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Review Article

Impurity Profiling of Active Pharmaceutical Ingredients (APIs)

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ABSTRACT

Impurity profiling plays a critical role in ensuring the safety, efficacy, and quality of Active Pharmaceutical Ingredients (APIs). Pharmaceutical impurities may arise from starting materials, intermediates, by-products, degradation pathways, or contamination during manufacturing and storage. Regulatory bodies such as ICH, FDA, and EMA mandate strict identification, quantification, and control of these impurities to safeguard patient health. This review provides a comprehensive overview of impurity types including organic, inorganic, and residual solvents—as well as factors affecting impurity formation, such as environmental conditions, formulation processes, and functional group reactivity. Various analytical and isolation techniques used in impurity profiling, including HPLC, GC, HPTLC, CE, NMR, MS, IR spectroscopy, gravimetric analysis, and chromatographic methods, are discussed along with their applications and limitations. The significance of impurity profiling in drug development, regulatory compliance, process optimization, and stability studies is highlighted. This review emphasizes that robust impurity profiling is essential for maintaining pharmaceutical quality standards and ensuring the therapeutic reliability of drug products.

INTRODUCTION

Since it is the source of active pharmaceutical ingredients (APIs) of particular quality, the bulk drug sector serves as the foundation for all other pharmaceutical enterprises. The quality of new medications has received a lot of attention during the past few decades. Producing high-quality goods is a key difficulty for both the pharmaceutical and bulk medication sectors. Strict quality control inspections are required to preserve the caliber and purity of each industry's product. The kind of crystallization and purifying technique, raw materials, and manufacturing procedure all affect how pure an active medicinal ingredient is. The idea of purity evolves throughout time and is inextricably linked to advancements in analytical chemistry. In addition

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to defining purity, the pharmacopoeias set extremely strict limitations on the amounts of certain contaminants. Because they simultaneously separate and measure the components, modern separation techniques undoubtedly play a major role in scientific research today. This makes it simpler to separate and characterize contaminants.

Pharmaceutical impurities are undesirable substances that either stay with the Active Pharmaceutical Ingredients (APIs) or emerge during the formulation process or as the APIs and formed APIs age [1-4]. Even minute levels of these undesirable substances may have an impact on the pharmaceutical goods' safety and effectiveness. Various pharmacopoeias, including the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP), are gradually adding restrictions on the permissible amounts of contaminants in APIs or formulations. The international Conference on ICH Harmonization has released rules regarding contaminants in novel medication ingredients, finished goods, and leftover solvents [5-7]. Furthermore, Ahuja and Gorog have Released books that address various issues of impurity containing legal specifications, sources, and kinds of contaminants, separation, description, and observation of contaminants in pharmaceutical goods. The profile of impurities is An explanation of the contaminants that have been detected and those that have not seen in a typical batch of API made by a certain regulated manufacturing procedure. It is among the most significant areas of action in modern industrial pharmaceutical evaluation.

The main reasons for the increasing interest of drug manufacturers and drug registration authorities in the impurity profiles of bulk drug substances are as follows:[8]

a. Knowing the structures of the impurities is crucial when developing a new drug or a new method for producing an existing drug. With this knowledge, synthetic organic chemists can frequently alter the reaction conditions to prevent the formation of the impurity or reduce its quantity to a manageable level.
b. With proposed structures for the impurities, they may be synthesized and so offer conclusive proof of the structures previously ascertained by spectroscopic techniques.
c. During the development of a selected technique for the quantitative assessment of the impurity and the use of this method as part of the quality control testing of each batch, the synthesized material can be employed as a "impurity standard."
d. Toxicological investigations can be conducted on the synthesized or separated substance in the event of significant impurities, considerably enhancing the safety of medication therapy.
e. The impurity profile of a drug substance serves as a useful fingerprint for drug regulators to determine the degree and consistency of the bulk drug substance's production process.

Regulatory Guidelines on Impurities in an Active Pharmaceutical Ingredient:

Monitoring contaminants in pharmaceutical goods is necessary for safety and efficacy reasons, as well as ethical, economic, and competitive ones. Even individuals in the pharmaceutical sciences and industry may have differing perspectives on monitoring and managing these contaminants. To ensure that everyone uses the same vocabulary while responding to inquiries about contaminants, a uniform nomenclature is required. The International Conference of Harmonization's (ICH) guidelines have been approved by the US

