rs) equation.

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

III. HPLC AND UPLC : COMPARISON

The characteristics of HPLC and UPLC and advantages of UPLC over HPLC are summarized in Table

Table 1: Comparison of HPLC and UPLC

CHARACTERISTICS	HPLC	UPLC
Particlesize	<4µm	1.7µm
Maximumbackpressure	35-40MPa	103.5MPa
Analyticalcolumn	Alltima C 18	AcquityUPLCBEHC 18
Columndimensions	150 X 3.2mm	150 X 2.1mm
Injectionvolume	20L	3-5L
Pressurelimit	upto4000psi	15000psi
Totalruntime	10min	1.5min

IV. UPLC :- ADVANTAGES:

Various advantages of UPLC are as follows:

- Decreases run time and increases sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- Maintaining resolution performance.
- Expands the scope of Multi-Residual Methods.
- The rapid resolving power of UPLC allows for the rapid determination of related and unrelated compounds.
- Rapid analysis of ultra-fine particle sizes through the use of innovative separation materials.
- Operation cost is reduced.
- Less solvent consumption.
- Reduces process cycle times, so that more product can be produced with existing resources.
- Increases sample throughput and enables manufacturers to produce more material Consistently meet or exceed product specifications, potentially eliminating Variability, failed batches, or the need to rework materials.
- Provides real-time analysis in steps with manufacturing processes.
- Ensures final product quality including final release. [7-9]

V. UPLC DISADVANTAGES:

Due to increased pressure it requires more maintenance because of increased pressure it reduces life of columns of these types. So far the same or even higher performance has been demonstrated using stable steps about 2 meters in size without the adverse effects of high pressure. Along with steps less than 2 m are generally non-producible and thus have limited use [7].

VI. APPLICATIONSOF UPLC:

i. Analysis of Natural Products & Traditional Herbal Medicines:

UPLC provides high-quality separations and detection capabilities to identify Active compounds in highly complex samples that are derived from natural products and traditional herbal medicines. Metabonomics-based analysis, using UPLC, exact mass MS, and Marker Lynx Software data processing for multi-variate statistical analysis, can help quickly and accurately characterize these medicine so their effect on human metabolism.

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ii. Identification of Metabolite:

Biotransformation of new chemical entities (NCEs) is essential for e-drugs Search. When a compound reaches the growth stage, metabolite identification becomes a regulated process. It is extremely important for the laboratory to be successful. Detection and identification of all circulating metabolites of a candidate drug. UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unparalleled sensitivity, , dynamic range, resolution and mass accuracy.

iii. Study of Metabonomics / Metabolomics :

Metabonomics / Metabolomics Metabonomics research is done in labs to accelerating the development of new drugs. Ability to compare and contrast extensively sampling groups provide insight into the biochemical changes that occur when a the biological system is being introduced into a new chemical business (NCE). Metabonomics provides a quick and solid way to experience these changes, improves understanding of potential toxins, and allows to monitor its effectiveness. In the correct implementation of metabonomic and metabolomic information helps the same discovery, development, and production processes in biotechnology as well
chemical industry companies. UPLC analysis rapidly generates and interprets information-rich data, allowing rapid and informed decisions to be made.

iv. ADME (Absorption-Distribution,-Metabolism-Excretion) Screening

ADME studies measure physical and biochemical properties – absorption, distribution, metabolism, elimination, and toxicity of drugs where such compounds exhibit activity against the target disease. Tandem quadrupole MS joins UPLC in the ADME trial of sensitivity and selectivity through rapid analysis of samples in the matrix to be achieved
small clearance, using MRM (multi-response monitoring) to recover automated compound optimization.

v. Bio-Analysis / Bio-Equivalence Studies:

Applications of UPLC/MS/MS in bio-equivalence and bio-analysis are: - In UPLC/MS/MS, LC and MS instruments and software combine in a sophisticated and integrated system for bio analysis and bio-equivalence studies, providing unprecedented performance and compliance support. UPLC/MS/MS delivers excellent chromatographic resolution and sensitivity. Increase the sensitivity of analyses, quality of data including lower limits of quantitation (LLOQ), and productivity of laboratory by coupling the UPLC System's efficient separations with fast acquisition rates of tandem quadrupole MS systems. Easily acquire, quantify and report full system data in a compliant environment using security-based data collection software. Ensure the highest quality results and reliable system operationin regulated environment.

vi. Dissolution Testing:

For quality control and release in drug manufacturing, dissolution testing is essential in the formulation, development and production process UPLC provides accurate and reliable online sample acquisition. It automates dissolution testing, from pill drop to test start, through data acquisition and analysis of sample aliquots, to the publication management of test results and distribution.

vii. Forced Degradation Studies

The FDA and ICH need stability test data to understand the quality of the API (active ingredient of a drug) or medicine product changes over time under the influence of environmental factors such as heat, light, pressure and humidity or humidity. UPLC combined with specific Photodiode array detector and MS detection will provide confidence in identifying harmful products and thus shortening the time required to develop sustainable methods.

Viii. Manufacturing / QA / QC:

Identity, purity, quality, safety and effectiveness are important factors to consider when producing a pharmaceutical product. . UPLC is used for highly controlled, quantitative analysis performed in QA / QC laboratories.

