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A Detailed Review on Analytical Methods to Manage the Impurities in Drug Substances

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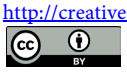

Abstract

Impurities, particularly those associated with the API (active pharmaceutical ingredient) and causing deterioration or interaction, may lower a drug's quality, safety, and efficacy. The successful reduction and control of impurities in pharmaceuticals rely on safety-based impurity limits. Many methods have been developed to define a practically safe dosage of impurities, with an emphasis on daily exposure limitations. They include permissible daily exposure (PDE), acceptable intake (AI), the threshold of toxicological control (TTC), and staged TTC. So, the less than lifetime (LTL) limits for mutagenic impurities were implemented, which are based on Haber's law, which stipulates that concentration and exposure durations are both crucial for estimating potential safety risks to people. Before moving on to further processes like analytical techniques and acceptance criteria, sources of impurities must be properly characterized so that regulatory requirements and management plans may be defined and adhered to. Pharmaceutical impurities and the current worldwide regulatory requirements for their control were discussed. The main aim of the study is to examine analytical methods to manage the impurities in drug substances. The study also focuses on the quality by Design approach for the analysis of impurities in pharmaceutical drug products and drug substances. In addition to this, the study also analyses strategies for the identification, control, and determination of genotoxic impurities in drug substances. Genotoxic impurities can be quantified at the trace level by using analytical techniques like LC-MS/MS, and GC-HS-MS/MS. Lastly, the study examines the importance of impurity analysis in pharmaceutical products".

Subject Areas

Analytical Chemistry

Keywords

Impurities, Drug Substances, Pharmaceutical Drug Products

1. Introduction

Drugs' identities, purities, physical properties, and efficacies, as well as their bioavailability and stability, are the area that needs to be determined by effective analytical methods. In the context of evaluating pharmaceuticals, namely the active pharmaceutical component, analytical method development and validation may be thought of as the process of demonstrating that analytical processes are appropriate for the purpose. Specific features of the compounds are analysed, using these appropriate analytical methods, and compared to acceptability criteria. Therefore, the development of analytical methods requires the assessment and selection of the most accurate estimation processes to establish a drug's composition (Dispas, 2018) [1].

For a drug development program to succeed, analytical methods must be developed and validated. At least three key reasons have been identified by researchers as to why analytical technique development is crucial for any business generating novel medication candidates. Since the quality of medicine is fundamental to the prospects of a pharmaceutical development program, organizations creating novel compounds need to give great attention to the research and development of appropriate analytical methods. Second, regulatory agencies all over the globe want proof of analytical technique suitability for its purpose before they will approve applications for clinical trials or marketing permits (Raman, 2011) [2]. Ultimately, people will be the ones getting the impurities in early phase clinical trials (first in human/Phase 1 studies), thus it is crucial to assure safety and perhaps show promising effectiveness in the novel medicines via the development and manufacturing quality of a drug.

The main aim of the study is to examine analytical methods to manage the impurities in drug substances. In addition, the study also focuses on a quality-by-design approach for the analysis of impurities in pharmaceutical drug products and drug substances. It also examines strategies for the identification, control, and determination of genotoxic impurities in drug substances. Lastly, it analyse the importance of impurity analysis in pharmaceutical products.

2. Analytical Methods to Manage the Impurities in Drug Substances

2.1. Quality by Design Approach (QbD) for the Analysis of Impurities in Pharmaceutical Drug Products and Drug Substances

According to Yabré (2020) [3], Good Manufacturing Practices (GMP) regulations stress the need for quality control in the pharmaceutical business. To guarantee quality and GMP compliance, this idea has to be present across the whole lifespan of a pharmaceutical product. The supplies, reagents, references, data, and deliverables of any type, as well as the laboratory's environment, equipment, procedures, and personnel, must all be managed. However, it is now generally acknowledged that risk management for a pharmaceutical product is an essential

part of any quality assurance system. "An effective quality risk management approach can further ensure the high quality of the drug (medicinal) product to the patient by providing a proactive means to identify and control potential quality issues during development and manufacturing" states ICH Q9. Subramanian (2020) [4] stated that in the event of a quality issue, implementing quality risk management may also enhance decision-making. This may be accomplished by instituting control mechanisms according to the level of risk involved in the operation. So, it is crucial to have a solid scientific understanding of the product's quality. Applying a systematic approach to pharmaceutical development, as made possible by the Quality by Design (QbD) strategy, is the only way to achieve such a degree of knowledge.

Quality control measures, whether performed during or after production, are an integral part of every manufacturing process' lifetime. For example, in the area of impurity control, analysts may encounter difficulties depending on the created product. The physico-chemical and chromatographic behaviour of analytes is a topic that has been studied in depth by scientists for a long time. The comprehensive knowledge of the retention or migration processes for liquid, gas, or supercritical fluid chromatography has made it considerably simpler to deal with complex samples.