Food and Drug Administration (US FDA). Regulators and industry representatives from the European Union (EU), Japan, and the United States worked together to develop the ICH guideline for impurities in pharmaceuticals, which has helped to guarantee that different regions have uniform requirements for the data that should be submitted to various regulatory agencies. The guidelines help FDA reviewers and field investigators interpret and apply regulations consistently. They also help sponsors of New Drug Applications (NDA) or Abbreviated New Drug Applications (ANDA) with the type of information that should be submitted with their applications. The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines "stability testing of new drug substances and products"- Q1A
2. ICH guidelines "Impurities in New Drug Substances"- Q3A
3. ICH guidelines "Impurities in New Drug Products"- Q3B
4. ICH guidelines "Impurities: Guidelines for residual solvents"- Q3C
5. US-FDA guidelines "NDAs -Impurities in New Drug Substances"
6. US-FDA guidelines "ANDAs – Impurities in New Drug Substances"
7. Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia

Need for Impurity profiling:

Any formulation's production process must include an analysis of the contaminants present in the raw materials utilized for formulation. These contaminants could affect how soluble APIs are. The presence of these undesirable compounds or substances may also have an impact on a drug's safety parameters by causing toxicities in the body or unfavorable drug responses, which compromises the safety as well as effectiveness of APIs.

DESIGNATION OF IMPURITIES

A. Common Terms of Impurities [09-16]

Following terms are used by various regulatory bodies and ICH to describe the impurities

a. Intermediate
b. Penultimate intermediate
c. By-products
d. Transformation products
e. Interaction products
f. Related products
g. Degradation products

a. **Intermediate:** the substances created either as part of the synthesis process or during the production of the desired item.

b. **Penultimate intermediate:** Before the final desired chemical is produced, it is the final compound in the synthesis chain.

c. **By-products:** the substance created during the reaction aside from the necessary intermediates. A number of side reactions, including overreaction, incomplete reaction demonization and rearrangement, and undesired interactions involving starting materials or intermediates and chemical reagents or catalysts, can cause them.

d. **Transformation products:** They have to do with both hypothesized and non-theorized products that might arise during a reaction. They resemble by-products, with the exception that These reaction products are better understood.

e. **Interaction products:** These items were created when different chemicals interacted, either on purpose or accidentally.

f. **Related products:** These may potentially have biological action and share chemical similarities with pharmacological substances.

g. **Degradation products:** They are created when an active component or other interesting substance breaks down due to outside influences like heat or light and dampness.

B. Classification of Impurity[16-20]

United States Pharmacopoeia (USP)

According to USP impurities are classified into three sections

1. Impurities in Official Articles
2. Ordinary Impurities
3. Organic Volatile Impurities

The ICH Terminology

Impurities in pharmacological substances made by chemical synthesis can be generally categorized into the following three groups, according ICH recommendations.

1. Organic Impurities (Process and drug-related)
2. Inorganic Impurities (Reagent, ligands, catalysts)
3. Residual Solvents (Volatile solvents)

1. ORGANIC IMPURITIES:

These kinds of contaminants develop while the medicinal substance is being manufactured or stored. These comprise the subsequent sub-impurities.

Starting Materials or Intermediate Impurities

These kinds of contaminants can be found in nearly all APIs if care is not taken at each stage of the therapeutic product's multistep manufacturing. Even though the final goods are always cleaned with solvents, if the producers are not extremely cautious about the contaminants, there may be a remnant of unreacted beginning ingredients.

By-products

It is extremely uncommon to obtain a single end product with full yield in synthetic organic chemistry; byproducts are always possible in addition to intended final result.

Degradation Products

Degradation of the final product during the production of bulk medications might also result in the formation of impurities. This generally happens as a result of incorrect formulation storage.

Other Types of Organic Impurities [21-24]

Synthesis Related Impurities

During the synthetic process, a new chemical entity is created from the raw material, solvent, intermediate, and byproduct. If an impurity is present in trace or considerable amounts in any of the substances engaged in the reaction during the synthesis process, the final product will eventually be contaminated with one or more undesirable elements. As a result, every stage of the synthesis process must be carried out with the utmost caution to reduce the amount of impurity that may occur.

Formulation Related Impurities:

Drug substances are exposed to a range of situations that cause them to degrade or undergo other responses. Hydrolysis can cause

solutions and suspensions to degrade. In addition to contributing to its impurity, the water utilised in the formulation creates an environment conducive to hydrolysis and catalysis.

Factors Affecting on Formulation Related Impurities

a. Environment related

I. When exposed to unfavourable temperatures, substances that are sensitive to heat or tropical temperatures cause the active component to degrade and impurities to develop. For instance, vitamins are heat-sensitive, and their breakdown results in a loss of efficacy.
II. When photosensitive materials are exposed to light or UV radiation, they degrade and produce impurities.
III. Humidity: Bulk powder and formulations with solid dose forms may suffer from it.

b. Formation of impurities on ageing:

Mutual interaction: When chemicals in a formulation interact with one another, it creates development of impurities.

