INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Review Article

Cutting-edge Techniques for Impurity Profiling in Pharmaceutical Analysis: A brief Overview

Sanket S. Gite[1], Nikit S. Thakare[2], Aditi A. Kokande[3], Aniket B. Bairagi[4], Shubham R. Jadhav[5], Himmat D. Sawant[6]

[1]Department of Pharmaceutical Quality Assurance, Divine College of Pharmacy, Satana, Nashik, Maharashtra, India- 423301

[2]Department of Pharmaceutical Chemistry, Divine College of Pharmacy, Satana, Nashik, Maharashtra, India-423301

ARTICLE INFO

Received: 20 Nov 2023
Accepted: 22 Nov 2023
Published: 27 Nov 2023

Keywords:
Pharmaceutical impurities, Impurity, Pharmacopeia, Companies, Manufacturing, Method, Synthesis, etc

DOI:
10.5281/zenodo.10213447

ABSTRACT

Purity profiling involves the meticulous gathering and analysis of information to ascertain the biological safety of specific impurities, underscoring its crucial role and extensive scope within pharmaceutical research. In the realm of pharmaceuticals, the term "impurity" lacks a precise definition. Impurity profiling encompasses the identification, structural clarification, and quantitative assessment of impurities and degradation products present in bulk medicinal materials and pharmaceutical formulations. Given that undisclosed and potentially harmful impurities pose a threat to health, their detection and quantification through meticulous methods are imperative to bolster the safety of drug therapy, rendering impurity profiling increasingly pivotal in modern pharmaceutical analysis. Impurities are commonly denoted by terms such as residual solvents, byproducts, transformation products, degradation products, interaction products, and related substances. Determining impurity identities involves employing diverse chromatographic techniques alongside compliance with CGMP (Current Good Manufacturing Practices), QC (Quality Control), QA (Quality Assurance), and water activity examinations. Furthermore, a pharmaceutical ingredient must meet the criteria for novel impurities. The process of segregating and characterizing impurities assumes great significance as it enables the accumulation and assessment of data that establishes biological safety, thus highlighting the indispensability and potential of drug impurity profiling in pharmaceutical research. To effectively segregate and quantify impurities, an array of instrumental analytical methods has been consistently employed

***Corresponding Author:** Nikit S. Thakare
Address: *Department of Pharmaceutical Chemistry, Divine College of Pharmacy, Satana, Nashik, Maharashtra, India-423301*
Email ✉: thakarenikit05@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The detection and regulatory assessment of organic impurities present a formidable challenge due to the myriad sources of such impurities, including microbiological contamination, API (Active Pharmaceutical Ingredient) breakdown products, and trace amounts of intermediates

INTRODUCTION

In the pharmaceutical industry, a constant principle dictates that products must exhibit the highest possible purity. Whether derived from natural sources or synthesized through chemical processes, pharmaceuticals are perpetually undergoing development. Purity has thus assumed a critical role in ensuring drug quality, as virtually no market-available drug remains safe when consumed in excessive amounts. Between the era of Paracelsus in the 16th century and Ehrlich's time, a transition occurred from utilizing entire natural products to refined extracts or chemically manufactured compounds [1]. The foundational core of all pharmaceutical sectors lies in the bulk drug industry, where active pharmaceutical ingredients (APIs) of specific grades originate. Over the past few decades, considerable emphasis has been placed on the quality of new drugs entering the market. This quality assurance challenge extends to both pharmaceutical and bulk medication domains, necessitating stringent quality control assessments to safeguard product integrity.

The purity of the active medicinal ingredient is influenced by factors such as crystallization methods, purification procedures, and the raw materials employed in synthesis. The concept of purity evolves in tandem with advances in analytical chemistry, with pharmacopoeias establishing stringent limits on contaminant levels alongside purity criteria. Current analytical methodologies, which simultaneously segregate and quantify components, hold a prominent position in scientific research, facilitating the distinction and characterization of contaminants. Pharmaceutical impurities, undesirable substances associated with Active Pharmaceutical Ingredients (APIs), emerge during formulation or with the aging of medications [2,3]. The term "impurities in pharmaceuticals" encompasses unwanted entities present in APIs during formulation or aging [4-7]. These impurities can infiltrate drug products during formulation or interact with packaging materials, augmenting the diverse impurities inherent in drug products, including the drug substance and inert components employed for formulation. The definition of impurities extends to "any component of the drug product that is not a drug substance or an excipient in the drug product," as outlined by ICH Q6A: Requirements [8]. The burgeoning interest in impurities within Active Pharmaceutical Ingredients (APIs) is evident. Recent regulatory mandates have compelled the need for both purity and impurity profiles. Within the pharmaceutical sector, an impurity is defined as any additional organic substance arising from synthesis or undesirable compounds that persist within APIs. These impurities can emerge during formulation or subsequent to the aging of both raw and formed APIs within pharmaceutical products. The identification of impurities, exemplified by 1-(1, 2, 3, 5, 6, 7-hexahydro-s-indacen-4-yl)-3-4[-1-hydroxy-1-methyl-ethyl]-furan-2-sulphonylurea, through a multidisciplinary approach, effectively underscores this characterization [9]. Even minute quantities of these undesired compounds can adversely affect the safety and efficacy of medicinal products. Regulatory authorities are now placing significant emphasis on impurity profiling, encompassing the identification and quantification of impurities present in medications. Various Pharmacopoeias, such as the British Pharmacopoeia (BP), United States Pharmacopoeia (USP), and Indian Pharmacopoeia (IP), are progressively incorporating limits on allowable levels of impurities within APIs and formulations. Additionally, the International Conference on Harmonization of Technical

Requirements for Registration of Pharmaceuticals for Human Use (ICH) has issued guidelines for validating methodologies to analyze impurities in new drug substances, products, residual solvents, and microbiological impurities [10-13].

I. DEFINITION OF IMPURITY AND IMPURITY PROFILING

Impurity

Pharmaceutical impurities are the unwanted chemicals that remain with active. pharmaceutical ingredients (APIs) or drug product formulations.

As defined by the United States Pharmacopeia (USP), impurity is "any component of a drug substance that is not the chemical entity defined as the drug substance and in addition, for a drug product, any component that is not a formulation ingredient".

