Review Article

STABILITY INDICATING BY LC-MS METHOD

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ABSTRACT

Liquid chromatography - Mass spectroscopy (LC-MS) is an analytical technique that couples high resolution chromatographic separation with sensitive and specific mass spectroscopic detection. There are the needs of stability study to develop stability test method, to study the degradation pathway of drugs, to determine storage condition, to study the drug stability in different biological fluids. In the pharmaceutical world, impurity is consider as any other organic material, decide the drug substance, or ingredients, arise out of synthesis or unwanted chemicals remains with active pharmaceutical ingredients (API'S). Degradation product, by-product, intermediates, starting materials, these are the types and sources of impurities. The impurities present in the drugs are adversely affecting the quality of the drug product. Thus is one of the hyphenated technique which revolutionized impurity profiling and degradation products formed during the formulation and production procedure. The technique is still fast developing, with high resolution and high sensitivity, particularly in mass spectrometry area. There are using chromatographic techniques like GC, HPLC, HPTLC, TLC & spectroscopic technique like MS, IR, NMR, and UV for isolation and identification of impurities which are present in drug substances and drug products. LC-MS having application in drug metabolism studies, analysis of drug and metabolites, analysis of chiral impurity, molecular weight determination, structural determination and some biochemical application.

Keywords: Hyphenated technique (LC-MS), Stability study, Degradation product, Impurities study, Analytical method development.

INTRODUCTION

The combination of high-performance liquid chromatography and mass spectrometry (LC/MS) has had a significant impact on drug development over the past decade. Continual improvements in LC/MS interface technologies combined with powerful features for structure analysis, qualitative and quantitative, have resulted in a widened scope of application. These improvements coincided with breakthroughs in combinatorial chemistry, molecular biology, and an overall industry trend of accelerated development. New technologies have created a situation where the rate of sample generation far exceeds the rate of sample analysis. As a result, new paradigms for the analysis of drugs and related substances have been developed The growth in LC/MS applications has been extensive, with retention time and molecular weight emerging as essential analytical features from drug target to product. LC/MS based methodologies that involve automation, predictive or surrogate models. An iterative cycle of "what is it?" and "how much is there?" continues to fuel the tremendous growth of LC/MS in the pharmaceutical industry. During this time, LC/MS has become widely accepted as an integral part of the drug development process. This review describes the utility of LC/MS techniques for accelerated drug development and provides a perspective on the significant changes in strategies for pharmaceutical analysis. This main aim of this review is to develop the method for the impurity profile determination in API using chromatography and mass spectrometry. It was shown that liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a suitable tool for the identification of organic impurities in the pharmaceutical substance of interest. Using these techniques, the methods for separation and identification were developed which allow determining both, process impurities and degradation impurities which were formed during the stability study of the tested substance. The mass spectra, fragmentation spectra and chromatographic data were used to identify the impurities[1].

Need of stability study

- To develop a stability test methods.
- To develop quantitative analytical procedure.
- To study degradation pathways of drugs.
- To determine storage condition.
- To detect drug stability in different biological fluids.
- To the relevant legal requirements concerned with the identity, strength, purity and quality.
- To minimize financial loss.

Application

- LC-MS in drug metabolism studies
- Analysis of drug & metabolites
- Analysis of chiral impurities in pharmaceuticals
- Molecular weight determination
- Structural determination e.g. Ginsenoside
- Biochemical application e.g. rapid protein identification using capillary LC/MS/MS

STUDYING THE DEGRADATION PRODUCT BY THE FORCE DEGRADATION METHOD

Degradation Product

An impurity resulting from a chemical change in the drug substance brought about during manufacture and/or storage of the new drug product by the effect of, for example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate container closure system .

Degradation Profile

Degradation profile is the description of the degradation products observed in the drug substance or drug product.

Specified Degradation Product

A degradation product that is individually listed and limited with a specific acceptance criterion in the new drug product specification. A specified degradation product can be either identified or unidentified.

Unidentified Degradation Product

A degradation product for which a structural characterization has not been achieved and that is defined solely by qualitative analytical properties (e.g., chromatographic retention time).

Unspecified Degradation Product

Unspecified degradation product is the degradation product that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion, in the new drug product specification.