C. Functional Group Related Impurities

I. Ester hydrolysis: This process occurs in medications such as aspirin, benzocaine, cocaine, cefoxime, and ethyl paraben.
II. Hydrolysis: Common medications that go through this process include benzyl penicillin, barbital, and chloramphenicol.
III. Degradation by oxidation: medications such as hydrocortisone, Methotrexate, nitroso/nitrile derivative, heterocyclic aromatic ring.
IV. Photolytic cleavage: When a product is exposed to light during production, storage in a hospital, or when it is being used by a customer.
V. Decarboxylation: When heated, some dissolved carboxylic acids, such p-amino salicylic acid, release CO2.

2. INORGANIC IMPURITIES

The manufacturing techniques that are utilised in the creation of bulk drugs also yield inorganic contaminants. Usually, they are recognised and known.

a. Reagent, Ligands, and Catalysts: These contaminants are uncommon. A issue will arise if the production process is not correctly followed.

b. Heavy Metals: When acidification or acid hydrolysis occurs, water is typically employed in various manufacturing processes that serve as the primary source of heavy metals such as Ar, Cd, Cr, Na, Mg, Mn, etc. Glass-lined reactors and demineralised water make it simple to prevent heavy metal contaminants.

c. Other Materials (Filter Aids, Charcoal): In bulk medicine production facilities, filters or filtering aids like centrifuge bags are frequently utilised. Activated carbon is also frequently utilised, which serves as a source of impurities. Therefore, it is crucial to regularly check the bulk medications for fibres and black particles in order to prevent contamination.

3. RESIDUALSOLVENTS

Organic or inorganic liquids used in the manufacturing process are known as residual solvents.

Completely eliminating these solvents during the work-up process is quite challenging. When producing pharmaceuticals in large quantities,

some solvents that are known to be harmful should be avoided.

4. FORMATION OF RELATED IMPURITIES (IMPURITIES IN DRUG PRODUCT):

A pharmacological agent's formulation may have a variety of contaminants in the therapeutic product. A medicinal substance passes through a number of factors that might cause deterioration or other negative consequences during formulation. Degradation in suspensions and solutions can result via hydrolysis. The water used in the formulation may not only include its own impurities but also provide the perfect conditions for hydrolysis and catalysis. Other solvents that may be used could have comparable results. The following categories might be used to group the pollutants connected to the formulation:

- Method related
- The following are the main environmental elements that are related to the environment and can lower stability:

I. Humidity
II. Light-especially UV light
III. Exposures to adverse temperatures

Method related:

When diclofenac sodium is sterilised by terminal autoclaving, a known contaminant called 1-(2, 6-diclorophenyl) During the manufacturing process, indolin-2-one is produced. For the intramolecular cyclic reaction of diclofenac sodium to produce the indolinone derivative and sodium hydroxide, the autoclave method's 123+2°C requirement was adhered to. It has been shown that the formation of this contaminant is influenced by the formulation's initial pH. The final product from the ampoule has an impurity content higher than the raw material limit set by the BP.

Environmental related:

The following are the main environmental elements that can lower stability:

Exposures to adverse temperatures: Numerous APIs exhibit heat- or tropical-temperature sensitivity. For instance, vitamins lose efficacy in vitamin products due to deterioration, especially in liquid formulations, as they are highly heat-sensitive medicinal compounds.

Radiation, particularly UV radiation: Several investigations have shown that ergometrine and methyl ergometrine injections are unstable in tropical climates with high light and heat levels, and there is relatively little active component in many field samples. Only half of the commercially available ergometrine injectable samples that were evaluated had an active ingredient level that satisfied the BP/USP criterion of 90% to 110% of the reported amount. After 42 hours in the sun, the ergometrine injection, which was made to order and had 0.2 mg/mL, almost entirely deteriorated.

Humidity: Humidity is considered to be harmful to both bulk powder and prepared solid dose forms for hygroscopic products. Classic examples include ranitidine and aspirin. **[25]**

Significance of impurity profiling

Safety

Impurities in APIs can have serious consequences for user safety. Finding and regulating contaminants is essential for lowering patient risk since many impurities are hazardous or poisonous at specific quantities, which can result in poor health.

Efficacy

Impurities can affect a drug's therapeutic effectiveness. For example, degradation products may alter the effectiveness of the API, leading to a decreased therapeutic response or even therapeutic failure.