Impurity Profiling

The description, characterization and quantitation of identified and unidentified impurities present in the drug substances is known as impurity profile.

An impurity profile is a description of both known and unknown impurities, Contaminants in pharmaceutical products.

Why Drug Companies and Regulators Care About Impurity Profiles in Medicines:

a. It's really important to know what unwanted stuff might be in a new medicine or when making an existing one. Scientists who make drugs can often change how they make the drug to stop or reduce these unwanted things. They need to understand what these unwanted things look like.

b. The impurities can be manufactured after having recommended structures for them, providing concrete proof of the structures that were previously established using spectroscopic techniques.

c. When creating a selective procedure for the quantitative analysis, the material created might be utilized as an "impurity standard." identifying the impurity and applying using this technique as a quality control measure testing of every batch.

d. In the event of significant contaminants, the synthetic or solitary materials could be exposed to Thus, toxicological research significantly contributes to the security of pharmacological therapy.

e. The impurity profile of a drug substance is a reliable fingerprint for drug regulators to identify the consistency and scale of the production process of the bulk drug substance [14].

II. PHARMACOPOEIAL AND REGULATORY GUIDELINES AND STATUS ON IMPURITY PROFILING

In earlier versions of several pharmacopoeias, they didn't pay much attention to checking for impurities in medications. But in more recent editions, they've started focusing on it for many medications and included it in their guidelines. They've set limits on how much contamination is allowed in both the active ingredients and the final drug products in documents like the IP, BP, and USP. The International Conference on Harmonization (ICH) has also provided guidelines on how to test for impurities in new drugs, including substances, final products, any leftover chemicals, and microbial impurities [15].

According to USP, improvements in analytical chemistry are inseparable from changes in the idea of purity over time. If an item that was once thought to be pure can now be classified as having inorganic, organic, isomeric, or polymeric components that are deemed impurities.

Creating quality standards for medicines can be challenging because some medicines might have too many impurities, while others might have too few. If a medication changes over time, the tests that show if it's still good also show if it's still pure [16]. The BP (British Pharmacopoeia) divides impurities into two categories: "Qualified Detectable Impurities" and other "Impurities." These are the impurities that are considered

acceptable by competent authorities. They can be naturally occurring or present in very small amounts (less than 0.1%), but they are still controlled through testing. The purpose of the pharmacopoeia's guidelines is to ensure that medicines meet a minimum acceptable standard for consumers. Many guidelines focus on reducing impurities and byproducts that can degrade medicines. However, it's not practical to test for every possible impurity, contaminant, or adulterant in every medicine's guideline [17].

Table 1 lists the impurity acceptability standard. Impurities in drug products must be monitored for safety, efficacy, and economic as well as competitive reasons. However, even in the pharmaceutical sciences and business, monitoring contaminants and regulating these impurities might imply different things to different individuals or to the same people at different times. To ensure that everyone uses the same word when addressing queries about impurity, a combined terminology is required. The guidance created under the direction of the ICH has been approved by the US Food and Drug Administration (US-FDA). The European Union (EU), Japan, and the United States worked together to produce the ICH guideline for impurities in pharmaceuticals, which has helped to ensure that different areas have dependable standards for the data that should be reported to various regulatory bodies. The guidelines support the FDA reviewers and field investigators in their consistent interpretation and application of regulations, as well as the 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 different regulatory requirements for impurities, ***Table 2*** [18].

Table 1 Acceptance criteria for Impurities (As per Indian Pharmacopoeia

Criterion	For Drug Substances	For Drug Products
Each identified specified impurity	0.5%	-
Each unidentified impurity	0.3%	-
Total impurity	1.0%	-
Each identified specific degradation product	-	1.0%
Each unidentified degradation product	-	0.5%
Total degradation product	-	2.0%

Guideline	Depiction
Q1A	ICH guidelines “stability testing of new drug substances and products”
Q3A	ICH guidelines “Impurities in New Drug Substances”
Q3B	ICH guidelines “Impurities in New Drug Products”
Q3C	ICH guidelines “Impurities: Guidelines for residual solvents”
US-FDA	“NDAs -Impurities in New Drug Substances”
USFDA	“ANDAs – Impurities in New Drug Substances”

Table 2 Regulatory guidelines

III. SOURCES OF IMPURITY IN MEDICINE

(Impurities in medications can come from three main sources:) Impurities related to the main active ingredients (APIs): These can happen due to how the chemical structure is arranged, how it's made into crystals, or the specific groups of atoms in the APIs. Impurities related to the manufacturing process: These can be caused by the chemicals used in making the medication, like reagents and catalysts. They can also result from various steps in the production process, like intermediates and byproducts, or from how the medication is made and mixed.

Impurities related to the stability of the medication: These occur as the medication sits over time. It can include the API breaking down or reacting with other substances in the medication [19].

1. Synthesis Related Impurity:

Pharmaceutical compounds typically contain impurities since the synthesis process contaminates the final product with raw ingredients, solvents, intermediates, and by-products [20].

By-product: In organic chemistry, it's tough to make compounds that are 100% pure because there's always a chance of getting extra substances called byproducts. These byproducts happen because lots of things can go a bit wrong during the chemical reactions. It could be reactions that don't finish completely, changes in the arrangement of atoms, molecules sticking together, or other unintended reactions between the chemicals you started with [21]. For instance, a by-product of diacetylated paracetamol production. In the case of p reducing paracetamol [22].

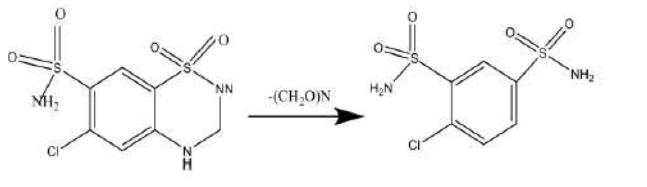

Figure 1 **Production of paracetamol from intermediate p-Aminophenol**

2. API Related Impurities:

They test for a substance called p-aminophenol in the raw materials used to make paracetamol because it's one of the ingredients in the process. It's important to check these raw materials because if they contain too much of this substance or other impurities, it could make the medicine less safe or effective, which is something we want to avoid [23]. There are numerous causes of contaminants in pharmaceutical goods, including.

