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A COMPREHENSIVE REVIEW ON STABILITY INDICATING METHOD DEVELOPMENT USING UHPLC

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ABSTRACT

Stability indicating methods (SIMs) are critical in the pharmaceutical industry to ensure the quality, safety, and effectiveness of drug products. Due to its improved speed, resolution, and sensitivity, the development of these methods on ultra-high performance liquid chromatography (UHPLC) platforms has attracted a lot of attention. The goal of the stability indicating approach is to resolve all of the main degradants and any probable process-related contaminants by achieving the right mass balance during the forced degradation. Stability indicating method is capable to measure the active substance and all its degradation products unequivocally in presence of excipients present in the respective formulation. The power of any stability indicating analytical method is to be proved in stress study by identifying its degradation products which in turn establishes the innate stability of the molecule and its degradation pathways. This review aims to provide an in-depth analysis of the latest advances and challenges encountered in the development of stability indicating methods using UHPLC.

KEYWORD:- Stability indicating method, UHPLC, degradation products, validation, pharmaceutical analysis.

1. INTRODUCTION[1]

Stability indicating methods are indispensable in the pharmaceutical industry to assess the stability of drug compounds and formulations under various stress conditions. A stability indicating methods major goal is to keep an eye on the outcomes of stability investigations in

order to ensure their safety, effectiveness, and quality. When investigating outcomes that are out of trend (OOT) or out of specification (OOS) in quality control processes, it also serves as a potent tool. UHPLC, as a powerful analytical technique, has gained significant attention due to its ability to separate complex mixtures with unparalleled speed and resolution. This review highlights the significance of UHPLC in stability indicating method development.

Principles of stability indicating methods:

Stability indicating methods are developed to differentiate between the active pharmaceutical ingredient (API), its degradation products, and other impurities that might arise during storage or use. The International Conference on Harmonization (ICH) guidelines, particularly ICH Q1A(R2), provide the framework for establishing stability testing protocols. A stability indicating method should meet various criteria, including selectivity, accuracy, precision, linearity, range, robustness, and sensitivity.

UHPLC: A Better system [2,3,4]

UHPLC is an evolution of traditional HPLC, offering improved efficiency, resolution, and speed due to the use of smaller particle size columns, higher pressure capabilities, and advanced detection systems. The UHPLC system is superior to HPLC system because the UHPLC system operates at high pressure up to 1000 bar or more than that, but a conventional HPLC system, compass a pressure up to 400 bars and it suffers problems like mobile phase swallowing and increases the time of analysis. But in UHPLC system, less solvent consumption and less time for analysis are required.

Ultra-high-performance liquid-chromatography (UHPLC) covers liquid Chromatography separations implementing columns enclose particles smaller than the 2.5–5 µm sizes typically used in high-performance liquid chromatography (HPLC). UHPLC Work on the same assumption as that of HPLC and of which governing principle is that, as Column packing particle size decrease, efficiency and thus resolution accretion. Separations Using column contain smaller particles display enhance efficiency per unit time, but the Efficiency cannot minimize at superior mobile phase flow rates or linear velocities. After Attribute, slighter particles, rapidity, and peak resolution can be absolute to new limits.

Instrumentation:

Ultra-high-performance liquid chromatography (UHPLC) encompasses LC Separations using columns containing particles smaller than the 2.5–5-µm sizes typically Used in HPLC. The

benefit of using columns containing smaller particles (typically sub-2 μm) is greater efficiency per unit time. Ultrahigh-pressure liquid chromatography (UHPLC) instruments from different Manufacturers and instruments with different configurations can produce significant Variations in chromatographic separation. The variety in instrument configuration increases The complexity of the method development process, which now requires a more thorough evaluation of the effect of instrument variations on the method. The studies presented here determined the typical inter instrument variations in dwell volume, extra column dispersion, and mixing efficiency as measured by mobile-phase compositional accuracy. Additionally, the dwell volume and extra column dispersion were independently and systematically varied to evaluate the resulting impact on resolution for a small-molecule test mixture during gradient elution. To account for these inter instrument variations, dwell volume and wash-out volume method translation and adjustment techniques were evaluated.

Applications of UHPLC:

1. Analysis of natural products and traditional herbal medicine.
2. Identification of metabolomics.
3. Study of metabolomics/metabolomics.
4. Bio analysis/bioequivalence studies.
5. Manufacturing/QA/QC.
6. Impurity profiling.
7. Forced degradation studies.
8. Dissolution testing.
9. Toxicity studies.

Advantages of UHPLC:

1. Decreases run time and increases sensitivity.
2. Reducing analysis time so that more products can be produced with existing resources.
3. Provides the selectivity, sensitivity and dynamic range of LC analysis.
4. Maintains resolution performance.
5. Fast resolving power quickly quantifies related and unrelated compounds.
6. Operation cost is reduced.
7. Less solvent consumption.
8. Very fast separations with good resolution. High-resolution separations of complex samples

9. Rapid development of stability-indicating HPLC methods
10. Higher sensitivity and precision performance.

Disadvantages of UHPLC:

1. Due to increased pressure requires more maintenance and reduces the life of the Columns of this type.
2. In addition, the phases of less than 2um are generally non-re-generable and thus Have limited use.