Stability

Impurities may have a negative impact on API stability. A drug's stability and shelf life can be reduced by reactive pollutants because they accelerate the drug's breakdown.

Regulatory Compliance

Regulatory bodies including the FDA, EMA, and ICH have strict guidelines for the amounts of impurities in APIs and final pharmaceutical products. Meeting specific requirements is necessary to obtain authorisation to sell pharmaceutical products on the market. Noncompliance may result in the removal of products from the market or a delay in the approval of medications. [26,27]

Isolation methods

Usually, pollutants must be isolated. However, because the impurities are directly characterised by instrumental processes, impurity isolation is avoided. Pollutants are usually isolated before being characterised using both chromatography and non-chromatographic techniques. The term "chromatographic reactor" refers to a method of product and reactant separation that uses a flow-through reactor with an analytical-scale column. The solution-phase hydrolysis kinetics of the prodrug Aprepitant (EmendTM), also referred to as Fosaprepitant dimeglumine, were investigated using an HPLC, chromatographic reactor method. Ofloratidine was identified as an impurity in loratidine, whereas celecoxib and amikacin are other examples. Below is a list of techniques that can be used to isolate contaminants.

Contaminants can be separated using a variety of methods. Three of the most used techniques include chromatography, TLC, and preparative HPLC. The exact method to be used will depend on the kind of impurity and/or degradant and how much of it is present in the original material that has to be separated. Extraction techniques are sometimes used to separate contaminants since therapeutic compounds and impurities do not dissolve in the same solvents. It is possible to remove certain contaminants with preference based on their acidity, basicity, or neutrality. The extraction procedure usually uses liquid-liquid extraction with an aqueous phase and a non-polar organic phase. Contaminants that are neutral, basic, or acidic can be extracted by suitably adjusting the pH of the aqueous phase. The method is successful when the polarity of a few impurities, or pKa, is sufficiently different from that of pharmaceutical compound 20. Chromatographic methods can be utilised if further separations are required. SPE and SFE are two other methods for separating pollutants. Samples are frequently cleaned up utilising some of the aforementioned techniques, such SPE and SFE, before inspection. One of the main applications of capillary electrophoresis is the analysis of pollutants.

In pharmacological chemicals and drug products, the structure of impurities—unknown degradation products—must explain if they exceed a level of more than 0.1%, according the most recent FDA and EMEA regulations. The most recent analytical techniques, such semi- and Analytical services, such as MALDI-TO, GC-MS, LC-MSMS, and high field NMR, provide fully preparative HPLC for both structural elucidation and the preparative separation of unknown contaminants. Software

tools that predict spectra assist specialists in their work.

Isolation Methods of Impurities

Solid-Phase Extraction Methods

Solid phase extraction (SPE) is one sample preparation method that is becoming more and more useful. A few examples are the use of expensive, breakable speciality glassware, imprecise phase separation, non-quantitative recoveries, and the removal of quantities of organic solvents. of the problems that SPE can prevent. SPE is more effective than liquid-liquid extraction and yields rapid, easy, and automatable quantitative extractions. Both the amount of time and solvent required in the lab are reduced.

SPE is commonly used to extract semi-volatile or non-volatile analytes from liquid samples. SPE is defined as useable solids that have previously separated into solvents. SPE cleaning materials and sample extraction are top-notch. They have a wide variety of sizes, adsorbents and chemistries. Selecting the ideal material for each sample and application is crucial. **[28]**

Liquid -Liquid Extraction Methods

Liquid-liquid extraction, also known as solvent extraction with partitioning, is a method of separating chemicals based on how soluble they are in two different immiscible liquids, such as water and an organic solvent. transferring a substance between different liquid phases. Liquid-liquid extraction is a crucial procedure in chemical laboratories. A separating funnel is used to carry out Procedure Can. This type of technique is often performed as part of the workup after a chemical reaction.

Accelerated Solvent Extraction Methods

Accelerated Solvent Extraction (ASE) is a better technique for solid and semisolid sample matrices at high pressure and temperature utilising conventional solvents. ASE systems come in two varieties: the fully automated ASE 350 and the entry-level ASE 150. Using Dionium When compared to techniques like Soxhlet and sonication, extractions that normally take hours may be completed in a matter of minutes using TM components and ASE with pH-hardened pathways.The many steps involved in sample preparation may now be automated thanks to ASE flow-through technology. As part of the solvent extraction process, solid sample filtering and cleaning can be finished in a single step. Because up to 90% less is needed for Accelerated Solvent Extraction It is less costly per sample than alternative techniques.