Stereochemistry: Impurities in APIs can be brought on by their stereochemistry. In simple terms, when it comes to medicines, having just one specific version of a molecule (called a single enantiomer) is often considered better. This single version can sometimes work more effectively and be safer compared to mixtures of different versions. For example, Omeprazole is sold as its specific S-conformation known as esomeprazole, Ofloxacin as levofloxacin in its S-conformation, and Albuterol as levalbuterol in its R-conformation. These single forms can provide better results in treating medical conditions.

Crystallization: When medicines are made, one of the ways they can get impurities is during a process called crystallization. How a medicine's molecules arrange themselves during this process can change how the medicine behaves. It can create different versions of the same substance, called polymorphs, which have unique properties like how they dissolve, their shape, and more. These differences can affect how well the medicine works in your body. Because of this, pharmaceutical companies have to pay a lot of attention to these differences in their products to meet regulations from health authorities [24].

3. Organic Impurities:

If sufficient attention is not taken in each phase of the multi-step synthesis, organic contaminants are the most prevalent impurities detected in any API.

If the makers are not extremely vigilant about the impurities, even though the end products are always cleansed with solvents, it is always possible that the residual unreacted beginning ingredients will remain. There is a limit test for p-aminophenol in a paracetamol bulk, which may be used as a beginning material by one manufacturer or as an intermediate by another [Fig. 2].

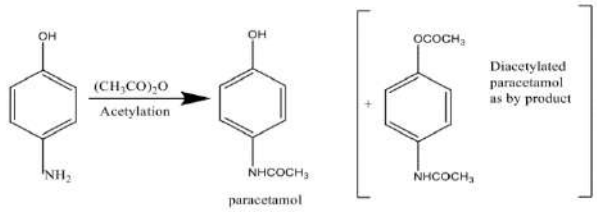

Figure 2 **Paracetamol is produced from the intermediate p-Aminophenol**

Oxidative degradation: The following substances are vulnerable to oxidative degradation: hydrocortisone, methotrexate, aminoazole, hydroxyl groups directly bonded to aromatic rings (e.g., phenol derivatives like catecholamines and morphine), conjugated dienes, heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (e.g., flavones).

Decarboxylation: When irloxacin undergoes a reaction when exposed to light, certain dissolved carboxylic acids, like p-amino salicylic acid, can lose carbon dioxide from their carboxyl group when they are heated during this reaction [25].

Hydrolysis: In some medicines that have an ester structure, like barbital, chloramphenicol, and others, a common thing that can happen is called hydrolysis. This mainly occurs in liquid forms of these medications [26]. Hydrolysis is when the ester part of the medicine breaks down when it comes into contact with water [27].

4. Photolytic Cleavage:

Pharmaceutical items are made as a solid or solution and then packaged after being exposed to light during manufacturing. When exposed to high energy UV radiation, the majority of substances breakdown as solutions (Ergometrine [28]. Nifedipine [29]. riboflavin, and phenothiazines are very labile to photooxidation.). It has been discovered that fluoroquinolone antibiotics are also photolytically cleavable [30]. The photocleavage reaction creates the ethylenediamine analogue of ciprofloxacin in ciprofloxacin eye drops [31].

Enantiomeric Impurities: In the world of medicines, having just one specific version of a molecule (called a single enantiomer) is often seen as better. It can potentially make the medicine work better, have fewer side effects, and be safer. However, in some cases, like with ofloxacin (in its R-form) and levofloxacin (in its S-form), both versions work similarly, so there's no big advantage to using just one. When it comes to controlling the unwanted version of a molecule in these medicines, it's treated like any other impurity.

Inorganic Impurities: It's also possible for inorganic contaminants to be produced during the manufacture of bulk medications. They include the following and are typically 11tilized1111 and named:

- ***Catalysts, ligands, and reagents:*** These contaminants are extremely unlikely to exist, although in some procedures they might if the producers don't take adequate precautions throughout production.
- ***Heavy metals:*** When we use acids or acid reactions, the water and sometimes the equipment, like stainless steel reactors, can introduce heavy metals into the process. To avoid this, we can use purified water (demineralized water) and equipment with a glass lining, which prevents these heavy metal contaminants from getting into the mix.
- ***Other materials (such as charcoal, filter aids, etc.):*** Activated carbon is frequently 11tilized in bulk drug manufacturing facilities in addition to filters or filtering aids like centrifuge bags. To prevent these

contaminations, it is crucial to regularly check the bulk medications for fibres and black particles.

In-Process Production Impurities:

- ***Contaminants connected to crystallization:*** Impurities are things that aren't supposed to be in a crystal when it forms. Even the liquid used to make the crystals can be seen as an impurity. Additives are impurities that are intentionally added to create a specific shape or structure in the crystals. When there are impurities or additives in the crystal-making process, it can really change how the crystals grow, stick together, and incorporate foreign particles into their structure [32].
- ***Solvents remain after processing:*** Residual solvents are chemicals used in making things or created during the production process. When producing large amounts of medicines, it's important to stay away from certain solvents that we know can be harmful [33]. Remaining solvents are broken down into three categories based on the potential risk to human health ***Table3***.

Category	Name of the solvent /limits	Unit/Specification
Class i	Benzene (2 ppm), carbon tetrachloride (4 ppm), methylene chloride (600 ppm), methanol (3000 ppm) pyridine (200 ppm) toluene (890 ppm)	More than this should be avoided
Class ii	N-Dimethylformamide (880 ppm), acetonitrile (410 ppm)	More than this should be avoided
Class iii	Acetic acid, ethanol, acetone (50mg)	Have permitted daily exposure of 50 mg or less per day, as per the ICH guideline.
Category	Name of the solvent /limits	Unit/Specification

Table 3 Classification of solvents on the basis of their limit in parts per million(ppm)

Synthetic by-products and intermediates impurities: In the process of creating new drugs or medicinal compounds, some unwanted substances can be created along the way. These impurities can form when making the initial materials, middle steps, or extra products. For example, impurities were found in the middle steps of making tablets using a specific chemical process called reductive amination. This was determined by analyzing the tablets and a substance called MDMA using a method called GC-MS [34].

Impurities generated during storage: Numerous contaminants might develop when medication products are being stored or transported. Stability studies must be conducted in order to forecast, assess, and guarantee the safety of pharmacological products [35].

