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Original Article

Impurity Profiling and its Significance Active Pharmaceutical Ingredients

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ABSTRACT

USFDA, and ICH and other regulatory bodies areplacing a strong emphasis on the detection of contaminants in pharmaceutical active ingredients (APIs) and purity standards that must be met. The process of acquiring and reviewing data to establish the biological safety of each impurity and, thus, its level of qualification the necessity and extent of medication impurity profiling in pharmaceutical research. To identify pollutants, a variety of chromatographic and spectroscopic methods can be applied either alone or in combination with other methods. Impurities can be found and characterized using a variety of techniques like TLC, HPLC, HPTLC, AAS, etc. The discipline of Profiling impurities has utilized conventional LC and HPLC. This method numerous uses might result from its sensitivity, affordability, ability to disentangle a variety of detectors and stationary phases. The most popular separating method is TLC among the several Planar Chromatographic Methods, for isolation of contaminants; as a result of its affordability and ease of use in comparison to HPLC. A popular method for isolating impurities is thin-layer chromatography (HPTLC), which has advanced throughout time. Headspace gas phase separation is a highly favored method for determining the presence of leftover solvents. Hyphenated approaches have revolutionized impurity profiling by enabling structural identification of impurities in addition to their separation.

Keywords: impurities, Analytical method development, Spectrophotometry, Chromatography

INTRODUCTION

Since it provides precise high-quality active compounds in pharmaceuticals (APIs), the majority drug industry acts as the basis for all other pharmaceuticals enterprises. The standard of pharmaceuticals that are introduced to the market has drawn a lot of interest during past few decades. Manufacturing superior products is the Producing high-quality good's is the main issue confronting the pharmaceutical and bulk drug industries. Vigorous quality control inspections are required to preserve the caliber and integrity of any industry's output. Raw materials used, manufacturing process, the type of crystallization, and the purifying procedure are some of the cans have an impact on a pharmaceutical ingredient's purity. The concept of pure is dynamic and inextricably linked to advancements in analytical chemistry. The pharmacopoeias set very strict limitations on the amounts of different contaminants in addition to requiring purity. It is evident that modern separation techniques are important to scientific study today since they allow for the simultaneous separation and quantification of the components, which facilitates the identification and isolation of contaminants. [1,2,3] The contaminants found in APIs are attracting more and more attention. As a result of several regulatory requirements, purity and impurity profiles have recently become crucial. Within Any organic material is considered an impurity in the field of pharmaceuticals that is not part of the drug substance or components, or as undesired compounds that are left behind with APIs. Contamination could appear in medications either in the process of formulating or as the prepared APIs and APIs themselves age. [4] Guidelines for techniques for evaluating contaminants in novel Microbiological pollutants, residual solvents, pharmacological substances, and goods have also ICH authorized the publication. [5]

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Figure1: Impurity profiling

Regulatory guidelines on contaminants in API's

Necessity to monitor contaminants in pharmaceutical items is by safety and efficacy concerns, in addition to Ethical, economic, and commercial considerations. Even among people working in pharmaceutical sciences & industry, monitoring and managing pollutants may mean different things to different individuals at different times, even to the same people. To ensure that everyone speaks with the same vocabulary while discussing impurity-related issues, a uniform nomenclature is required. The ICH provided recommendations, and the US FDA has approved it. Together, regulators and industry representatives from the EU, Japan, and the United States developed the ICH guideline for impurities in pharmaceuticals. This guideline has helped to ensure that different regions have uniform requirements for the data that should be submitted to various regulatory agencies. The guidelines not only provide sponsorship of NDA and ANDA with guidance on what material to include in their applications, but they also assist field investigators and FDA reviewers inconsistently interpreting and applying regulatory requirements. [6]

The following are several rules and regulations pertaining to impurities

"Stability testing of new drug substances and products"- Q1A "Impurities in New Drug Substances"- Q3A.
"Impurities in New Drug Products"- Q3B.
"Impurities: Guidelines for residual solvents"- Q3C.
"NDAs -Impurities in New Drug Substances" .
"ANDAs – Impurities in New Drug Substances" .
Australian regulatory guideline for TGA & prescription medicines

Stability Testing of New Drug Substances and Products (Q1A)

This guideline outlines procedures to ensure that drug substances and products keep quality, safety, and efficacy over the course of time. It explains stress testing, conditions for storage, and shelf-life determination for various climates. Stability studies are critical for defining right storage and packaging conditions. The data generated ensures that the product meets requirements throughout its intended usage.

Impurities in New Drug Substances (Q3A)

Q3A deals with the detection, qualification, and control of contaminants in pharmacological compounds. It establishes thresholds for reporting, identification, and qualification depending on the drug's maximum daily dose. These recommendations promote safety by regulating chemical byproducts, degradation materials, and pollutants. Regulatory acceptability is contingent on meeting these impurity limitations.

Impurities in New Drug Products (Q3B)

This guideline concentrates on contaminants that occur during drug product manufacture or storage. It specifies the allowable levels and types of impurities, which include products of degradation and

interaction-based contaminants. Q3B assures that contaminants in the finished product do not endanger patient safety. Reporting thresholds are consistent with daily consumption and manufacturing process features.