Identified Degradation Product

Identified degradation product is the degradation product for which a structural Characterization has been achieved.[2]

FORCED DEGRADATION

Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behavior can be used to improve a drug product. The available regulatory guidance provides useful definitions and general comments about degradation studies. However, guidance concerning the scope, timing, and best practices for degradation studies is very general. Various issues related to stress testing are addressed in numerous guidance documents but not always in the context of stress testing. For example, the available guidance discusses issues such as stereochemical stability, degradation product identification thresholds, polymorphism and crystal forms, stability of (parenteral) combination products, and mass balance but does not address these issues in the context of degradation studies. The FDA and International Conference on Harmonization (ICH) guidance provides very little information about strategies and principles for conducting forced degradation studies, including problems of poorly soluble drugs and exceptionally stable compounds. In particular, the issue of how much stress is adequate in stress testing is not addressed specifically. Overstressing a molecule can lead to degradation profiles that are not representative of real storage conditions and perhaps not relevant to method development. Therefore, stress-testing conditions should be realistic and not excessive. In this regard, it is the amount of stress that is important and not necessarily the extent of degradation. Indeed, some compounds may not degrade significantly after considerable exposure to stress conditions[2].

Overview of regulatory guidance

According to the available guidance forced degradation studies are carried out for the following reasons:

- Development and validation of stability-indicating methodology
- Determination of degradation pathways of drug substances and drug products
- Discernment of degradation products in formulations that are related to drug substances versus those that are related to non–drug substances (e.g., excipients)
- Structure elucidation of degradation products
- Determination of the intrinsic stability of a drug substance molecule.

Degradation studies have several defining characteristics

- They are carried out in solution and/or the solid state
- They involve conditions more severe than accelerated testing (e.g., >40°C; ≥75% relative humidity in excess of ICH light conditions; high and low pH, oxidation, etc.)
- They are typically carried out on one batch of material
- They include conditions that analyze thermolytic, hydrolytic, oxidative, and photolytic degradation mechanisms in the drug substance and drug product (as appropriate)
- They are not part of the formal stability program.[11, 12]

Reporting Degradation Products Content of Batches

Documentation should include

1) Analytical results should be provided in the registration application for all relevant batches of the new drug product used for clinical, safety, and stability testing, as well as batches that are representative of the proposed commercial process.

2) Quantitative results should be presented numerically, and not in general terms such as “complies”, “meets

limit" etc. Any degradation product at a level greater than (>) the reporting threshold, and total degradation products observed in the relevant batches of the new drug product, should be reported with the analytical procedures indicated.

3) Below 1.0%, the results should be reported to the number of decimal places (e.g., 0.06%) in the applicable reporting threshold; at and above 1.0%, the results should be reported to one decimal place (e.g., 1.3%). Results should be rounded using conventional rules. Tabulation (e.g., spreadsheet) of the data is recommended.

4) Degradation products should be designated by code number or by an appropriate descriptor, e.g., retention time. If a higher reporting threshold is proposed, it should be fully justified. All degradation products at a level greater than (>) the reporting threshold should be summed and reported as total degradation products.

5) Chromatograms with peaks labeled (or equivalent data if other analytical procedures are used) from representative batches, including chromatograms from analytical procedure validation studies and from long-term and accelerated stability studies, should be provided.

6) The applicant should ensure that complete degradation product profiles (e.g., chromatograms) of individual batches are available, if requested. For each batch of the new drug product described in the registration application,

· Batch identity, strength, and size

· Date of manufacture

· Site of manufacture

· Manufacturing process

· Immediate container closure

· Degradation product content, individual and total

· Use of batch (e.g., clinical studies, stability studies)

· Reference to analytical procedure used

· Batch number of the drug substance used in the new drug product

· Storage conditions for stability studies

IMPURITY STUDIES

Impurity

Impurity is the any component of the new drug product that is not the drug substance or an excipient in the drug product.

Impurity Profile

A description of the identified and unidentified impurities present in a drug product.

Impurities can be described as shown in table 1.

Table 1: Description of Impurity types and Impurity sources

Impurity types	Impurity sources
1. Process-related drug substance	- Organic - Starting material - Intermediate - By-product
2. Process-related drug product	- Organic or inorganic - Reagents, Catalysts
3. Degradation	- Organic substance or drug product - Degradation products
4. Degradation drug product	- Organic - Excipient interaction

According to ICH guidelines impurities has been classified as shown in table 2.