Metal impurities: In the APIs and excipients, metal behaves as an impurity. Three categories can be used to categories metals, as shown in ***Table 4*** [36].

Class	Category	Example
Class i	metals of significant safety concern	Ur (uredinium), Pt (platinum), Rh (rubidium), Mo (molybdenum), V (vanadium) Cr (chromium) and Ni (nickel)
Class ii	metals with low safety concern	Cu (copper) and Mn (manganese)
Class iii	metals with minimal safety concern	Fe (iron) and Zn (zinc)

Table 4 Classification of metals on the basis of their safety concern.

Leachable / Extractables: When looking at the substances that can come out of materials or get into them, like chemicals from containers, it's really important to think about rules, safety, and science. This includes planning out how to study and figure out what these substances are, how much of them there are, and keeping an eye on them over time [37].

IV. IMPURITIES RELATED TO FORMULATION

The version of a drug that a scientist creates can contain impurities that form differently from the main drug impurities.

- ***Impurity Forms During Formulation:***

Method related: When a parenteral medication is made, a recognized impurity called 1-(2, 6-diclorophenyl) indolin-2-one is produced if the diclofenac sodium dosage form has undergone autoclave final sterilization [38]. When diclofenac sodium is processed in certain conditions with sodium hydroxide, it can turn into another chemical called an indolinone derivative. The initial acidity of the mixture affects how much of this undesired substance is produced. Sometimes, the amount of this substance can be higher than what's allowed in the final product stored in an ampoule according to the British Pharmacopoeia (BP) guidelines.

Environmental related: The following are the main environmental elements that can weaken stability

Exposures to adverse temperatures: Some things, like vitamins, can be easily damaged by hot weather or tropical conditions. When they get too hot, they lose their effectiveness, especially if they're in liquid form. So, it's important to store them in a cool place to keep them working well.

Light-especially UV light: Ergometrine and methyl ergometrine injections can get damaged in hot and sunny tropical places because of the heat and light. Sometimes, when you take samples in these areas, you might find that they have very little of the actual medicine in them. Only 50% of the marketed samples of ergometrine injections that were tested [39]. Normally, medicines should have between 90% to 110% of the amount they claim to have. But when a special ergometrine injection (which is supposed to have 0.2 milligrams per milliliter) was left in direct sunlight for 42 hours, it pretty much fell apart and didn't have the right amount anymore.

Humidity: Humidity is thought to be harmful to both bulk powder and manufactured solid dosage forms for hygroscopic products. Ranitidine and aspirin are two examples from the past.

Dosage form factors related: Pharmaceutical companies usually test their products to make sure they're stable before selling them. However, sometimes the way a medicine is made or packaged can cause problems, even after these tests. In the US, they had to recall 60-milliliter bottles of a skin solution called fluocinonide because it was getting weaker and had harmful things in it, which made it less effective [40]. In simple terms, liquid medicines can easily get spoiled or contaminated by bacteria, fungi, and yeast. Several factors can make this happen, like the amount of water in the medicine, its acidity, and how different ingredients interact with each other. Even the container can play a role.

If these microbes grow in the medicine, it can become unsafe to take. Also, if preservatives are not used correctly or if the container allows air and moisture to get in, the medicine's quality and safety can be affected. This means that liquid medicines can go bad and be harmful if they are not handled and stored properly [41].

V. FORMATION OF IMPURITIES ON AGING:

Mutual interaction amongst ingredients:

The majority of vitamins are exceedingly labile and become unstable in various dose forms as people age [42]. In liquid medicines with vitamins like folic acid, pantothenic acid, cyanocobalamin, and thiamine, if these vitamins break down, they don't create harmful substances, but the vitamins become less effective than what's required by medical standards. When you add nicotinamide to a mixture with four vitamins (nicotinamide, pyridoxine, riboflavin, and thiamine) for B-complex injections, it makes the thiamine vitamin deteriorate to a low level within a year of storage [43].

The pH level of the vitamin B-complex injections for sale was found to be between 2.8 and 4.0. When they studied a new formula made with just distilled water and compared it to the usual formula with disodium edetate and benzyl alcohol, they found that both had similar problems that caused the vitamins to break down.

Functional group-related typical degradation:

Ester hydrolysis: The following were examples. Aspirin, benzocaine, cefotaxime, cocaine echothiopate, ethyl paraben, cefpodoxime Proventil.

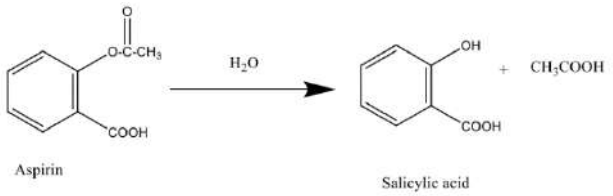

Figure 3 **Form action of Salicylic acid impurity from Aspirin**

Hydrolysis: Liquid medicines that contain certain types of drugs tend to break down over time, and this process is called hydrolysis. Some examples of these drugs are barbital, chloramphenicol, chlordiazepoxide, oxazepam, benzylpenicillin, and chloramphenicol [44].

Oxidative degradation: Certain substances like hydrocortisone, methotrexate, aminoazole, as well as molecules with certain structures like vitamin A and unsaturated fatty acids, can break down due to a process called oxidative degradation. This can also happen to compounds with specific structures like heterocyclic aromatic rings, nitroso and nitrite derivatives, aldehydes (found in flavorings), and hydroxyl groups attached directly to an aromatic ring.

Photolytic cleavage: Pharmaceuticals are made as a solid or solution, packaged, and stored in pharmacies all while being exposed to light. or institutions for future use, or kept by the client awaiting use. These substances are extremely susceptible to photo-oxidation: ergometrine [45], nifedipine [46], nitroprusside, riboflavin, and phenothiazines. When certain molecules are exposed to sunlight, they can produce tiny particles called free radicals, which can cause more chemical reactions to happen. When you shine strong UV light on most substances in liquid form, they tend to break down. Some antibiotics called fluoroquinolones have been found to break down when exposed to this kind of light [47].

