IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
June 2023 Vol.:27, Issue:3

Stability Indicating RP-UPLC Method - Development and Validation

IJPPR
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ISSN 2349-7203
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Submitted: 22 May 2023
Accepted: 29 May 2023
Published: 30 June 2023

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Validation, Linearity, Precision, Range

ABSTRACT

The discovery, development, and production of APIs or medicines depend heavily on the development and validation of analytical methods. It is possible to use streamlined procedures to confirm that an analysis procedure, accurately and consistently, will deliver a reliable measurement of an active ingredient in a compounded preparation through the process of demonstrating that an analytical method is appropriate for use to measure the concentration of an Active Pharmaceutical Ingredient in a particular compounded dosage form. The validation of analytical methods is crucial for the development of analytical methods and involves rigorous testing for robustness, linearity, accuracy, precision, range, detection limit, and specificity. In conclusion, developing and validating analytical methods enables ensuring that a pharmaceutical preparation's potency may be measured in an accurate and trustworthy manner.

INTRODUCTION: -

By exceeding traditional high-performance liquid chromatography (HPLC), the ultra-performance liquid chromatography (UPLC) equipment revolutionized high-performance chromatography. By utilizing 95% less solvent and cutting sample run times by a factor of 10, UPLC greatly boosts lab productivity. Utilizing cutting-edge sub-two-micron particles that shorten chromatographic run times and improve resolution, UPLC achieves speed. The UPLC system was created as a whole to benefit from the features of both ultra-high pressure and small particle separation, producing a performance that is incredibly better in every way. [1]

The UPLC system enables chromatographers to work at increased productivity, flow rate, and backpressure while reducing substantial time and expense per sample from the analytical process. The UPLC photodiode array detector (pda) identifies and quantifies the sample substance at lower concentrations, detects contaminants with the highest level of sensitivity, and compares spectra across a wide range of concentrations. [1]

Sub-2 micron particles are used in Ultra-Performance Liquid Chromatography (UPLC) to achieve remarkable improvements in better linear solvent velocity resolution, sensitivity, and analytical speed. Particle size reduction to less than 2 micrometers necessitates the use of equipment capable of operating at pressures between 6000 and 15000 psi. The peak width produced by the plc system is about 10 minutes. occurs for an 8–10 minute separation on the order of 1-2 seconds. This method is used in the current work to study metabolism in vivo, namely the analysis of drug metabolites in bile. A decrease in peak width and a concurrent increase in peak capacity greatly minimize the spectral overlap that leads to MS. A drop in peak width boosts analytical sensitivity by up to three to five times.[remaining repeat and check]

- **CHEMISTRY OF SMALL-SIZE PARTICLES: -**

The chemistry of the particles utilized in this method's course leads to its improved effectiveness and capacity to operate at amplified linear velocity, so offering both speed and resolution. Efficiency is one of the crucial separation factors in UPLC since, like HPLC, it depends on selectivity and retention. The basic resolution (Rs) equation shown below can help you understand this.

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

- **HPLC AND UPLC: COMPARISON**

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

Table 1: Comparison of HPLC and UPLC

CHARACTERISTICS	HPLC	UPLC
Particle size	<4µm	1.7µm
Maximum backpressure	35-40MPa	103.5MPa
Analytical column	Altima C 18	Acquity UPLC BEHC 18
Column dimensions	150 X 3.2mm	150 X 2.1mm
Injection volume	20L	3-5L
Pressure limit	upto4000psi	15000psi
Total runtime	10min	1.5min

- **UPLC:- ADVANTAGES:**

The following are some of the benefits of UPLC: It Shortens run time and improves sensitivity.

Offers the LC analysis's selectivity, sensitivity, and dynamic range.

- Upholding resolution effectiveness.
- Widens the application of multi- residual methods.
- UPLC's quick resolving ability enables the quick identification of related and unrelated molecules.
- Through the application of cutting-edge separation materials, ultra-fine particle sizes can be quickly analyzed.
- Operation costs are decreased.
- Less use of solvents.

- Shortens process cycle times, enabling the production of more products using already available resources.

- Improves sample throughput, allowing producers to generate more product. Meet or surpass product criteria consistently, potentially reducing variability, failed batches, or the need to rework materials.

- Offers real-time analysis that corresponds to manufacturing procedures.

- Assures the quality of the finished product, including its release. [7-9]

- **UPLC DISADVANTAGES:**

It requires more maintenance due to the higher pressure, which also shortens the lifespan of these sorts of columns. Without the negative consequences of high pressure, steady steps of 2 meters in size have so far been used to exhibit the same or even higher performance. Steps shorter than 2 m are typically impractical for production and so have restricted usage [7].