Table 2: Classification of impurities

Common name	United state pharmacopeia	ICH terminology
By-Product	Impurities in official articles	Organic impurities
Degradation product	Ordinary impurities	Inorganic impurities
Interaction product	Organic volatile impurities	Residual solvents
Intermediates	Toxic impurities	
Related products		

ICH limits for impurities

According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic.

- Each specific unidentified impurity at or above 0.1%

- Any unspecific impurity, with limit of not more than 0.1%

- Total impurities

SOURCES OF IMPURITIES

From the preceding discussion, it is clear that impurities can originate from several sources; such as:

1. Crystallization-related impurities

The pharmaceutical industry is required to take a strong interest in polymorphism and solvatomorphism as per the regulations laid down by the regulatory authorities.

Polymorphism is the term used to indicate crystal system where substances can exist in different crystal packing arrangements, all of which have the same elemental composition.

2. Stereochemistry-related impurities

It is of paramount importance to look for stereochemistry related compounds; that is, those compounds that have similar chemical structure but different spatial orientation, these compounds can be considered as impurities in the API's. Chiral molecules are frequently called enantiomers. The single enantiomeric form of chiral drug is now considered as an improved chemical

entity that may offer a better pharmacological profile and an increased therapeutic index with a more favorable adverse reaction profile. However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard.[5, 6]

3. Residual solvents

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into three classes. Especially, solvents in;

Class I, *viz* benzene (2 ppm limit), carbon tetrachloride (4 ppm limit), methylene chloride (600 ppm), methanol (3000 ppm, pyridine (200 ppm), toluene (890 ppm) should be avoided.[4]

4. Synthetic intermediates and by-products

Impurities in pharmaceutical compounds or a new chemical entity (NCE) can originate during the synthetic process from raw materials, intermediates and/or by-products. For example, impurity profiling of ecstasy tablets by GC-MS, and MDMA samples, produced impurities in intermediates via reductive animation route.[18]

5. Formulation-related impurities

Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub-potency.[20] In general, liquid dosage forms are susceptible to both degradation and microbiological contamination. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients, and the primary container are critical factors.

6. Impurities arising during storage

A number of impurities can originate during storage or shipment of drug products. It is essential to carry out stability studies to predict, evaluate, and ensure drug product safety.[6]

7. Method related impurity

A known impurity, 1-(2, 6-dichlorophenyl) indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium, if it is terminally sterilized by autoclave.

8. Mutual interaction amongst ingredient

Most vitamins are very labile and on aging they create a problem of instability in different dosage forms, especially in liquid dosage forms. Degradation of vitamins does not give toxic impurities; however, potency of active ingredients drops below Pharmacopoeial specification.

9. Functional group related typical degradation

Ester hydrolysis can be explained with a few drugs *viz.,* aspirin benzocaine, cefotaxime, ethyl paraben, and cefpodoxime proxetil. Hydrolysis is the common phenomenon for ester type of drugs, especially in liquid dosage forms *viz* benzylpenicillin, oxazepam and lincomycin.[4]

Table 3: Goals of impurity investigations

Process-related impurities	Degradation–related impurities
Identify significant impurities	Identify potential degradation product through stress testing and actual degradation products through stability studies
Determine origin of impurities and method For elimination or reduction	Understand degradation pathway and method to minimize degradation
Establish a control system for impurities involving: 1) Processing/manufacturing conditions 2) Suitable analytical methods / specifications	Establish a control system for impurities involving: 1) Processing / manufacturing conditions 2) Suitable analytical methods/ specifications 3) Long term storage conditions including packaging 4) Formulation

ANALYTICAL METHOD DEVELOPMENT

Method development usually requires the choice of columns, mobile phase, detectors, and method of quantization etc.