Impurities: Guidelines for Residual Solvents (Q3C)

Q3C controls the amount of residual solvents used in medicine manufacture to ensure low toxicity. It categorizes solvents based on their health risks and establishes daily exposure limits. Test techniques and controls are intended to ensure compliance. These rules strive to strike a compromise between the security of products and manufacturing feasibility.

NDAs – Impurities in New Drug Substances

Impurities in new drug applications (NDAs) submitted to regulatory bodies are covered by this rule. According to predetermined thresholds, it necessitates thorough impurity profiling and risk evaluation. Data on possible effects of contaminants on product efficacy and safety must be included in NDAs. Acceptable impurity management techniques are necessary for regulatory approval.

ANDAs – Impurities in New Drug Substances

This rule guarantees that generic medications preserve impurity profiles similar to reference medications for abbreviated new drug applications (ANDAs). For ANDAs to provide safety and bioequivalence, impurity limits must fall within permissible bounds. Manufacturers must use validated techniques to control and justify contaminants. This rule guarantees that generic medications adhere to the same safety requirements as name-brand medications.

Australian TGA Guidelines for Prescription Medicines

In Australia, the Therapeutic Goods Administration (TGA) sets standards for the effectiveness, safety, and quality of prescription medications. It consists of risk-based evaluation, stability studies, and impurity control in accordance with international standards such as ICH. According to the standards, medications must have complete data in order to be authorized for sale. To preserve product quality and safeguard the public's health, TGA makes sure that compliance is maintained. [7]

Classification and source of impurities in API asper ICH guidelines Organic impurities

These Impurities connected to the procedure be intermediates from chemical reactions, starting ingredients, or by-products. Degradation products that arise from the chemical instability of the API are also considered organic contaminants.

Oxidative damage

Oxidative degradation can affect adinazolam, conjugated dienes hydrocortisone, methotrexate, heterocyclic aromatic rings, hydroxyl groups firmly attached to aromatic rings (e.g., phenols derivatives like catecholamine's & morphine), nitrous and nitrite derivatives, and aldehydes (flavoones).

Decarboxylation

In the case of rufloxacin photoreaction, carboxylic acids, including p-aminosalicylic acid, when heated, the carboxyl group releases carbon dioxide.

Hydrolysis

For medications of the ester class, hydrolysis is a frequent occurrence, particularly in liquid dosing forms. Lincomycin, ethyl paraben, barbitol, cefpodoxime proxetil, benzyl penicillin, and chloramphenicol few examples. Furthermore, the benzocain hydrolysis scheme. [8,9]

Inorganic impurities

It is possible for inorganic contaminants to originate from the bulk medication manufacturing processes. They typically consist of the following and are recognized and identified;

Reagents, catalysts &ligands

Despite being rare, these pollutants may interfere with a number of processes if the producers don't take the necessary precautions throughout production. [10]

Heavy metals

Reactors are the main suppliers of heavy metals (assuming metal such as stainless reactors are used) and the water used in the processes, where acid hydrolysis or acidification occurs. It is simple to prevent these heavy metal contaminants by employing glass-lined reactors and demineralized water.

Other components

Centrifuge bags and other filters and filtering aids are often used in bulk medication production. in conjunction with activated carbon. To prevent these contaminations, it is crucial to regularly check for fibers and black particles in the bulk medications. Additionally, the manufacturing techniques used to produce bulk medicines may introduce inorganic contaminants. The leftover unreacted starting components are typically recognized and identified, unless the producers exercise extreme caution when it comes to contaminants. In synthetic the field of organic chemistry, it is incredibly rare to produce a single final product containing a 100% yield; by-products are always possible. For instance, diacetylated paracetamol is used in the bulk form of paracetamol. [11]

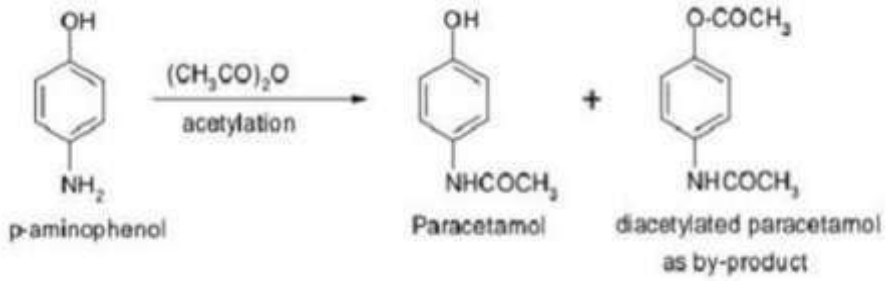

Figure 2: Production of Paracetamol from intermediate p-aminophenol Residual solvent impurities

These are liquids, either organic solvents or inorganic solvents, that are employed in the formulation process. Complete removal of these solvents through the workup process is quite challenging. When making bulk medications, there are certain solvents that should be avoided because they are known to be harmful. Remaining depends on the potential harm to human well-being. [12]

Enantiomeric contaminants

Nowadays, it is thought that a chiral drug's single enantiomeric is a superior chemical compound that may have a greater therapeutic index, improve therapeutic characteristics, and improve adverse reaction probability. The absence benefits of a single isomer are suggested by the same pharmacokinetic profiles of ofloxacin (R-isomeric form) and levofloxacin (S-isomeric form). The one that in drug control is regarded by makers of single enantiomeric drugs (eutomers) in the same way as other organic contaminants. [13]

Formulation related impurities

There may be contaminants in the prepared version of the API that are produced using various techniques, in addition to bulk drug related contaminants.