❖ **APPLICATIONS OF UPLC:**

- **Analysis of Natural Products & Traditional Herbal Medicines:**

.To find Active components in extremely complex samples originating from natural goods and traditional herbal medicines, UPLC offers high-quality separations and detection capabilities. UPLC, precise mass MS, and Marker Lynx Software data processing for multi-variate statistical analysis are used in a metabonomics-based investigation to swiftly and accurately characterize these drugs and their impact on human metabolism.

- **Identification of Metabolite**

For the search for e-drugs, biotransformation of new chemical substances (NCEs) is crucial. The process of metabolite identification gets regulated after a chemical enters the development stage. For the laboratory to be effective, it is crucial. detection and identification of every medication candidate's circulating metabolite. By providing unmatched sensitivity, dynamic range, resolution, and mass accuracy, UPLC/MS/MS solves the intricate analytical needs of biomarker discovery.

- **Study of Metabonomics / Metabolomics**

Molecular genetics or metabolomics Research on metabonomics is carried out in laboratories to hasten the creation of novel medications. A thorough comparison and contrast of sampling groups can shed light on the biochemical alterations that take place when a biological system is incorporated into a new chemical enterprise (NCE). Metabonomics offers a quick and reliable approach to noticing these changes, enhances knowledge of potential toxins, and enables monitoring of their efficacy. Companies in the biotechnology and chemical industries benefit from the proper application of metabolomic and metabonomic information in the research, development, and production stages. Rapid generation and interpretation of information-rich data by UPLC analysis enable quick and well-informed decision-making.

- **ADME (Absorption-Distribution,- Metabolism-Excretion) Screening :**

Potential for the desired ailment. Tandem quadrupole MS joins UPLC in the ADME trial of selectivity and sensitivity by analyzing samples quickly in the matrix to achieve minimal clearance and recovering automated compound optimization using MRM (multi-response monitoring).

- **Bio-Analysis / Bio-Equivalence Studies:**

The following are examples of UPLC/MS/MS's use in bioanalysis and bioequivalence: For bioanalysis and bio-equivalence investigations, UPLC/MS/MS combines LC and MS equipment and software into a complex and integrated system, offering unmatched performance and compliance support. Excellent chromatographic resolution and sensitivity are provided by UPLC/MS/MS. By combining the effective separations of the UPLC System with quick acquisition rates of tandem quadrupole MS systems, you may improve the sensitivity of analyses, the quality of data, including lower limits of quantitation (LLOQ), and laboratory productivity. With the use of security-based data-collecting tools, it is simple to gather, quantify, and report whole system data in a legal setting. Ensure the best possible outcomes and dependable system performance in a controlled environment.

- **Dissolution Testing:**

Dissolution testing is crucial in the formulation, development, and production processes for quality assurance and medication release. Online sample capture is precise and dependable thanks to UPLC. It automates the entire dissolution testing process, from the administration

of the pills to the start of the test, data collection and analysis of sample aliquots, to the publication management and distribution of test findings.

- **Forced Degradation Studies:**

To understand how the quality of the API (the active component of a drug) or medicinal product changes over time under the effect of external conditions including heat, light, pressure, and humidity, the FDA and ICH need data from stability tests. The time needed to develop sustainable solutions will be shortened thanks to the use of UPLC in conjunction with a specialized Photodiode array detector and MS detection.

- **Manufacturing / QA / QC:**

When making a pharmaceutical product, identity, purity, quality, safety, and effectiveness are crucial considerations. In QA/QC laboratories, UPLC is utilized for rigorously controlled, quantitative analysis.

- **Method Development / Validation:**

Validation, as defined by the FDA, is the establishment of documented proof that offers a high level of assurance that a certain process will consistently result in a product fulfilling its intended standards and quality features. It takes a lot of time and effort to develop and validate a method since there are many different mobile phase, temperature, pH, column chemistry, and gradient profile combinations that must be considered.

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

❖ **UPLC columns:**

A wide variety of column temperatures and pHs can be investigated because of the high stability.

Use HPLC techniques on the UPLC before scaling to UPLC with the help of the UPLC Column Manager, which makes it simple to evaluate column temperatures from 10 °C below room temperature to 90 °C.

Use the UPLC Calculator to get information on converting current chromatographic analyses to UPLC procedures at your fingertips.

- **Impurity Profiling**

High-resolution chromatography that can reliably and repeatedly separate and identify all known impurities of the active molecule is necessary for impurity profiling. Specific criteria for high-impact analysis while preserving high peak adjustment are addressed by the UPLC System and Columns. The UPLC-PDA detector comprises two analytical flow cells, one for high chromatographic correction and the other for high sensitivity, both of which can be configured quite differently depending on the needs of the application. To enhance data processing and reporting, UPLC also uses the most recent high-value acquisition algorithms and bespoke calculations. Additionally, it accurately finds pollution on computers, even at very low levels.

- **Compound Library Maintenance**

Chemists can quickly receive complete, high-quality data about their compounds by using the fast-scanning MS in combination with the UPLC System's remote status monitoring software. This facilitates informed decision-making more quickly and better supports the demands of the contemporary drug discovery process when combined with sophisticated open-access software.