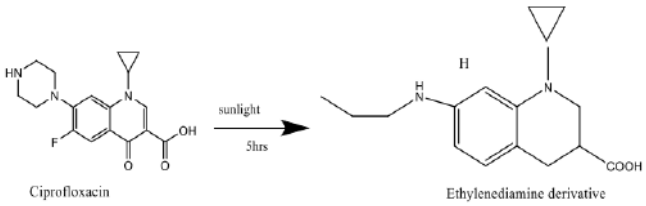

Figure 4 **Photolytic cleavage of Ciprofloxacin in eye drops preparation .**

Decarboxylation: When you heat certain chemicals like p-amino salicylic acid that are dissolved in a solution, they can lose a carbon dioxide molecule that was originally attached to them. This process is called "decarboxylation." It can also happen to substances like irloxacin when they undergo a reaction due to exposure to light [48].

VI. CHARACTERIZATION OF IMPURITIES:

When we find impurities in a new medication, the first step is to figure out how much of the impurity is present. We use a reference standard, which is a known substance, to measure this. If our analysis shows that the impurity is higher than 0.1%, we have to follow FDA guidelines and include it on the medication label.

These impurities can come from various sources like raw materials, things that happen during the manufacturing process, or other substances added to the medication. To understand these impurities better, we can use different methods, some involving chromatography (a technique to separate and analyze compounds) and others not.

In simpler terms, when we find impurities in a new medication, we need to know how much is there. We use known substances to measure it. If it's

more than 0.1%, we have to mention it on the label as per FDA rules. These impurities can come from different places, and we use different methods to study them, including one called chromatography.

The following separation methods are most frequently used:

a. Accelerated Solvent Extraction Method (ASE): There's a method called ASE that speeds up the process of getting impurities out of a substance. It's faster than other methods like sonication or Soxhlet extraction. ASE works better because it uses higher pressure and temperature, which help to get impurities out more effectively. Plus, it's cheaper because it uses 90% less solvent (the liquid used to extract impurities).

b. Supercritical Fluid Extraction (SFE): When a substance is heated or compressed above a certain point, it becomes a supercritical fluid. This special substance can act like a gas, moving through materials, and also like a liquid, dissolving solids. Using supercritical fluid is great for extracting things from a mixture because it helps stuff move quickly from the sample to the solvent. Carbon dioxide is a popular choice for this because it becomes supercritical at a relatively low temperature. This method can be used for different types of analysis, like high-performance liquid chromatography (HPLC), gas chromatography (GC), and supercritical fluid chromatography (SFC). SFC, in particular, has some advantages over HPLC and GC. It can work with substances that don't easily turn into a gas and can be sensitive to heat, it uses a universal flame detector, and it creates narrower peaks in the data, which can be helpful for analysis. However, HPLC and GC are still widely used in most cases, except for specific situations like chiral separations and studying big molecules like hydrocarbons [49].

c. Column Chromatography: Column chromatography is a method that separates different parts of a mixture as it flows through a column filled with a special material. The components in the mixture move through the column at different speeds because some like the material in the column (stationary phase) more than the liquid moving through it (mobile phase). This difference in attraction helps to separate them effectively.

However, the downside is that this process can be quite slow because the liquid moves through the column gradually due to gravity. The big advantage of column chromatography is that it can be adjusted to fit the specific needs of a project. This is really useful when you're trying to separate and purify a mixture after a series of chemical reactions. But keep in mind, it can take some time to set up and use this method correctly [50].

d. Thin Layer Chromatography (TLC): Thin-layer chromatography (TLC) is a method used to find even tiny amounts of different substances in a sample. It's handy for creating a type of analysis that can tell us if a product is stable or not over time. However, it has its downsides - it can't always give us exact amounts, and it can vary from one test to another. TLC can be used as a simple way to figure things out quickly. It can also be turned into a more precise method when used with something like high-performance thin-layer chromatography (HPTLC), which can handle tricky substances that don't show up easily. The idea behind TLC is that different substances interact differently with the materials on the plate, and this helps us detect and identify them. It's often used to see how much a product breaks down or changes under different conditions, especially during testing and stress experiments [51].

Utilizing the HPTLC approach, numerous stabilities indicating methods have been reported. tablets containing medications like telmisartan and ramipril [52], Prasugrel [53], Drotaverine and Cyclofenil in tablets [54], Pseudoephedrine and Cetirizine in pharmaceutical formulations [55].

e. Gas Chromatography (GC): Timing APIs is crucial for finding contaminants, especially the

ones that can easily turn into gas and withstand high temperatures. It's used to check for things like leftover solvents in medicine or to understand the starting materials used in making drugs. Gas chromatography (GC) is a method that has some advantages. It's faster, can handle more samples, uses less expensive equipment, and gives a clearer signal. However, it needs careful attention when setting up and using the machine.

GC is only good for substances that can become a gas without falling apart and can evaporate at a safe temperature (not too hot to damage the machine). It's mainly used for substances that can turn into a gas or something similar. But there are some challenges with GC. It's tough to measure and inject really small samples (about 0.3 microliters) without them evaporating. Sometimes, sample loss can happen if there's a problem with the rubber seal used for injecting the sample.

Sometimes, tiny bits of rubber can stick to the column and cause strange signals called "ghost peaks" in gas chromatography (GC). To avoid this, you can inject the sample right into the hot part of the machine's injector.

When GC is used with something called an internal standard (a known substance added to the sample), it can be just as accurate and precise as high-performance liquid chromatography (HPLC). In simple terms, GC can give very reliable results if you use it right, especially when you use an internal standard. Various articles of study focused on stability stating that there are available GC techniques, for example; identifying divalproex sodium contaminants in pharmaceutical formulations [56], fluconazole [57].

f. High Performance Liquid Chromatography (HPLC): HPLC is a versatile method of analysis as it is not limited to volatile or stable sample and separation is based on the fact that certain compounds have different migration rates on a particular stationary and mobile phase [58]. component separation using the HPLC technique and any suitable detector, such as a refractive index detector [59], PDA detector [60], An accurate, precise, and reliable method for quantitative analysis of pharmaceutical products and impurities is provided by fluorescence detectors, electrochemical detectors, electrical conductivity detectors, light scattering detectors, evaporative light scattering detectors, Corona Charged Aerosol Detector (CAD), Nano Quantity Aerosol Detector (NQAD), etc. HPLC also involves monitoring of stability of pure drug substance and in case of drug formulations. It can be used to measure degradation products, such as salicylic acid, betamethasone dipropionate, and their related chemicals in diplomatic lotion using a stability-indicating technique and HPLC [61], norfloxacin [62], allantoin [63].

g. Flash Chromatography: Gravity-fed chromatography is a slow and not very effective method. We can replace it with something called flash chromatography, which is a combination of medium pressure and short column chromatography. In flash chromatography, we use air pressure to push the solvent through the column faster, which speeds up the purification process.