Impurities connected to the method

During the formulation process, certain impurities may arise from exposure to heat, light, changes in pH, solvents, etc. For example, impurity 1-(2,6-Dichlorophenyl)-indolin-2-one may occur when autoclaving Diclofenac sodium.

Impurities related to the environment

As a result of exposure to extreme temperatures (e.g., vitamins as medication particularly in liquid formulations, substances are highly susceptible to heat degradation, which usually results in a loss of efficacy in vitamin products. As a result of light exposure, particularly UV radiation (for example, methylergometrine and ergometrine are unstable under tropical conditions with heat and light). Humidity (Hygroscopic products, such as aspirin and ranitidine, are thought to be negatively impacted by humidity)

Crystallization related impurities

A substance that is imp is any substance that is not the crystallizing material; for example, the solvent required to produce the crystals maybe termed an impurity. Are contaminants that are purposely added to achieve a certain morphological impact. Foreign ion absorption in the crystalline material structure as well as crystal development, nucleation, and agglomeration can all be dramatically impacted whether a crystallization system contains additives or contaminants.

Stereochemistry related impurities

Searching for chemicals that are connected to stereochemistry which means that those that differ in their spatial orientation yet share a similar chemical structure is crucial. These compounds could be considered contaminants in APIs., it is thought that a chiral drug's single enantiomeric form may provide, improved adverse reaction profile, a higher therapeutic index, and a superior pharmacological profile.

Terms commonly used in impurities.

Various regulatory authorities and the ICH use the following names to describe contaminants; Intermediate
Penultimate intermediate By-products
Transformation products Interaction products
Related products

Intermediate

The compounds produced during synthesis of the desired material or as apart of the route of synthesis.

Penultimate Intermediate

It is the last compound in the synthesis chain prior to the production of the final desired compound.

By-products

The compound produced in the reaction other than the required intermediates. They can occur through a variety of side reactions, such as overreaction, incomplete reaction, demonization and rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts.

Products

They are related to theorized and nontheorized products that can occur in a reaction. They are similar to by-products except that more is known about these reaction products.

Interaction Products

These products formed either intentionally or unintentionally interaction between various chemicals involved.

Related Products

These are chemically similar to drug substance and may even possess biological activity.

Degradation Products

These are created when a active component or other item of interest breaks down due to the influence of outside variables including moisture, heat, and light.

ICH Limits for Impurities

The detection of impurities below no 0.1% level is seen to be important, unless there are potential contaminants that expected to exist exceptionally strong as well as harmful, in compliance with the ICH’s guidelines regarding contaminants in novel pharmaceuticals. per the ICH guidelines, the highest qualifying daily dose threshold <2 g/day, 0.1% or 1 mg/day, however is lower; >2 g/day, 0.05%—that must betaken into account.

Organic Contaminants

Every particular detected contaminant;
Every distinct unknown impurity at or above 0.1%
Any non-specific impurity, up to a maximum of 0.1%
Total contaminants Solvent residue
Inorganic impurity

Significance of impurity profiling Safety

The presence of impurities in APIs can have significant safety implications. Many impurities are toxic or harmful at certain concentrations, which can

lead to adverse health as a result, identifying and controlling contaminants is critical for reducing patient risk.

Efficacy

A drug's therapeutic efficacy maybe impacted by impurities. Degradation products, for instance, may change the API's efficacy, resulting in a reduced therapeutic response or possibly therapeutic failure.

Stability

API stability may be adversely affected by impurities. Reactive contaminants can shorten a drug's stability and shelf life by causing it to break down more quickly.

Regulatory Compliance

Strict rules are set forth by regulatory authorities, including the FDA, EMA, and ICH, regarding the quantities of impurities in APIs and finished medicinal products. Getting permission to sell pharmaceutical items on the market requires meeting certain regulations. Products may be taken off the market or medication approval processes maybe delayed as a result of noncompliance. [14, 15]