With simple-to-use equipment, a user-friendly software interface, and quick, reliable analyses employing UV or MS for nominal and accurate mass measurements, UPLC and UPLC/MS systems and software provide adaptable and open operation for medicinal chemistry labs.[7-16]

- **VALIDATION STUDIES:**

It is necessary to disclose and appropriately discuss any pertinent data gathered during validation as well as the formulas used to determine validation features. Throughout the verification investigation, reliable, well-documented reference materials should be consulted. Depending on the intended application, different levels of cleanliness are needed. This document examines many aspects of authentication in several categories by the parent document and for clarification. These parts' categorization reveals the method by which the analytical process can be created and put to the test.

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

- **ACCURACY**:

Accuracy must be attained within the analytical procedure's defined range. [3]

- **PRECISION**:

A precision study is part of the validation process for assay and quantitative impurity determination tests. [3]

- **INTERMEDIATE PRECISION:**

Intermediate precision describes variances found within laboratories, such as those caused by different days/dates, analysts, people, or equipment. [3].

- **LINEARITY AND RANGE:**

Linearity (analytical range) refers to the range in which findings can be acquired without the requirement for dilution, i.e., the range in which the analyte concentration and the signal have a direct proportional connection. [3][6].

LIMIT OF DETECTION:

The lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an accurate value is the detection limit of a specific analytical process. The smallest concentration or amount of an analyte product that may be accurately measured in a particular type of sample or medium and reliably detected is known as the limit of detection. The LOD is described by the United States Pharmacopoeia (USP) as being two or three times the background noise. This is based on the presumption that the data from a normal distribution will make up around 100% of the data when the noise is multiplied by three. [3][13].

- **LIMIT OF QUANTITATION:**

The lowest analyte concentration that may be quantitatively detected in a sample with a certain degree of accuracy and 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 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].

Precisely measured using quantity. One of the criteria of quantitative assays for low amounts of chemicals in sample matrices is the quantitation limit, which is especially useful for identifying large/trace contaminants and/or degradation products. [3][5].

- **REPEATABILITY**:

A minimum of 9 determinations covering the procedure's indicated range (for example, 4 concentrations with 4 replicates each) or a minimum of 5–6 determinations at 100% of the test concentration should be used to determine repeatability [3].

- **ROBUSTNESS**:

The robustness of an analytical method or procedure is a measure of its ability to be unaffected by the minute but intentional changes in method parameters and offers a clue as to its dependability under typical conditions.[3].

- **ASSAY:**

The application of an analytical technique to an analyte of known purity (such as reference material) can be used to determine the accuracy of a drug substance in several different ways today. Once precision, specificity, and linearity have been established, accuracy can be inferred. [3].

❖ **Drug Products:- METHODS FOR DETERMINING ACCURACY :**

Application of the analytical process to synthetic mixtures of the drug product's constituents that have known additions of the drug substance to be examined; System Suitability Parameters: Testing system appropriateness parameters is a crucial step in many analytical processes. The tests are founded on the idea that the tools, electronics, analytical processes, and test samples make up a whole system that can be assessed and examined as such. The parameters for system suitability testing that should be chosen for a certain technique depend on the kind of method that is being validated. [3]

- **FORCED DEGRADATION:**

It is a new drug substance or drug product degrading under settings that are more extreme than accelerated conditions. It is necessary to show the specificity of stability indicating

methods or procedures, and it also offers information on the drug substance's degradation pathways and byproducts and aids in illuminating their structural details. Studies on forced degradation help enhance a drug's formulation and packaging by illuminating the molecule's chemical behavior. Additionally, the regulatory guideline is highly vague and makes no mention of how forced deterioration studies are performed. [4]

- **METHODOLOGY:-**

The sample was subjected to various stress conditions, such as heat, light, humidity, oxidation, and acid/base/water hydrolysis, to enjoy conducting placebo/blank interference and forced degradation trials, the specificity will be proven.

Blank interference: Prepare blank solutions by the test protocol and analyze them by the test procedure.

Preparing comparable placebo solutions to the test concentration (deducting the weight of the that it was degraded and maintained at its highest active component) and analyzing them by the test technique.

Studies on Force Degradation sure purity.[5]

- **CONCLUSION: -**

The specificity will be demonstrated through the use of forced deterioration and placebo/blank interference trials.

Complete interference by the test protocol, prepare blank solutions and analyze them as directed by the test process.

Creating placebo solutions that are comparable to the test concentration (after subtracting the weight of the active ingredient) and evaluating them in line with the test method.

Research into Force Degradation To make that the sample was deteriorated and kept at its optimum purity, it was exposed to a variety of stress conditions, including heat, light, humidity, oxidation, and acid/base/water hydrolysis.[5].

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