In flash chromatography, we use tiny silica gel particles to help move the solvent quickly across the stationary phase, which is the part that separates the substances we want. This method is much more efficient than the old gravity-fed approach [64].

h. Capillary Electrophoresis (CE): Electrophoresis is a method used to separate charged molecules based on their physical characteristics. It makes these molecules move in different directions. However, traditional electrophoresis has some downsides. For one, you need to finish the separation before you can see the molecules. Additionally, using high voltage can risk damaging the samples due to heat. Capillary

electrophoresis is a solution to these problems. It uses a very narrow tube called a capillary, which has a high surface area compared to its volume (because it's so thin). This capillary helps dissipate heat effectively, preventing the samples from getting too hot. It's particularly useful for finding different drug-related impurities in various types of samples because it allows for efficient separation and detection of molecules [65].

Capillary electrophoresis (CE) offers some advantages over reversed-phase high-performance liquid chromatography (RP-HPLC). It provides a different way to separate substances and is more flexible, with high separation efficiency, fast method development, and good resolution. CE is particularly useful when you need to separate impurities that are very polar and don't retain well in RP-HPLC. CE is well-known for its effectiveness in separating charged pharmaceuticals and contaminants. Many studies and examples show its use in analyzing impurities, including a method for determining metformin hydrochloride in tablets, which is used to check the quality and stability of the medication. In simple terms, CE is a valuable tool for separating and identifying different substances, especially those that RP-HPLC might struggle with [66].

It can also be coupled with a mass spectrometer to create a system called CE-MS using a variety of interfaces, such as a triple tube nebulizer [67,68].

Micellar electrokinetic chromatography (MEKC) is a technique within capillary electrophoresis (CE) that allows the separation of analytes, including those that are electrically neutral. It achieves this by adding something called an ionic micelle to the running solution, and it doesn't require any major changes to the CE instrument. The idea behind MEKC is that these ionic micelles help the analytes move differently under electrophoresis conditions, along with interactions between the micelles and the analytes in the buffer solution. So, in a way, MEKC uses a similar principle as regular chromatography. MEKC is a powerful method, especially for separating small molecules, whether they are charged or not. It can quickly and efficiently separate samples, which is handy for various applications, like checking the stability and content of pharmaceutical products, such as fluticasone nasal sprays used to reduce cholesterol levels. MEKC can be used for stability testing, determining the quality of the product, and making sure it contains the right amount of active ingredients [69-71].

Various other instrumental methods are also used for the purpose of characterization of impurities in any sample. These methods include:

- Nuclear Magnetic Resonance (NMR) Spectroscopy
- Mass Spectroscopy (MS)
- IR Spectroscopy
- Hyphenated Techniques

VII. APPLICATIONS OF ISOLATION AND CHARACTERIZATION OF IMPURITIES:

Drug impurity profiling is a necessary step to make sure that pharmaceutical products are safe and effective. This process involves monitoring and designing drugs to meet regulatory requirements. It's essential for ensuring the quality, stability, and safety of pharmaceutical chemicals, whether they are made synthetically or derived from natural sources or produced using recombinant techniques. This includes a wide range of substances like alkaloids, amines, amino acids, painkillers, antibiotics, drugs for seizures, antidepressants, sedatives, cancer-fighting drugs, local anesthetics, large molecules, steroids, and more. All of these substances have various purposes in the field of medicine [72].

CONCLUSION

When it comes to pharmaceutical ingredients, they need to pass various tests like CGMP, QC, QA, and water activity tests. They also have to meet specific criteria for new impurities. Separating and characterizing these impurities is crucial for

collecting information that ensures the safety of these ingredients. This highlights the importance of studying and profiling drug impurities in pharmaceutical research. Scientists use a variety of analytical instruments to separate and measure these contaminants. Organic impurities can come from different sources, such as microbial contamination, the breakdown of the active ingredient, or leftover substances from the manufacturing process. Identifying and dealing with these organic impurities is a challenging task, even though there are guidelines from organizations like the International Council for Harmonization (ICH). In essence, there's a pressing need for consistent standards and requirements for impurities in pharmaceuticals to ensure their safety and quality.

REFERENCES

1. Barber HJ. Historical Aspects of Chemotherapy: Six Essays. May and Baker; 1978.
2. Ahuja S, Alsante KM, editors. Handbook of isolation and characterization of impurities in pharmaceuticals. Academic press; 2003.
3. Roy J. Pharmaceutical impurities—a mini-review. AAPs PharmSciTech. 2002 Jun;3(2):1-8.
4. Ahuja S, Alsante KM, editors. Handbook of isolation and characterization of impurities in pharmaceuticals. Academic press; 2003.
5. S. Ahuja. Impurities Evaluation of Pharmaceuticals, Marcel Dekker, Inc. New York, 2006.
6. Ahuja S, Scypinski S, editors. Handbook of modern pharmaceutical analysis. Academic press; 2001 Aug 9.
7. Roy J. Pharmaceutical impurities—a mini-review. AAPs PharmSciTech. 2002 Jun;3(2):1-8.
8. Keitel S. Impurity Profiles in Active Pharmaceutical Ingredients. In EU/Swiss medic GMP Workshop Beijing University 2006 Sep (Vol. 9).
9. Alsante KM, Boutros P, Couturier MA, Friedmann RC, Harwood JW, Horan GJ, Jensen AJ, Liu Q, Lohr LL, Morris R, Raggon JW. Pharmaceutical impurity identification: a case study using a multidisciplinary approach. Journal of Pharmaceutical Sciences. 2004 Sep;93(9):2296-309.
10. International Conference on Harmonization (2000) Draft Revised Guidance on Impurities in New Drug Substances. Federal Register Q3A(R) 65 (140): 45085.
11. International Conference on Harmonization (2000) Draft Revised Guidance on Impurities in New Drug Products. Federal Register Q3B(R) 65 (139): 44791.
12. International Conference on Harmonization (1997) Impurities, Q3C- Guidelines for Residual Solvents, Q3C. Federal Register 62(247): 67377.
13. International Conference on Harmonization (1999) Specifications, Q6A: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products. Chemical substances 65 (146):67488.
14. Görög S, Babjak M, Balogh G, Brlik J, Csehi A, Dravecz F, Gasdag M, Horvath P, Lauko A, Varga K. Drug impurity profiling strategies. Talanta. 1997 Sep 1;44(9):1517-26.
15. Guideline IH. Impurities in new drug substances Q3A (R2). In Proceedings of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use, Geneva, Switzerland 2006 Oct 25 (Vol. 25).
16. Deshpande MM, Bhalerao MH, Pabale PD. A Review on Impurity Profiling, Degradation Studies, and Bioanalytical Methods of Anti-diabetic Drugs.
17. Pharmacopoeia I. Ghaziabad: Indian Pharmacopoeia Commission. Govt. of India-