Isolation methods

Isolating contaminants is typically required. However, impurity isolation is avoided because instrumental procedures directly characterize the impurities using them. Both chromatography and non-chromatographic methods are typically used to isolate pollutants prior to their characterization. The use of an using a flow-through reactor with an analytical-scale column and, at the same time, is known as a way for the product and reactant separation "chromatographic reactor. “The prodrug Aprepitant (EmendTM), also known as Fosaprepitant dimeglumine, had solution-phase hydrolysis kinetics that was studied by means of an HPLC, chromatographic reactor approach. 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. There are several techniques for separating contaminants. Preparative HPLC, TLC, and chromatography are three of the most widely used methods. The type of impurity and/or degradant, as well as how much of it is in the original material that needs to be isolated, will determine the precise technique to be employed. Because impurities and medicinal substances do not dissolve in the same solvents, extraction procedures are occasionally utilized to isolate contaminants. Based on the impurities' acidity, basicity, or neutrality, it is feasible to extract certain impurities with preference. Liquid-liquid extraction is typically used in the extraction process, with an organic phase that is non- polar and an aqueous phase. Aqueous phase pH can be adjusted appropriately to extract neutral, basic, or acidic contaminants. When there the polarity of a few impurities, or pKa, differs enough from that of pharmacological substance 20, the approach works effectively. Chromatographic techniques can be used to achieve more separations if needed. Additional techniques for isolating contaminants include SPE, and SFE. Prior to examination, samples are often cleaned up using some of the above-mentioned procedures, such as SPE and SFE. The analysis of contaminants is a major use of capillary electrophoresis. According to the mostrecent FDA and EMEA recommendations, in pharmacological compounds and drug products, the structure of impurities—unknown degradation products—must explain if they surpass a level of more than 0.1%. The most up-to-date analytical methods, such as semi- and completely preparative HPLC, are offered by analytical services for both preparative separation of unknown contaminants and structural clarification (e.g., MALDI-TO, GC-MS, LC-MSMS, and high- field NMR). Specialist’s work is supported by software technologies that forecast spectra.

Isolation methods of impurities Solid-Phase Extraction Methods

One technique of sample preparation that is becoming more and more helpful is solid phase extraction (SPE). The employment of costly, breakable specialist glassware, imperfect phase separation, recoveries that are not quantitative, and the elimination of amounts of organic solvents are just a few of the issues that can be avoided with SPE. Compared to liquid-liquid extraction, SPE is more efficient and produces quantitative extractions that are quick, simple, and automatable. Less time is spent in the lab and less solvent is used. SPE is usable solids which have already separated into solvents and is

frequently used for create extract semi-volatile or non-volatile analytes from liquid samples. SPE cleaning supplies are excellent, and extracting samples. They come in large range of sizes, chemistries, and adsorbents. It's critical to choose the best material for every sample and use. [16]

Liquid -Liquid Extraction Methods

A technique for separating substances according often called solvent extraction plus partitioning, liquid-liquid extraction refers to their relative solubility in a pair of distinct Immiscible liquids consist of water and an organic solvent. The process of moving material from one liquid phase to others. The process of liquid-liquid extraction essential in chemical labs. Procedure Can is accomplished using a separating funnel. As part of the workup, this kind of procedure is frequently carried out following a chemical reaction.

Accelerated Solvent Extraction Methods

A superior method for Sample matrices that are solid and semisolid under high pressure and temperature using common solvents is called Accelerated Solvent Extraction (ASE). There are two types of ASE systems: the fully automated ASE 350 the ASE 150 entry-level. With use of Dionium TM components and ASE with pH-hardened routes, in only a few minutes, extractions that typically take hours can be finished produces outcomes in a far shorter amount of time when compared to methods like Soxhlet and sonication. The ASE flow-through technology has made it possible to automate the several procedures required in sample preparation. Solid sample filtration and cleanup can be completed in a single stage as a component of the extraction of solvents. Because Accelerated Solvent Extraction uses up to 90% less solvent than other methods, it is less expensive per sample.

Supercritical Fluid Chromatography

One of chromatography in the normal phase utilized for the examination and purifying of thermally unstable, low-to-moderate molecular weight substances called supercritical fluid chromatography, or SFC. Chiral compound separation is another application for it. The principles of SFC are similar to those of HPLC; however, the entire chromatographic stream channel must be pressurized because carbon dioxide is typically employed as the mobile phase.

Column chromatographic technique

One popular method in chemistry for removing particular molecules from chemical mixtures is column chromatography. It is commonly used on scales ranging from micrograms to kilograms in preparative applications. A glass tube with a tap at the bottom makes up a conventional preparative chromatography column. These tubes are usually 50 mm in diameter and range in height from 50 cm to 1 m. There are two ways to setup the column: moist and dry. The mixture's constituents can separate due to their unique conversations with the stationary phase. as a result of their varying rates of passage through it. One by one, they then elute from the column. The eluent is gathered throughout the chromatography procedure. One by one, they then elute from the column. Several fractions of the eluent are recovered during the chromatography process. By examining each fraction for dissolved substances using eluent composition can be examined using techniques such as analytical chromatography, which UV absorption, and fluorescence. certain instances, colored compounds—or those that glow when exposed to ultraviolet light—can be seen as bands that move through the glass column. The molecules contained in each fraction are identified through analysis, and the process can be tracked as the fractions go through the column.

Flash chromatography

A quick chromatographic method called flash chromatography makes it easier to separate substances in a mixture according to how differently they bind to a stationary phase. This method works especially well for isolating contaminants from different samples and purifying organic molecules. Flash chromatography incorporates the concepts of both normal-phase and reversed-phase chromatography, allowing for versatility in separating polar and non-polar substances.

Key Principles and Mechanisms

Stationary Phase: Typically, the preferred stationary phase is silica gel, that can be modified to meet

different separation requirements. (e.g., reverse phase with C18 chains for more polar molecules).

Mobile Phase: One or more solutions make up the mobile phase. polarity and solubility of the target chemicals and contaminants can be used to optimize the solvent selection, which is crucial.