Supercritical Fluid Chromatography

Supercritical fluid chromatography, or SFC, is a kind of normal phase chromatography used for the analysis and purification of thermally unstable, low-to-moderate molecular weight compounds. Another use for it is the separation of chiral compounds. SFC's tenets are comparable to characteristics of HPLC; however, since carbon dioxide is usually used as the mobile phase, the entire chromatographic stream channel needs to be pressurised.

Column chromatographic technique

Column chromatography is a widely used technique in chemistry for extracting specific compounds from chemical mixtures. In preparative applications, it is frequently employed on scales from microgrammes to kilogrammes. A traditional preparative is a glass tube with a tap at the bottom. column for chromatography. These tubes range in height from 50 cm to 1 m and typically have a diameter of 50 mm. The column

can be set up in one of two ways: wet or dry. Because of their different speeds of transit through the stationary phase and their distinct interactions with it, the mixture's constituents may separate. They then elute from the column one by one. Throughout the chromatography process, the eluent is collected. They then elute from the column one by one. Throughout the chromatography process, many eluent fractions are recovered. Eluent composition may be analysed by looking for dissolved materials in each fraction utilising methods like analytical chromatography, fluorescence, and UV absorption. In certain cases, coloured compounds or those that glow in the presence of UV light—can be observed as bands that flow across the glass column. Each fraction's molecules are recognised by analysis, and as the fractions pass through the column, the process may be monitored.

Flash chromatography

Flash chromatography is a fast chromatographic technique that facilitates the separation of compounds in a mixture based on how differently they bind to a stationary phase. This technique is very effective for purifying organic molecules and separating impurities from various samples. Flash Chromatography combines the ideas of reversed-phase and normal-phase chromatography, enabling flexible separation of polar and non-polar materials.

Key Principles and Mechanisms

Stationary Phase: Silica gel, which may be altered to satisfy various separation needs, is often the recommended stationary phase. For more polar compounds, for example, reverse phase with C18 chains.

Mobile Phase: The mobile phase consists of one or more solutions. The target's polarity and solubility It is important to optimise the choice of solvent by using chemicals and pollutants.

Pressure Application: Unlike traditional column chromatography, which uses gravity as its driving force, flash chromatography uses positive pressure to speed up the elution process. This is suitable for rapid screening and purification since it reduces the separation time.

Column Design: Flash columns accelerate the separation process since they are usually broader and shorter than regular columns. The resolution and effectiveness of the separation can also be impacted by the size of the particles in the stationary phase.

Applications in Impurity Profiling

Flash chromatography has gained appeal for impurity profiling in a variety of industries, including environmental analysis, natural commodities, and pharmaceuticals. Among the notable uses are:

Pharmaceutical Industry

It is used to identify and categorise contaminants in pharmaceutical materials to ensure compliance with regulations. Because of the rapid separation, impurity detection and quantification may be finished quickly.

Natural Product Isolation

When bioactive molecules are extracted from natural sources, flash chromatography helps purify substances while profiling any impurities that can affect biological activity.

Environmental Samples

Flash chromatography, which aids in the analysis of environmental samples, such as soil and water,

for the presence of pollutants and dangerous compounds, enables effective monitoring and assessment.

Method Development

The rapidity of flash chromatography enables for rapid technique development and optimization, useful in research contexts for examining various separation conditions.

TLC (Thin layer chromatography)

Thin-layer chromatography (TLC) is frequently employed early in the drug development process because to its low cost, user-friendliness, and ability to process several samples simultaneously. Given the lack of knowledge on potential contaminants or degradants in medicinal substances and goods, its great sensitivity and rapid It is particularly helpful when separated. TLC plate technology has become more useful because to recent advancements in equipment like densitometers and high-performance thin-layer chromatography (HPTLC). As a result, TLC is now used in conjunction with HPLC and is crucial for the quantitative analysis of drug contaminants. [29] Pharmaceutical contaminants have been identified using a variety of sophisticated chromatographic methods. In one investigation, chromatograms for diclofenac, together with its impurities and breakdown products, were analysed using UV scanning at 248 nm. Ciprofloxacin, chlorpromazine, trifluoperazine, promazine, and doxepin were assessed by another group without the use of a spraying method. Anjaneyulu et al.'s HPTLC technique was successful in identifying contaminants in alprazolam. To find contaminants in pantoprazole and omeprazole, Agbaba et al. developed an additional HPTLC method. Naidong et al. pre-treated the chromatographic layer with sodium EDTA to improve selectivity. Tetracycline and chlortetracycline impurities were measured by fluorimetric scanning at 400 nm. Kochana et al. developed a technique that combines TLC and solid-phase extraction. Ecstasy pills contain 3,4-methylenedioxymethamphetamine (MDMA), which may be profiled via fluorescence scanning. Furthermore, Bagócsi et al. detected norethisterone impurities using over-pressurized liquid chromatography (OPLC), a version of HPTLC, with fluorimetric scanning after sulphuric acid spraying, demonstrating better efficiency than normal pharmacopoeial testing. **[30,31]**