New drug development requires meaningful and reliable analytical data to be produced at various stages of the development.

a) Sample set selection for analytical method development

b) Screening of Chromatographic conditions and Phases, typically using the linear solvent- strength model of gradient elution

c) Optimization of the method to fine-tune parameters related to ruggedness and robustness

The impurities can be identified predominately by following methods

- Reference standard method
- Spectroscopic method
- Separation method
- Isolation method
- Characterization method

1. Reference standard method

The key objective of this is to provide clarity to the overall life cycle, qualification and governance of reference standards used in development and control of new drugs. Reference standards serve as the basis of evaluation of both process and product performance and are the

benchmarks for assessment of drug safety for patient consumption. These standards are needed, not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates, and excipients.

2. Spectroscopic methods

The UV, IR, MS, NMR and Raman spectroscopic methods are routinely being used for characterizing impurities.

3. Separation methods

The Capillary electrophoresis (CE), Chiral Separations, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), TLC, HPTLC, HPLC are regularly being used for separation of impurities and degradation products.

4. Isolation methods

It is often necessary to isolate impurities. But if the instrumental methods are used, isolation of impurities is avoided as it directly characterizes the impurities. Generally, chromatographic and non chromatographic techniques are used for isolation of impurities prior its characterization. The term 'chromatographic reactor' refers to the use of an analytical-scale column as both a flow-through reactor, and simultaneously, as separation medium for the reactant(s) and product(s).

A list of methods that can be used for isolation of impurities is given below.

- Solid-phase extraction methods
- Liquid-liquid extraction methods
- Accelerated solvent extraction methods
- Supercritical fluid extraction
- Column chromatography
- Flash chromatography
- TLC
- GC
- HPLC
- HPTLC
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

5. Characterization methods

Highly sophisticated instrumentation, such as MS attached to a GC or HPLC, are inevitable tools in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices. For characterization of impurities, different techniques are used; which are as follows:

NMR

The ability of NMR to provide information regarding the specific bonding structure and stereochemistry of molecules of pharmaceutical interest has made it a powerful analytical instrument for structural elucidation. The ability of NMR- based diffusion coefficient determination to distinguish between monomeric and dimeric substances was validated using a standard mixture of authentic materials containing both monomers and dimers. Unfortunately, NMR has traditionally been used as a less sensitive method compared to other analytical techniques.

MS

Advances in the design and efficiency of the interfaces, that directly connect separation techniques with Mass Spectrometers have afforded new opportunities for monitoring, characterizing, and quantification of drug-related substances in active pharmaceutical ingredients and pharmaceutical formulations. If single method fails to provide the necessary selectivity, orthogonal coupling of chromatographic techniques such as HPLC-TLC and HPLC-CE, or coupling of chromatographic separations with information rich spectroscopic methods such as HPLC-MS or HPLC-NMR may need to be contemplated, but hopefully only as a development tool rather than a tool for routine QC use.

Hyphenated Methods:

- LC-MS-MS
- HPLC-DAD-MS
- HPLC-DAD-NMR-MS
- GC-MS
- LC-MS

An example of reverse-phase LC-MS analysis in gradient elution with two distinct soft ionization techniques is the Atmospheric pressure ionization with electrospray source (API-ESI) and the chemical ionization of d-allethrine.[20] The popularity of LC-MS-MS systems for complex mixture analysis of thermally labile and biologically relevant molecules, *viz* mosapride, is largely attributed to the "soft" Nature of atmospheric pressure chemical ionization (APCI), and atmospheric pressure ionization (APPI),HPLC-DAD-MS (HPLC) coupled with a diode array UV detector and a mass spectrometer, and such other techniques are almost routinely used. NMR has now been added to this combination to provide HPLC-DAD-NMR-MS capabilities in a commercial instrument. In GC-MS of methamphetamine and in LC-MS of risperidone, and cetrizine tablets a number of other chromatographic and spectroscopic configurations are found to be perfectly suitable for initial characterization of the impurities. The goal for investigation of impurities is outlined in Table below. A common goal for investigation of both process and product degradation related impurities is to determine which of the many potential impurities are, in fact, produced in the manufacturing process and which occur under a given set of storage condition.

ANALYTICAL METHOD VALIDATION

The registration application should include documented evidence that the analytical procedures have been validated and are suitable for the detection and quantitation of degradation products (see ICH Q2A and Q2B guidelines on analytical validation). In particular, analytical procedures should be validated to demonstrate specificity for the specified and unspecified degradation products. As appropriate, this validation should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis, and oxidation. Degradation product levels can be measured by a variety of techniques, including those that compare an analytical response for a degradation product to that of an appropriate reference standard or to the response of the new drug substance itself. Reference standards used in the analytical procedures for control of degradation products should be evaluated and characterized according to their intended uses. Differences between the analytical procedures used during development and those proposed for the commercial product should also be discussed.