Pressure Application: Positive pressure is used in flash chromatography to quicken the elution process, in contrast to conventional column chromatography, which relies on gravity as its driving force. This shortens the separation time, which makes it appropriate for quick screening and purification.

Column Design: Because flash columns are typically shorter and wider than normal columns, they speed up the separation process. The size of the particles in the stationary phase can also affect the separation's resolution and efficacy.

Applications in Impurity Profiling

For the purpose of impurity profiling, flash chromatography has acquired popularity in a number of industries, including medicines, natural goods, and environmental analysis. Among the noteworthy applications are:

Pharmaceutical Industry

In order to guarantee adherence to regulatory requirements, it is utilized to separate and describe contaminants in pharmaceutical substances. Impurity identification and quantification may be completed quickly because to the fast separation.

Natural Product Isolation

Flash chromatography aids in the purification of substances while profiling any contaminants that may impact biological activity during the extraction of bioactive chemicals from natural sources.

Environmental Samples

Effective monitoring and evaluation are made possible by the use of flash chromatography, which helps with the examination of environmental samples, including soil and water, for the presence of pollutants and hazardous materials.

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)

Early in the drug development process, Thin-layer chromatography (TLC) is often used because it is affordable, ease of use, and capacity to handle numerous samples at once. When little is known about possible contaminants or degradants in drug substances and products, its high sensitivity and quick separation make it especially useful. Current developments in TLC plate technology, such as high-performance thin-layer chromatography (HPTLC), & improvements in instruments like densitometers have enhanced its utility. As a result, TLC has become a complementary tool to HPLC, play an essential role in the quantitative investigation of medication impurities. [17] Several advanced chromatographic techniques have been employed for identifying impurities in pharmaceuticals. One study used UV scanning at 248 nm to analyze chromatograms for diclofenac, as well as its contaminants and degradation products. Another group measured ciprofloxacin, chlorpromazine, trifluoperazine, promazine, and doxepin without using a spraying technique. An HPTLC method developed by Anjaneyulu et al. was effective in detecting impurities in alprazolam. Agbaba et al. introduced another HPTLC approach to identify impurities in pantoprazole and omeprazole. To enhance selectivity, Naidonget al. pre-treated the chromatographic layer with sodium- EDTA. Fluorimetric scanning at 400 nm was utilized to quantify impurities in tetracycline and chlortetracycline. Kochana et al. designed a method combining solid-phase extraction with TLC and fluorescence scanning to profile impurities in 3,4-methylenedioxymethamphetamine (MDMA) found in Ecstasy tablets. Additionally, Bagócsi et al. employed over-pressurized liquid chromatography (OPLC), a variation of HPTLC, with fluorimetric scanning following sulfuric acid spraying, to detect norethisterone impurities, showing improved efficiency compared to standard pharmacopoeial tests. [18,19]

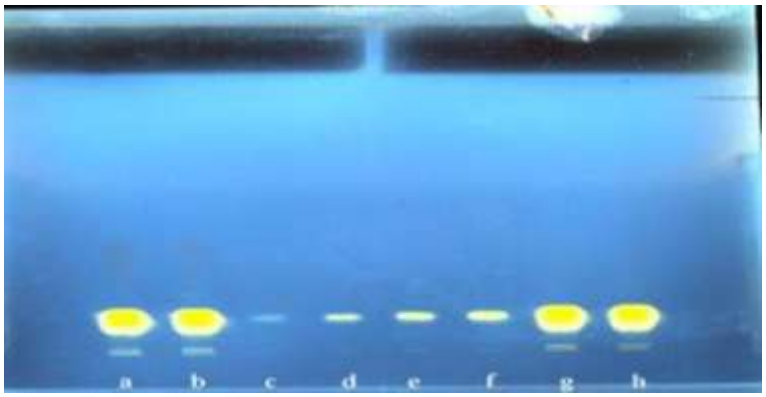

Figure 4: Impurity profiling of chromatography pharmaceutical's ingredients by thin layer

Gas chromatography

In analytical chemistry, GLC, often know as gas chromatography (GC), is a popular technique for isolating & evaluating substances that evaporate without breaking down. GC is commonly used to separate the various constituent of an ixture (The relative proportions of these elements can also be calculated.) or to assess the purity of a specific material. GC may be detecting a chemical in some circumstances. It can be used to extract chemicals from a mixture.

HPLC

This is also known as high-pressure liquid chromatography, is a famous method in branch analytical chemistry and biochemistry for separating, recognizing, and measuring compounds depending on their distinct polarities and interaction with a column's static phase. This system is made up of a pump that drives the mobile phase and analyte across the column, which is usually filled with stationary phases composed of hydrophobic alkyl chains. The system's detector measures the analyte's retention period, which varies depending on the chemical. In some configurations, extra data, such as UV/Vis spectral information, can be sent. The duration of the analyte's retention in the column is determined by its contact during the stationary phase. The composition & proportion of solvents in a mobile phase, as well as mobile phase's flow rate.