Gas chromatography

GLC, sometimes referred to as gas chromatography (GC), is a widely used method in analytical chemistry for separating and assessing compounds that evaporate without degrading. GC is frequently used to separate the different components of a mixture (the relative amounts of these components can also be computed.) or to evaluate a particular material's purity. In certain situations, GC may be identifying a chemical. Chemicals can be extracted from a mixture using it.

HPLC

This technique, often referred to as high-pressure liquid chromatography, is well-known in the fields of analytical chemistry and biochemistry for the separation, identification, and measurement of chemicals based on their unique polarities and interactions with the static phase of a column. This setup consists of a pump that propels the analyte and mobile phase through the column, which is typically packed with stationary phases made of hydrophobic alkyl chains. The analyte's retention duration, which varies based on the chemical, is measured by the system's detector. Additional data, such UV/Vis spectral information, can be sent in some systems. The analyte's interaction during the stationary phase determines how long it

is retained in the column. the amount and makeup of solvents in a mobile phase, together with the flow rate of that phase.

HPTLC

This technique, often referred to as high-pressure liquid chromatography, is well-known in the fields of analytical chemistry and biochemistry for the separation, identification, and measurement of chemicals based on their unique polarities and interactions with the static phase of a column. This setup consists of a pump that propels the analyte and mobile phase through the column, which is typically packed with stationary phases made of hydrophobic alkyl chains. The analyte's retention duration, which varies based on the chemical, is measured by the system's detector. Additional data, such UV/Vis spectral information, can be sent in some systems. The analyte's interaction during the stationary phase determines how long it is retained in the column. the amount and makeup of solvents in a mobile phase, together with the flow rate of that phase.

Capillary electrophoresis (CE)

Ammonium ions are the first noteworthy use of this technique because it is particularly well-suited to the separation and assessment of charged contaminants. is significant because ammonium salts, which frequently show up as impurities, are not usually found using methods like TLC or HPLC and do not leave a residue in tests of ignition. To find this cation, Gong et al. used non-aqueous CE in conjunction with indirect UV detection, employing imidazole as the UV probe. Similarly, Schöftner et al. separated and identified tetraalkyl ammonium salts as contaminants in colesevelam using indirect UV detection (with creatinine as the probe). HCl, contrasting the effectiveness of the technique with ion chromatography. Phosphate and its contaminants were found by indirect UV detection utilising sodium chromate as the probe. Ibandronate and its related substances, which have salt-like characteristics, were also examined. [33] Basic chemicals are easier to protonate in low pH environments, which makes them better for capillary electrophoresis-based impurity monitoring. For instance, Quaglia et al. successfully separated and measured five contaminants in an aqueous buffer using trimethyl-cyclodextrin at pH 2.5. direct UV detection of fenticonazole. Similar to this, Sabbah & Scriba profiled contaminants in 3,4-diaminopyridine and 4- aminopyridine using low pH buffers and UV detection. Saavedra et al. showed that CE could effectively separate an alprazolam degradant that HPLC was unable to separate. Additionally, the Box-Behnken experimental design was used to optimise the distinction of ethambutol hydrochloride and its degradant, 2-amino-1-butanol. While CE can occasionally be used in conjunction with chromatographic methods, its limitations in examining uncharged molecules frequently render it less practical as a full substitute for HPLC in pharmaceutical impurity detection. [34]

Electrons Paramagnetic Resonance

A method for looking for chemical entities such as any or all unpaired electrons, inorganic complexes, organic and inorganic free radicals, and transition metal ions is called paramagnetic resonance of electrons. Although EPR activates the spins of electrons rather than atomic nuclei, the basic NMR and physics concepts are comparable. NMR is a more widely used technique for EPR since the majority of stable substances have all of their electrons linked. Because typical chemical solvents and matrices cannot create EPR spectra, the great specificity of the EPR approach is offset by its limitation to paramagnetic species.