VALIDATION METHODS

Definition

Establishing documented evidence which provides high degree of assurance that specific process, specific method, specific equipment will consistently produce a product meeting its pre determined specification and quality attributes. According to ICH, typical analytical performance characteristics that should be considered in the validation of all the types of methods are:

1. Limit of Detection

The Limit of Detection (LOD) of an individual analytical method is the lowest concentration/amount of analyte in a sample.

2. Limit of Quantification

The Limit of Quantification (LOQ) of an individual analytical method is the lowest concentration/ amount of an analyte in a sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. .

3. Linearity

The Linearity of an analytical method is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

4. Range

The Range of an analytical method is the between the upper and lower concentration (amounts) of analyte the sample (including those concentrations) for which it has been demonstrated that analytical procedure has a suitable level of precision, accuracy and linearity.

5. Robustness

Robustness is the measure of the analytical method to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of its reliability during normal usage.

6. Ruggedness

The Ruggedness is the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as different analyst, instruments, days, reagents and, columns.[20]

APPLICATIONS

1. LC-MS in drug metabolism studies

A good drug should ideally be metabolically stable and show a good pharmacokinetic profile with high bioavailability and long half-life. Some metabolites may also be more pharmacologically active or more toxic than the parent drug. Characterization of the major and active metabolites helps in the discovery and design of new drug candidates with improved pharmacological activity, metabolic stability and toxicology profile. LC-MS is the method of choice for the study of drug metabolism because of its sensitivity and specificity.

a) In vivo Drug Metabolism

The liver is the primary organ that metabolizes drugs. Preliminary studies of drug metabolism have therefore commonly been carried *out in vitro* with liver microsomal preparations or hepatocytes. These provide good initial indication of the metabolic fate of a drug.

b) In Vitro Drug Metabolism

In vivo metabolism studies involve analysis of drugs and metabolites in blood, urine and faeces. These samples contain a larger amount of endogenous compounds that could co-elute and interfere with the LC-MS analysis. A good sample preparation technique coupled with efficient chromatographic separation is therefore essential for the successful application of LC-MS *to in vivo* metabolism studies.

2. High–throughput LC-MS analysis of drug metabolites

High-throughput approaches to sample preparation coupled with LC-MS greatly improved the speed and sensitivity of analysis necessary for the accelerated drug discovery process.

3. Analysis and identification of impurities and degradation product in pharmaceuticals

Drug Regulation Authorities require the purity of a pharmaceutical to be fully defined and the presence of impurities be fully tested and evaluated. This is important to ensure that the observed pharmacological and toxicological effects are truly those of the pharmaceutical and not due to the impurities. The identification of degradation products will aid in the understanding of potential side effects associated with degradation and in

the design of a more favorable formulation and synthesis of new drugs with greater stability. The major applications of LC-MS in pharmaceutical analysis have been in drug metabolism studies, the analysis and identification of impurities and degradation products in pharmaceuticals and the isolation and characterization of potential drug substances from natural or synthetic sources.

4. Analysis of chiral impurities in pharmaceuticals

The separation and detection of chiral impurities in pharmaceuticals are of great importance because the D-isomer of a drug can have very different pharmacological, metabolic and toxicological activity from the L-isomer. E.g.LC-MS has been used for the analysis of chiral amino acids in the identification of chiral impurities present in diastereomeric peptide drugs.

CONCLUSION

Stability indicating LC-MS method for determination of drug in presence of degradation products and its process related impurities was studied. The Stability study need for study of degradation pathway of drugs, for develop quantitative analytical procedure, for determination of storage condition, for biochemical fluids. Also with the help of these hyphenated techniques we can minimize financial loss. The information presented could be very useful for quality monitoring of drug substances and its dosage forms. Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biological safety which revels the need and scope of impurity profiling of the drug in pharmaceutical research and LC-MS technique having some important application like drug metabolism studies, molecular weight and structural determination, and biochemical application, and also for identification of compound.

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