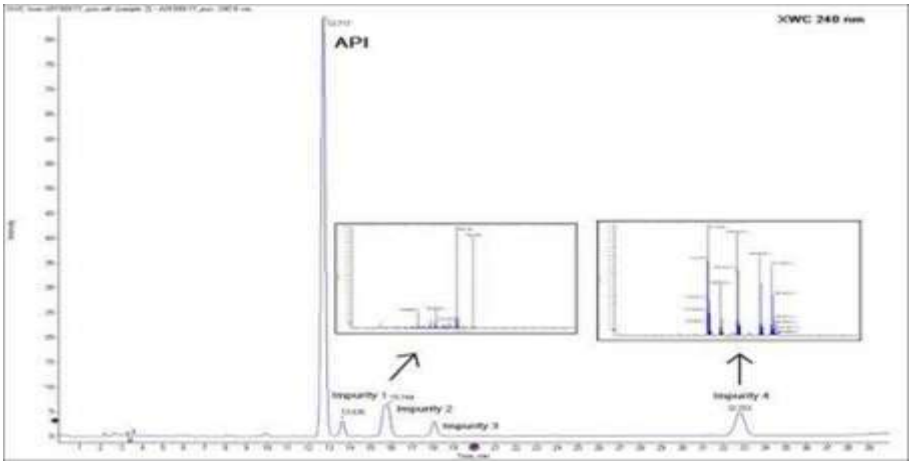

Figure 5: Impurity profiling by HPLC method

HPTLC

An advanced chromatographic technique called great-Performance Thin-Layer Chromatography (HPTLC) provides great resolution, sensitivity, and efficiency for identification, separation & quantification contaminants found in various materials, especially in the study of food and medicines. Its benefits include cost-effectiveness, the capacity to analyze several samples at once with little preparation, and the utilization of various imaging techniques, such UV and densitometry, which improve impurity identification. HPTLC is crucial tool for combining both quantitative and qualitative analysis. since it

makes it possible to compare the impurity profiles of several batches and ensure that regulations are followed. The process entails choosing suitable stationary and mobile phases, precisely applying samples, and developing plates in a controlled environment. HPTLC is still a flexible and effective tool for impurity profiling, despite certain obstacles including the requirement for standardized procedures and method development. Ongoing developments indicate that HPTLC will eventually have more automated and sophisticated detection capabilities. [20]

Capillary electrophoresis (CE)

The first notable use of this technic involves ammonium ions, as it is especially well-suited to the separation and evaluation of charged pollutants. is important because ammonium salts, which often appear as impurities, are not typically detected by techniques like TLC or HPLC, nor do they leave a residue in ignition tests. Gong et al. utilized non-aqueous CE coupled with indirect UV detection, using imidazole as the UV probe, to identify this cation. Similarly, Schöftneret al. applied indirect UV detection (using creatinine as the probe) to separate and identify tetraalkyl ammonium salts as impurities in colesevelam hydrochloride, comparing method's efficiency to ion chromatography. Ibandronate and its related compounds, which exhibit salt-like properties, were also analyzed, with phosphate and its impurities detected through indirect UV detection using sodium chromate as the probe. [21] Low pH conditions facilitate the protonation of basic compounds, making them more preferable for impurity profiling via capillary electrophoresis. For ex, Quaglia et al. employed an aqueous buffer at pH 2.5 containing trimethyl-cyclodextrin to effectively separate and quantify five impurities in fenticonazole using direct UV detection. Similarly, Sabbah & Scriba used low pH buffers in conjunction with UV detection to profile impurities in 3,4-diaminopyridine and 4-aminopyridine. Saavedra et al. demonstrated that CE could successfully separate an alprazolam degradant that could not be separated using HPLC. Furthermore, the differentiation of ethambutol hydrochloride and its degradant, 2-amino-1-butanol, was optimized using the Box-Behnken experimental design. Although CE can complement chromatographic techniques in some cases, its limitations in analyzing uncharged molecules often make it less viable as a complete alternative to HPLC for impurity analysis in pharmaceuticals.[22]

Electrons Paramagnetic Resonance

Paramagnetic Resonance of Electrons is a technique for searching chemical entities that include any or all unpaired electrons, inorganic complexes / organic and inorganic free radicals including transition metal ion. While electron spins rather than atomic nuclei's spins areactivated in EPR, the fundamental physics ideas are similar to those of NMR. Since most stable compounds have all of their electrons coupled, NMR is a more commonly employed technology when it comes to EPR. The EPR method's high specificity is compensated by its restriction to paramagnetic species, as EPR spectra are not produced by common chemical solvents or matrices.

Gravimetric Analysis

Particularly helpful for the separation and measurement of impurities, gravimetric analysis is a traditional, extremely accurate analytical method that calculates an analyte's amount based on its mass. Gravimetric analysis's basic method is converting the target material—typically an impurity—into a stable, pure state that can be precisely weighed. This approach has uses in a number of industries, including environmental research, pharmaceuticals, food safety, & components testing. They are frequently used in impurity profiling, especially for inorganic and metallic impurities. Its simplicity, dependability, and high degree of accuracy account for its continuing significance; nonetheless, in order to achieve precision, meticulous control over the experimental circumstances is necessary. The method of gravimetric analysis often comprises of multiple essential processes, which are dissolving, precipitation, filtering, drying, and weighing. In order to guarantee that the analyte or impurity is completely accessible for chemical reaction, the sample must first be dissolved in the correct solution. Subsequently, the impurity is precipitated as an insoluble solid by adding an appropriate reagent to the solution. Reagents like sodium sulfate or chloride, for instance, can be added to remove metal impurities like lead or silver from the solution by creating insoluble salts like barium sulfate or silver chloride. After the precipitate