Differences between HPLC and UHPLC

These are the top 5 differences between HPLC and UHPLC:

1. Particle sizes – In HPLC particle sizes of the stationary phase are typically in the order of 3-5 μm, whilst UHPLC is characterized by particles of 2 μm or less.
2. Column dimensions – As with particle sizes there is a corresponding reduction in Column dimensions with UHPLC. A typical HPLC column has an internal diameter of 4.6 mm and a length of 250 mm, whilst a UHPLC column has internal diameters of 2.1 mm or less and is much shorter, 100 mm for example.
3. Flow rates – UHPLC runs at much lower flow rates than HPLC, for example 0.2 –0.7 ml/minagainst 1-2 ml/min respectively.
4. Backpressure – With the smaller particles and reduced column diameter then this Manifest itself in to higher backpressures in UHPLC compared to HPLC. HPLC Instruments typically operate at maximum pressures of 400-600 bar, whilst UHPLC instruments can operate at up to 1500 bar in the case of the Thermo Scientific TM VanquishTM Horizon UHPLC System.
5. Detection parameters – Narrow peaks are produced with UHPLC, requiring a detector that can keep pace and provide the required number of data points per peak for Detection. Most modern detectors, though, are capable of detection speeds of up to 250 Hz, which is sufficientfor both HPLC and UHPLC

These are the common differences between HPLC and UHPLC, but they can also be broadly separated by application area. HPLC is commonly used in routine environments, whilst UHPLC is more common in research and development, but this is not exclusive.

2. Method development strategies:[5]

2.1. Selection of stationary phase: The choice of an appropriate stationary phase is critical for achieving optimal resolution and selectivity. Different types of stationary phases, such as reversed-phase, ion-exchange, and HILIC, are explored in stability indicating method development.

2.2. Mobile phase optimization: The composition of the mobile phase affects separation efficiency and peak shape. The review discusses the optimization of mobile phase components, including solvents, buffers, and additives, for achieving robust and reproducible methods.

2.3. Column Selection and Dimensions: The impact of column dimensions (length, diameter, particle size) on separation efficiency and analysis time is explored. UHPLC's compatibility with shorter columns and smaller particles is highlighted.

2.4. Detection wavelength: The selection of appropriate detection wavelengths for UV-visible and other detectors is crucial to achieve maximum sensitivity and selectivity, especially whendealing with complex mixtures and impurities.

3. Challenges in Stability Indicating Method Development using UHPLC:[6]

3.1.Degradant identification: Accurate identification of degradation products is challenging due to their low concentrations and co-elution with the main compound. Advanced techniqueslike tandem mass spectrometry (MS/MS) are discussed.

3.2.Forced degradation studies: Conducting stress tests to induce degradation and assess method selectivity is a critical aspect. The review addresses various stress conditions like heat,light, pH, and oxidation.

3.3.Peak Assignment and Integration: Proper peak assignment and integration in complex chromatograms are vital for accurate quantification. Automation and advanced software toolsfor peak deconvolution are explored.

4. Method validation:[7,8]

4.1. Specificity and Selectivity: Ensuring that the method can differentiate the analyte from impurities and degradation products is a key validation parameter. Chromatographic peak purity assessment techniques are discussed.

4.2. Linearity and Range: The establishment of linearity and a suitable analytical range is crucial to ensure accurate quantification over a broad concentration range. Approaches for determining linearity are covered.

4.3. **Accuracy and Precision:** The review outlines the methods to assess accuracy and precision, including inter-day and intra-day variations, considering UHPLC's rapid analysis capabilities.

4.4. **Robustness and System suitability:** Factors affecting method robustness and system suitability, such as changes in flow rate and column temperature, are explored.

Application in pharmaceutical analysis[9]

The review emphasizes the practical application of stability indicating UHPLC methods in pharmaceutical analysis, including drug formulation analysis, degradation kinetics studies, and batch-to-batch consistency assessment.

Advancements in Stability Indicating UHPLC Methods:[9]

Recent years have witnessed significant advancements in stability indicating method development using UHPLC:

1. **Column technology:** Novel UHPLC column phases and particle technologies have improved peak separation, resolution, and analysis speed.
2. **Stationary phase selectivity:** The availability of a wide range of stationary phases allows method developers to choose the most suitable phase for a particular analysis, enhancing selectivity.
3. **Gradient elution:** Advanced gradient programming enables efficient separation of complex mixtures, resulting in sharper peaks and improved resolution.
4. **Detection systems:** Modern detectors, such as diode array detectors (DADs) and mass spectrometers, offer enhanced sensitivity, selectivity, and the ability to characterize impurities based on their spectra.
5. **Automated method development:** Software tools that aid in method development, such as Design of Experiments (DoE) and Quality by Design (QbD) approaches, streamline the optimization process.
6. **Hyphenated techniques:** Coupling UHPLC with mass spectrometry (UHPLC-MS) allows for accurate identification and quantification of impurities, even in complex matrices.

As UHPLC technology continues to evolve, future prospects for stability indicating method development are discussed, including hyphenation with mass spectrometry, miniaturization, and automation.

CONCLUSION

The development of stability indicating methods using UHPLC has emerged as an essential tool in pharmaceutical analysis. This review highlights the methodologies, challenges, and advancements in this field, showcasing the crucial role of UHPLC in ensuring the safety and efficacy of drug substances and products. With the advancement of analytical instrumentation and techniques, UHPLC-based stability indicating methods are poised to make significant contributions to pharmaceutical analysis and regulatory compliance.

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