Ministry of Health and Family Welfare. 2014:1948.
18. Dhangar KR, Jagtap RB, Surana SJ, Shirkhedkar AA. Impurity profiling of drugs towards safety and efficacy: theory and practice. Journal of the Chilean Chemical Society. 2017 Jun;62(2):3543-57.
19. Beckett, A.H; Stanlake, J.B. Practical Pharmaceutical Chemistry. CBS Publishers and distributors: New Delhi, 2002.
20. Ahuja S, Scypinski S, editors. Handbook of modern pharmaceutical analysis. Academic press; 2001 Aug 9.
21. Damle MC et al., Development and Validation of Stability Indicating HPTLC Method for Determination of Ofloxacin and Ketorolac Tromethamine in Combination, J. Adv. Sci. Res. 2011; 2 (3): 77-82.
22. Connors KA, Amidon GL, Stella VJ. Chemical stability of pharmaceuticals: a handbook for pharmacists. John Wiley & Sons; 1986 Oct 13.
23. Nagpal S, Upadhyay A, R Bhardwaj T, Thakkar A. A review on need and importance of impurity profiling. Current Pharmaceutical Analysis. 2011 Feb 1;7(1):62-70
24. Görög S, editor. Identification and determination of impurities in drugs. Elsevier; 2000 May 19.
25. Condorelli G, De Guidi G, Giulfrido S. Molecular mechanisms of photosensitization induced by drugs XII. Photochemistry and photosensitization of rufloxacin: An unusual photodegradation path for the antibacterials containing a fluoroquinolone like chromophore. Photochem Photobiol 1999;70:280-6
26. Connors KA, Amidon GL, Stella VJ. Chemical stability of pharmaceuticals: a handbook for pharmacists. John Wiley & Sons; 1986 Oct 13.
27. Hoerle SL, Evans KD, Snider BG. HPLC Determination of Impurities in a 3 rd Generation Cephalosporin. InSomerset, New Jersey: Eastern Analytical Symposium 1992 Nov 16.
28. Roy J, Bhuiyan K, Faruque A. Injectable ergometrine: Stability and packaging for developing countries. Indian Drugs1997; 34:634-6.
29. Kumar V, Sunder N, Potdar A. Critical factors in developing pharmnaceutical formulations-An overview. Part 2. Pharm Technol 1992;16:86-8
30. Smith A, Pennefather PM, Kaye SB. Fluoroquinolones - place in ocular therapy. Drugs 2001;61:747-61
31. Pilaniya K, Chandrawanshi HK, Pilaniya U, Manchandani P, Jain P, Singh N. Recent trends in the impurity profile of pharmaceuticals. Journal of Advanced Pharmaceutical Technology & Research. 2010 Jul;1(3):302.
32. Hatakka H, Alatalo H, Palosaari S. Effect of Impurities and additives on crystal Growth Available from: http://www2.lut. fi/~hhatakka/docit/impure.html.[Last accessed on 2010].
33. Jacobs P, Dewe W, Flament A, Gibella M, Ceccato A. A new validation approach applied to the GC determination of impurities in organic solvents. Journal of pharmaceutical and biomedical analysis. 2006 Feb 13;40(2):294-304.
34. Gimeno P, Besacier F, Bottex M, Dujourdy L, ChaudronThozet H. A study of impurities in intermediates and 3, 4 methylenedioxymethamphetamine (MDMA) samples produced via reductive amination routes. Forensic Sci Int 2005;155:141-57.
35. Ahuja S. Impurities evaluation of pharmaceuticals. Informa Health Care; 1998 Mar 27.
36. Pilaniya K, Chandrawanshi HK, Pilaniya U, Manchandani P, Jain P, Singh N. Recent trends in the impurity profile of pharmaceuticals.