has formed, any soluble pollutants are removed by carefully washing it after it has been filtered out of the solution—typically by vacuum filtration. Following filtration, any water or other volatile materials are burned or dried out of the precipitate, leaving behind a clean, stable solid that can be weighed. Finally, gravimetric analysis is particularly useful for impurity profiling because it can isolate and quantify particular impurities, especially inorganic substances like metals and metal oxides. By weighing the precipitate with a high-precision analytical balance, the analyst can calculate the impurity's mass. The great precision and direct measurement of contaminants without the requirement for calibration standards—which are frequently needed in experimental techniques like spectroscopy or chromatography—are the method's main strengths. For example, the concentration of metal contaminants in pharmaceutical formulations, where trace levels of metals like lead, mercury, or arsenic need to be strictly controlled due to their toxicity, is often determined using gravimetric measurement. Similar to this, gravimetric procedures are used in environmental studies to measure pollutants in water and soil samples, such as heavy metals (including chromium and cadmium), by turning them into insoluble compounds that can be filtered and weighed. [23]

Infrared Spectroscopy

A potent analytical method for identifying and quantifying chemical compounds, as well as for locating and analyzing contaminants in intricate mixtures, is infrared (IR) spectroscopy. Its foundation is the idea that certain infrared light frequencies are absorbed by molecules through vibrations, creating a distinct spectral pattern that serves as a molecular fingerprint. Because of its sensitivity to both organic and inorganic compounds, including those found in trace amounts in IR spectroscopy, the method is especially useful in impurity profiling. Impurities are identified by comparing the sample's absorption spectrum with reference spectra of pure substances. Impurities may be seen in the spectrum if there are variations, such as extra peaks or changes in peak locations. One popular infrared (IR) technique that improves detection sensitivity and enables quick, non-destructive investigation at high resolution is FTIR. Because it can find groups off functional like hydroxyl (-OH), carbonyl (C=O), or amine (- NH), which are frequently indicators of impurities, FTIR is very helpful for impurity profiling. The capacity of IR spectroscopy to evaluate solid, liquid, and gas samples without requiring a lot of preparation is one of its key benefits in impurity profiling. Because of this, it is helpful for quality control in sectors like pharmaceuticals, where the existence of contaminants may the safety and effectiveness of items. For instance, IR spectroscopy It is commonly used to check undesirable by- products or degradation products in raw materials, intermediates, and final products. [24]

Characterization methods

Extremely complex instruments, such MS coupled to a GC or HPLC, are essential for identifying minute elements (drugs, contaminants, breakdown products, and metabolites) in a variety of matrices. Different techniques are used to characterize contaminants. These strategies include the following:

NMR

Because NMR may reveal details about the precise stereochemistry and bonding structure of compounds of pharmacological relevance, it is an extremely useful analytical tool for elucidating structural mysteries. Using a common combination of real materials that contained both monomers and dimers, the ability of NMR-based diffusion coefficient measurement to discriminate between monomeric and dimeric compounds were demonstrated. Regretfully, in comparison to other analytical methods, NMR have historically been regarded as less sensitive approach. In contrast to MS, which needless than 1 mg, conventional NMR sample requirements are in the range of 10 m. [25]

MS

Over the last few decades, Technological advancements have had an increasing influence on the development of medicines process. Innovations in the structure and functionality of interfaces connecting differentiation techniques to MS was important to achieving this goal. When a single strategy does not provide sufficient selectivity, orthogonal coupling of chromatographic techniques, like HPLC-TLC and HPLC-CE, or combining chromatographic

differences with spectroscopic methods, such as HPLC-MS or HPLC-NMR, may be necessary. However, instead of using these combined methods for normal quality control, they are better suited as development tools. [26]

CONCLUSION

A substance under investigation's impurity profile provides the most comprehensive description of the impurities that are present in it. The quality criteria for producers are provided by the formulation of for the quantities of contaminants in medicinal substances and products. It is crucial to demonstrate that a novel chemical entity's impurity profiling is qualified. Because high dosage drugs have a qualifying threshold of 0.1% or less, the pharmaceutical analyst must carefully consider their analytical technology. High selectivity procedures, such as hyphenated techniques, maybe required, particularly during the development stages. Development scientists should be aware of the significance of qualifying impurity profiles in order to make Impurities in sets utilized in safety research are properly taken into account. This topic of impurity identification and quantification has advanced since the introduction of impurity limit tests. Impurity characterization and separation are necessary for data collection and analysis to establish biological safety, emphasizing the significance and extent of drug impurity profiling in pharmaceutical research.