Gravimetric Analysis

Gravimetric analysis is a conventional, highly precise analytical technique that determines an analyte's quantity based on its mass. It is especially useful for the separation and measurement of contaminants. The fundamental technique of gravimetric analysis is transforming the target substance, usually an impurity, into a steady, pure condition that is weighable with accuracy. Numerous sectors, such as food safety, medicines, environmental research, and component testing, employ this method. They are widely employed in impurity profiling, particularly for metallic and inorganic contaminants. Its ongoing importance may be attributed to its simplicity, reliability, and high degree of accuracy; nevertheless, careful control over the experimental conditions is required to obtain precision. Gravimetric analysis frequently involves many crucial procedures, namely are weighing, filtering, drying, dissolving, and precipitation. The sample must first be dissolved in the appropriate solution to ensure that the analyte or contaminant is fully accessible for chemical reaction. An suitable reagent is then added to the solution to precipitate the impurity as an insoluble solid. For example, metal contaminants like lead or silver can be eliminated from the solution by adding reagents like sodium sulphate or chloride, which produce insoluble compounds like barium sulphate or silver chloride. Once the precipitate has formed and been filtered out of the solution (usually by vacuum filtration), any soluble contaminants are eliminated by thoroughly washing it. Any water or other volatile elements are burnt or dried out of the precipitate after filtering. Leaving behind a weighable, stable, and clean solid. Finally, because gravimetric analysis can identify and measure specific impurities, particularly inorganic materials like metals and metal oxides, it is very helpful for impurity profiling. The analyst can determine the mass of the impurity by weighing the precipitate using a high-precision analytical balance.

The method's primary advantages are its high accuracy and direct assessment of contaminants without the need for calibration standards, which are sometimes required in experimental techniques like spectroscopy or chromatography. For instance, the metal concentration Gravimetric measurement is frequently used to identify impurities in pharmaceutical formulations, where tiny quantities of metals like lead, mercury, or arsenic must be rigorously managed due to their toxicity. Similar to this, environmental studies employ gravimetric techniques to quantify contaminants in soil and water samples, such as heavy metals (such as cadmium and chromium), by converting them into insoluble compounds that can be filtered and weighed. [35]

Infrared Spectroscopy

Infrared (IR) spectroscopy is a powerful analytical technique for detecting and analysing pollutants in complex mixtures as well as for identifying and quantifying chemical components. Its premise is that molecules absorb certain infrared light frequencies through vibrations, resulting in a unique spectral signature that functions as a fingerprint for molecules. The technique is particularly helpful in impurity profiling because to its sensitivity to both organic and inorganic chemicals, even those present in tiny levels in IR spectroscopy. The absorption spectrum of the sample is compared to reference spectra of pure substances in order to identify impurities. If there are differences in the spectrum, such as additional peaks or shifts in peak positions, impurities may be visible. FTIR is a widely used infrared (IR) method that increases detection sensitivity and permits rapid, non- destructive research at high resolution. Due of its ability to identify non-functional groups such as hydroxyl (-OH),

carbonyl (C=O), or amine (FTIR is highly useful for impurity profiling because NH) are often indicative of contaminants. One of the main advantages of IR spectroscopy in impurity profiling is its ability to assess solid, liquid, and gas samples with minimal pretreatment. As a result, it is beneficial for quality control in industries like pharmaceuticals, where the presence of pollutants may affect the efficacy and safety of products. For example, infrared spectroscopy It is often used to inspect raw materials, intermediates, and finished products for unwanted byproducts or degradation products. [36]

Characterization methods

For the identification of minute elements (drugs, pollutants, breakdown products, and metabolites) in a range of matrices, extremely sophisticated equipment are necessary, such as MS linked to a GC or HPLC. Contaminants are characterised using a variety of methods. Among these tactics are the following:

NMR

NMR is a very helpful analytical method for solving structural riddles since it may provide information about the exact stereochemistry and bonding structure of compounds with medicinal significance. Using a typical blend of actual materials containing both monomers and dimers, It was shown that NMR-based diffusion coefficient measurement could distinguish between monomeric and dimeric substances. Unfortunately, NMR has traditionally been thought of as a less sensitive method when compared to other analytical techniques. Conventional NMR sample needs are in the range of 10 m, whereas MS requires less than 1 mg. [37]

MS

The creation of medications has been increasingly impacted by technological breakthroughs over the past few decades. To accomplish this, innovations in the design and operation of interfaces linking differentiation strategies to MS were crucial. When just one If the strategy doesn't offer enough selectivity, it can be required to combine chromatographic differences with spectroscopic techniques like HPLC-MS or HPLC-NMR or to orthogonal couple chromatographic techniques like HPLC-TLC and HPLC-CE. However, these integrated techniques are better suited as development tools rather than for routine quality control. [38]

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. According to ICH, the maximum daily dose qualification threshold to be considered is as follows as shown in table no.2-5;< 2g/day 0.1 % or 1 mg per day intake (whichever is lower) >2g/day 0.05%

The Role of API Impurity Profiling in Drug Development [39]

The creation of safe and efficient medications is crucial in the pharmaceutical sector. The careful examination and management of contaminants in active pharmaceutical ingredients (APIs) is an essential part of this procedure. In order to guarantee the effectiveness, safety, and quality of pharmaceutical goods, API impurity profiling is essential. The need for trustworthy API impurity suppliers and manufacturers in India is growing as the pharmaceutical industry changes.