Journal of Advanced Pharmaceutical Technology & Research. 2010 Jul;1(3):302.
37. Markovic I. Evaluation of safety and quality impact of extractable and leachable substances in therapeutic biologic protein products: A risk-based perspective. Informa 2007;6:487-91.
38. Roy J, Islam M, Khan AH, Das SC, Akhteruzzaman M, Deb AK, Alam AH M. Diclofenac sodium injection sterilized by autoclave and the occurrence of cyclic reaction producing a small amount of impurity. J Pharm Sci. 2001;(90):541-544.
39. Roy J, Bhuiyan K, Faruque A, Sobahan M, AlFarooque M. Injectable ergometrine: stability and packaging for developing countries. Indian Drugs. 1997;34(11):634-636.
40. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA. Impurity profile: Significance in Active Pharmaceutical Ingredient. Eurasian journal of analytical chemistry. 2007 Mar 1;2(1).
41. Hoq MM, Morsheda SB, and Gomes DJ. Development of appropriate preservative system for liquid antacid: bacterial contaminants in antacid samples. Bang J Microbiology. 1991;8(1):5-9.
42. Buhler V. Vademecum for Vitamin Formulation. Stuttgart, Germany: Wiss. Verl-Ges; 1988.
43. Roy J, Mahmud M, Sobhan A, Aktheruzzaman M, Al-Farooque M, Ali AE. Marketed vitamin B-complex injectables: stability and mutual interaction. Drug development and industrial pharmacy. 1994 Jan 1;20(13):2157-63.
44. Connors KA, Amidon GL, Stella VJ. Chemical stability of pharmaceuticals: a handbook for pharmacists. John Wiley & Sons; 1986 Oct 13.
45. Hoerle SL, Evans KD, Snider BG. HPLC Determination of Impurities in a 3 rd Generation Cephalosporin. InSomerset, New Jersey: Eastern Analytical Symposium 1992 Nov 16.
46. Kumar V, Sunder N, Potdar A. CRITICAL FACTORS IN DEVELOPING PHARMACEUTICAL FORMULATIONS: AN OVERVIEW. II. Pharmaceutical technology. 1992;16(4).
47. Roy J. Pharmaceutical impurities—a mini-review. AAPs PharmSciTech. 2002 Jun;3(2):1-8.
48. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA. Impurity profile: Significance in Active Pharmaceutical Ingredient. Eurasian journal of analytical chemistry. 2007 Mar 1;2(1).
49. Bart, C. J. Separation Techniques - Additives in Polymers: indusrtrial analysis and applications. John Wiley and Sons. 2005, p. 212.
50. Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem., 1978, 43(14), 2923-2925.
51. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. Journal of pharmaceutical and biomedical analysis. 2002 Jun 15;28(6):1011-40.
52. Pal K, Raju MB, Kumar KP, Chakravorthy R. Simultaneous Determination of Telmisartan and Ramipril in Tablet Dosage Form by Spectrophotometry. Parameters. 2010 Sep 28;97(101.60):97-103.
53. Borole, T.C.; Mehendre, R.; Damle, M.C.; Bothara K.G. Development and validation of stability indicating HPTLC method for determination of Prasugrel. J. Chem. Pharm. Res., 2010, 2(4), 907-913.
54. Wankhede, S.B; Mahale, D.K; Chitlange, S.S. Stability-indicating HPTLC method for simultaneous determinationof Drotaverine and Aceclofenac in tablet formulation. Der. Pharma. Chemica, 2010, 2(5), 107-117.
55. Makhija, S.N; Vavia, P.R. Stability indicating HPTLC method for the simultaneous

determination of pseudoephedrine and cetirizine in pharmaceutical formulations. J. Pharm. Biomed. Anal., 2001, 25, 663-667.

56. Subasranjan, A.; Suresh, P.; Srinivasulu, C.; Hemant, R. A validated stability-indicating gas chromatography method for determination of divalproex sodium impurities in pharmaceutical preparation. Drug Test. Anal., 2010, 2(4), 182-1.
57. Hunt-Fugate, A.K; Hennessey, C.K; Kazarian, C.M. Stability of fluconazole in injectable solutions. American J Hospital Pharm., 1993, 50(6), 1186-1187.
58. Pal K, Raju MB, Kumar KP, Chakravarthy R. Simultaneous Determination of Telmisartan and Ramipril in Tablet Dosage Form by Spectrophotometry. Parameters. 2010 Sep 28;97(101.60):97-103.
59. Raymond S. Chromatographic detectors design: Function and operation. Chromatographic science series. 1995;73:201-4.
60. Jadhav Ankush P, Kedar Tejashree R, Gaikwad Vishal S, Jadhav Ravindra T. RECENT APPROACHES FOR IMPURITY PROFILING: A REVIEW.
61. Shou M, Galinada WA, Wei YC, Tang Q, Markovich RJ, Rustum AM. Development and validation of a stability-indicating HPLC method for simultaneous determination of salicylic acid, betamethasone dipropionate and their related compounds in Diplomatic Lotion®. Journal of pharmaceutical and biomedical analysis. 2009 Oct 15;50(3):356-61.
62. Rao RN, Nagaraju V. Separation and determination of synthetic impurities of norfloxacin by reversed-phase high performance liquid chromatography. Journal of pharmaceutical and biomedical analysis. 2004 Mar 10;34(5):1049-56.
63. Zaidi ZR, Sena FJ, Basilio CP. Stability assay of allantoin in lotions and creams by high-pressure liquid chromatography. Journal of Pharmaceutical Sciences. 1982 Sep 1;71(9):997-9.
64. Still WC, Kahn M, Mitra A. Rapid chromatographic technique for preparative separations with moderate resolution. The Journal of Organic Chemistry. 1978 Jul;43(14):2923-5.
65. Nagpal S, Upadhyay A, R Bhardwaj T, Thakkar A. A review on need and importance of impurity profiling. Current Pharmaceutical Analysis. 2011 Feb 1;7(1):62-70.
66. Hamdan II, Jaber AB, Abushoffa AM. Development and validation of a stability indicating capillary electrophoresis method for the determination of metformin hydrochloride in tablets. Journal of pharmaceutical and biomedical analysis. 2010 Dec 15;53(5):1254-7.
67. Sauber, C.; Ross, G. Impurity Profiling with Capillary Electrophoresis/Ion Trap Mass Spectrometry. Application Note, Agilent Technologies, USA
68. Blomberg LG, Wan H. Determination of enantiomeric excess by capillary electrophoresis. ELECTROPHORESIS: An International Journal. 2000 Jun 1;21(10):1940-52.
69. Terabe S. Capillary separation: micellar electrokinetic chromatography. Annual Review of Analytical Chemistry. 2009 Jul 19;2:99-120.
70. da Silva Sangoi M, Magalhães da Silva L, D'Avila FB, Dalmora SL. Determination of fluticasone propionate in nasal sprays by a validated stability-indicating MEKC method. Journal of chromatographic science. 2010 Sep 1;48(8):641-6.
71. . Bretnall AE, Hodgkinson MM, Clarke GS. Micellar electrokinetic chromatography

stability indicating assay and content uniformity determination for a cholesterol-lowering drug product. Journal of pharmaceutical and biomedical analysis. 1997 May 1;15(8):1071-5.

72. Gupta Raghav1 , Dr. Jain Suresh 1, Gupta Ankit1. a review on the impurity profile of pharmaceuticals. international journal of drug formulation and research.2014,april 15.

HOW TO CITE: Sanket S. Gite, Nikit S. Thakare, Aditi A. Kokande, Aniket B. Bairagi, Shubham R. Jadhav, Himmat D. Sawant, Cutting-edge Techniques for Impurity Profiling in Pharmaceutical Analysis: A brief Overview, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 11, 571-587. https://doi.org/10.5281/zenodo.10213447