REFERENCE

1. K. M. Alsante,P. Boutre, M. A. Couturier, Pharmaceutical Impurity Identification a Case Study Using a Multidisciplinary Approach, Journal of Pharmaceutical Sciences,2004:93(9):225-229.
2. N. R. Rao, S. S. Manikiran, N. L. Prasanthi, Pharmaceutical Impurities, Indian Journal of Pharmaceutical Education and Research,2010:44(3):301-310.
3. S. B. Bari, B. R. Kadam,Y. S. Jaiswal,A. A. Shirkhedkar, Impurity Profile and Significance in Active Pharmaceutical Ingredients, Eurasian Journal of Analytical Chemistry,2007:2(1):32-53.
4. Federal Register, International Conferences on Harmonization, Data, v4,2006.
5. Federal Register, International Conferences on Harmonization, Impurities in New Medicinal Products, Data, v4, 2006
6. V. S. Tegeli, G. K. Gajeli, G. K. Chougule, Significance of Impurity Profiling, International Journal of Drug Formulation and Research,2011: 2(4):174-195.
7. S. J. Ingale, C. M. Sahu, R. T. Paliwal, Advance Approaches for the Impurity Profiling of Pharmaceutical Drugs, International Journal of Pharmaceutical and Life Sciences,2011:2(7):955-962.
8. J. S. Peter, A. Ahmed,W. Yan, An HPLC Chromatographic Reactor Approach for Investigating the Hydrolytic Stability of a Pharmaceutical Compound, Journal of Pharmaceutical and Biomedical Analysis,2006:41(3):883-884.
9. T. Radhakrishna, J. Satyanarayana, A. Satyanarayana, Determination of Loratadine and its Related Impurities by HPLC, Journal of Indian Drugs,2002: 39(6):342-343.
10. T. Radhakrishna, J. Satyanarayana, A. Sataynarayana, HPLC Method for the Degradation of Celecoxib andIts Related Impurities, Journal of Indian Drugs,2002:40(3):166-168.
11. N.H. Zawilla, B. Li, J. Hoogmartens, E. Adams, Improved RP-LC Method Combined with Pulsed Electrochemical Detection for the Analysis of Amikacin, Journal of Pharmaceutical and Biomedical Analysis,2007:43(8):168-173.
12. R. Cornelis, Critical Evaluation of Scientific Literature on Trace Elements in Food and Diet, Journal of Pure and Applied Chemistry,2005:77(4):435-459.
13. N. Grekas, Organic Impurities in Chemical Drug Substances, Journal of Pharmaceutical Technology Europe,2005:17(10): 24-32.
14. A. Choudhary, P. Kaushik, HPTLC a Versatile Tool for the Analysis of Herbal Drugs, Asian Journal of Pharmaceutical Sciences,2020:15(4):1-10.
15. V. Patel, M. Patel, Recent Advances in HPTLC, International Journal of Pharmaceutical Sciences and Research,2013: 4(12):537-543.
16. M. Soni, R. Tiwari, HPTLC a Tool for Quality Control of Herbal Drugs, Journal of

Pharmaceutical and Biomedical Analysis,2011:55(5):988-996.
17. M. Bhalekar, P. Waghmare, HPTLC a Promising Analytical Technique for the Estimation of Impurities in Pharmaceuticals, International Journal of Research in Pharmaceutical Sciences,2013:4(1):120-124.
18. P. J. Derrick, P. Ratz, The Application of Fourier Transform Infrared Spectroscopy to the Analysis of Pharmaceutical Materials, Journal of Applied Pharmaceutical Science, 2000: 3(4):130-137.
19. A. M. Murray, D. J. Kiemle, Application of FTIR Spectroscopy for the Determination of the Quality of Pharmaceuticals, International Journal of Scientific Development and Research,2005:8(1):54-60.
20. H. M. Hwang, S. W. Lee, Application of FTIR Spectroscopy for the Detection of Environmental Pollutants, Journal of Environmental Science and Technology,2010:44(1):170-176.
21. M. Alam,M. Ali,A Comprehensive Review on High-Performance Thin Layer Chromatography (HPTLC) Method for Quality Control of Herbal Medicines, Journal of Pharmaceutical Sciences and Research,2020:12(3):411-421.
22. M. Nisha, M. Ismail, R. Ismail, F. Duncan, L. Maili, Impurity Profiling in Bulk Pharmaceutical Batches Using 19F NMR Spectroscopy and Distinction between Monomeric and Dimeric Impurities by NMR-Based Diffusion Measurements, Journal of Pharmaceutical and Biomedical Analysis,1999:13(4):511-512.
23. Shrinivas R. Mane, Sanjay K. Bais, Swapnil Waghmare, Synthesis of Schiff Base from O-Vanillin and Phenyl Urea by Using Catalyst Chloroacetic Acid, International Journal of Pharmacy and Herbal Technology,2024:2(03): 2231-2235.
24. M. G. Quaglia, E. Donati, E. Bossu,N. Desideri, F. Campana, Determination of Fenticonazole and its Impurities by Capillary Electrophoresis and High-Performance Liquid Chromatography, Journal of Separation Science,2001:24(5):392-396.
25. Shrinivas R. Mane, Sanjay K. Bais, Aditya A. Mali, Microwave Assisted Synthesis of Benzoic Acid, International Journal of Pharmacy and Herbal Technology,2024:2(3):1817-1818.
26. Shrinivas R. Mane, Sanjay K. Bais, Sarfaraz Kazi, Gauri Anuse, Microwave Assisted Synthesis of Benzocaine, International Journal of Pharmacy and Herbal Technology,2024:2(3):2076- 2082

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