Xi. Method Development / Validation:

According to FDA, validation is defined as establishing documented evidence that provides a high level of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Method development and validation is a time-consuming and complicated process: labs need to **assess** multiple combinations of mobile phase, temperature, pH, column chemisty, and gradient profiles to arrive at a reliable, robust, separation for every activity.

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The following parts of UPLC are important to give the required information.

UPLC columns:

High stability allows for a wide range of column temperatures and pH's to be explored.

UPLC Column Manager: Easily evaluate column temperatures from 10 °C below the room temperature to 90 °C; enables to use HPLC methods on the UPLC before scaling to UPLC.

UPLC Calculator: Put information at fingertips about how to transition existing chromatographic analyses to faster UPLC methods.

x. Impurity Profiling

Impurity profiling requires high-resolution chromatography able to be reliable and reproduce to separate as well as detecting all of the known impurities of the active compound. UPLC System and Columns specifically address requirements for high impact analysis while maintaining high peak adjustment. The UPLC-PDA detector consists of two analytical flow cells which are available in great flexibility depending on the needs of the application, one for the high chromatographic correction and second for high sensitivity. UPLC too includes the latest high-value acquisition algorithms and custom calculations to improve data processing and reporting. It also confidently detects pollution on computers and even at trace levels..

xi. Compound Library Maintenance

The use of the fast-scanning MS along with the throughput of the UPLC System's remote status monitoring software allows chemists to obtain high-quality comprehensive data about their compounds in the shortest possible timeframes. This, combined with intelligent open access software, allows making informed decisions faster, and better supporting the needs of the modern drug discovery process.

xii. Open Access

UPLC and UPLC/MS systems and software enable versatile and open operation for medicinal chemistry labs, with easy-to-use instruments, a user-friendly software interface, and fast, robust analyses using UV or MS for nominal and accurate mass measurements.[7-16]

VII. VALIDATION STUDIES:

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate. with positive, well-documented reference materials should be used throughout the verification study. The level of cleanliness required depends on the intended use. In accordance with the parent document, and for the purpose of clarification, this document looks at various aspects of authentication in different categories. The classification of these sections indicates the process by which the analysis process can be developed and tested.

Validation of proposed RP-UPLC method by using the following Parameters:

ACCURACY: Accuracy should be achieved across the specified range of the analytical procedure. [3]

PRECISION: Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision. [3]

Intermediate Precision: Intermediate precision explains within-laboratories variations: different day/dates, different analysts /person, different equipments, etc. [3].

LINEARITY AND RANGE: Linearity (analytical range) is an assessment of the range by which results can be obtained without any need for dilution, reflecting the range in which there it is a direct proportional relationship between analyte concentration and signal. [3][6]

LIMIT OF DETECTION:

The detection limit of an individual analytical procedure is the lowest valueof analyte in a sample which can be detected but not necessarily quantitated as an exact value. The Limit of Detection is defined as the smallest concentration or amount of an analyte product that can be reliably detected in a given type of sample or medium by a specific measuring process. The United States Pharmacopeia (USP) defines the LOD as 2 or 3 times of the baseline noise. This is derived from the assumption that 3 times the noise will contain approximately 100% of the data from a normal distribution. [3][13].

LIMIT OF QUANTITATION:

The Limit of Quantification (LOQ) is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision is the lowest analysis value in the sample

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that can be determined by quantity with appropriate accuracy and precision. The quantitation limit is one of the parameters of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of large/trace impurities and/or degradation products. [3][5].

REPEATABILITY:

Repeatability should be checked using: a) a minimum of 9 determinations covering the stated range for the procedure (e.g., 4 concentrations/4 replicates each); or b) a minimum of 5-6 determinations at 100% of the test concentration. [3].

ROBUSTNESS:

The robustness of an analytical method or procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.[3].

ASSAY:

For Drug Substance several different methods of determining there accuracy are available nowadays by application of an analytical method or procedure to an analyte of known purity (e.g. reference material) accuracy may be inferred once precision, specificity and linearity have been established. [3].

Drug Products:- Methods for determining Accuracy :

a) Analytical procedure application of the to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;

b) System Suitability Parameters: System suitability parameter testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated and analyzed as such. System suitability testing parameters to be established for a particular method depend on the type of method being validated. [3]

VIII. FORCED DEGRADATION:

It is a degradation of new drug substance or drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods or procedures and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Forced degradation studies show the chemical behavior of the molecule which aids in the development of its formulation and packaging. In addition, the regulatory guidance is very general and does not explain about the performance of forced degradation studies. [4]

Methodology: The Specificity shall be demonstrated by performing Placebo / Blank interference and forced degradation studies.

1. Blank interference: As per the test procedure prepare blank solutions and analyze them as per the test procedure.
2. Placebo interference (For Drug products): Prepare equivalent placebo solutions to the test concentration (Subtract the weight of active ingredient) and analyze it as per the test method.
3. Force Degradation studies : The sample forcefully Degraded under the various stress conditions like heat, Light, , humidity, oxidation and acid/base/water hydrolysis and ensure the degradation and for its peak purity.[5].

CONCLUSION:-

The study in this review shows that the RP-UPLC method can be used for the assessment of drugs by accessing it's purity, solubility, stability and lipid-formulation release profile with no interference of excipients or related substances of API (active pharmaceutical ingredient). Along with validation parameters like accuracy, precision, linearity, range, LOD, LOQ and forced degradation make the method systematic for research and analysis purpose, and has various advantages over HPLC method.

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