Starting materials, intermediates, byproducts, degradation products, and contaminants added

during manufacture are some of the sources of impurities in APIs. Even in tiny concentrations, these contaminants can have a major effect on the final therapeutic product's safety and effectiveness. As a result, thorough impurity profiling is required by regulatory bodies all over the world during the medication development and licensing process.

We at Aquigen Bio Sciences are aware of the function of API contaminants. The crucial role that API impurity profiling plays in drug development will be examined in this blog article, along with its significance, methods, legal requirements, and difficulties that pharmaceutical firms and API impurity providers confront. Now let's get started.

The Importance of API Impurity Profiling Ensuring Drug Safety and Efficacy

API impurity profiling is crucial for ensuring the safety and efficacy of pharmaceutical products. Impurities can potentially cause adverse effects, alter the drug's pharmacological activity, or interfere with its intended therapeutic action. By identifying and characterizing impurities, pharmaceutical companies can assess their potential impact on patient health and take appropriate measures to control or eliminate them.

Meeting Regulatory Requirements

Strict regulations for pharmaceutical impurity control have been set by regulatory bodies including the FDA, EMA, and WHO. Pharmaceutical businesses and providers of pharmaceutical APIs are required by these laws to adequately characterise and quantify contaminants in APIs and final medicinal formulations. For pharmaceutical goods to continue being marketable and to receive regulatory approval, adherence to these rules is crucial.

Optimizing Manufacturing Processes

Impurity profiling offers important information on the production of APIs. Pharmaceutical businesses may improve purification techniques, optimise their synthetic pathways, and increase overall process efficiency by discovering and characterising contaminants. This results in more affordable production techniques as well as higher-quality APIs.

Forced Degradation Studies

To find possible degradation products and evaluate the API's stability under different stress scenarios, forced degradation tests are carried out. These investigations aid in the development of suitable handling and storage settings as well as the prediction of the medication product's long- term stability.

Challenges in API Impurity Profiling Analytical Challenges

It can be difficult to identify and describe contaminants at trace levels, necessitating the use of extremely sensitive and precise analytical techniques. These techniques frequently take a lot of time and money to develop and validate.

Structural Complexity

Certain impurities could have intricate structures that are challenging to understand using conventional analytical methods. In these situations, sophisticated spectroscopic techniques and knowledge of structure elucidation are required.

Genotoxic Impurities

Because genotoxic contaminants may result in chromosomal abnormalities or genetic mutations, they provide a unique problem. These

contaminants must be strictly controlled and monitored, which frequently calls for extremely sensitive analytical techniques.

The Impact of Impurity Profiling on Drug Development Timeline

Early-Stage Development

Impurity profiling starts early in the drug development process, when the API is being synthesised and optimised. The best synthetic approach may be chosen and process development guided by early impurity discovery and characterisation.

Preclinical and Clinical Studies

Ensuring the safety and quality of API batches used in preclinical and clinical investigations requires thorough impurity analysis. Further toxicological evaluations could be necessary if the impurity profile significantly changes throughout these phases.

Regulatory Review and Approval

The regulatory assessment process can be greatly impacted by the completeness and calibre of impurity profiling data. Drug clearance delays or requests for more research may result from inadequate or missing impurity information.

CONCLUSION

Impurity profiling is crucial for: Ensuring Drug Safety and Efficacy: Identifying and characterizing impurities allows companies to assess their potential impact (adverse effects, altered pharmacological activity) and take control measures. Meeting Regulatory Requirements: Strict guidelines from bodies like the FDA and ICH mandate the characterization and quantification of contaminants in APIs and finished products for regulatory approval and marketability. Optimizing Manufacturing Processes: Information from profiling helps in optimizing synthetic pathways, improving purification techniques, and increasing overall process efficiency, leading to higher- quality and more affordable APIs.Regulatory bodies require thorough impurity profiling during drug development and licensing. The process starts early in development and its completeness significantly impacts the regulatory review and approval timeline. Various analytical techniques, from traditional chromatography (HPLC, TLC, GC) to advanced methods (HPTLC, CE, NMR, MS, FTIR, Gravimetric Analysis), are used to isolate and characterize these impurities.

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