Muchakayala (2022) [5] stated that approaches to developing analytical methods are built on a foundation of prior information. In development, the One-Factor-at-a-Time (OFAT) method is often used, along with Quality-by-Testing, which may be thought of as a trial-and-error method (QbT). This approach is often used since it is (erroneously) thought to get a good solution quickly. Despite its potential usefulness for straightforward issues, such a method may be too simplistic for more complicated samples or more laborious separations (*i.e.*, impurities analysis). Bastogne (2022) [6] analyzed that a QbT methodology does not characterize analytical methodologies adequately has been shown time and time again. Peak retention/migration time is affected by several variables that are not well understood or controlled, including mobile phase, pH, gradient duration (and so on), and technique uncertainties. On top of that, it would be impossible to conform to USP standards or pharmaceutical recommendations about risk management with this kind of development, much alone conduct a systematic evaluation of robustness throughout the development process. According to Katakam (2020) [7] the authors' recommended, Analytical Quality by Design (AQbD).

2.2. Overcoming Barriers to Implement Quality by Design (QbD) in Analytical Method Development

Teng (2022) [8] stated that choosing appropriate parameters for a separative approach is the first step in its creation. While the explanation following will center on liquid chromatography for ease of writing, the principles discussed are not limited to this method alone. There are unique challenges associated with chromatographic factor screening that have discouraged the widespread use of the

Design of experiment (DoE). Some of the first researched major components, such as the stationary phase and solvent, are qualitative, which is one of the problems. As a result, it becomes prohibitive to develop sparse and efficient experimental designs because of the sheer volume of additional factors required for statistical modelling. Another problem is that simple models cannot account for all column-to-factor interactions since the chromatographic interactions of continuous components (pH, etc.) may be vastly different for various stationary phases. Pasquini (2021) [9] examined that the ability to limit the number of tests is lost, however, when dealing with complicated models that include higher- order interactions. For this reason, the authors suggest determining qualitative parameters like stationary and mobile phases based on a scientific understanding of the compounds and any relevant contaminants before beginning screening. It is quite improbable that these values would shift during typical operations. Then, quantitative parameters like pH, mobile phase composition, or gradient duration may be investigated during technique optimization for robustness. Preliminary evaluations of qualitative aspects may be carried out if required. Peak forms, analysis time, and selectivity are often optimized when deciding which column(s) to use. Researchers must know how the stationary phase, mobile phase, and target molecule interact with one another.

2.3. Difficulties Related to Impurities Analysis

According to Subramanian (2022) [10] for a design of experiment (DoE), it may be challenging to get significant levels of certain impurities (e.g., degradation and manufacturing contaminants). The material may degrade throughout the course of the several runs that make up the DoE. There might be noise in the retention time measurements and difficulty with the identification of the compounds if the peak width and height changed between runs. It is common practice to adjust a drug's composition during the course of its lifetime. During stability over the time overall impurities may increase. This may cause the presence of extra compounds in the chromatogram, increasing the likelihood of co- elution for a particular chromatographic condition. Similarly, to the active pharmaceutical ingredient (API), impurities typically have molecular structures that are close to or connected to the API, and hence they frequently exhibit similar chromatographic performance. Manoel (2020) [11] examined that when selecting the stationary phase, it is critical to make use of all available data to choose a column that will allow for full separation. To put it another way, these gaps seldom widen to the extent that would be optimal. Fortunately, even though their behaviour remains linked, they often remain disjointed even when subjected to differences in the separative processes.

Pasquini (2020) [12] analyzed that "one of the key concerns is the concentration levels and specifically the gap between API and impurities concentration. Furthermore, the impurity level could be quite low". This necessitates the use of a stability signaling approach that can keep tabs on them. However, the DoE was established to create just such a procedure. In certain cases, it may be feasible to

spike the sample to raise the impurity concentration.

Pielenhofer (2023) [13] stated that developing DS that expresses a high likelihood of satisfying specification(s) with high resilience is important to produce a flexible technique that can overcome the obstacles outlined above. This implies that the approach may be simply upgraded by expanding the range of chromatographic variables that define the DS. Not knowing all multivariate combinations and interactions of input components considerably increases the expense of establishing an OFAT (and, by extension, developing a completely new assay due to a new impurity or a change in formulation).

According to Niedermeier (2020) [14] quality control for both raw materials and final goods relies heavily on the identification of contaminants. As a result of the need for selectivity and sensitivity in the detection of contaminants, separation methods are often used. It is recommended to take a systematic approach to the creation of methods to cope with the similar chemical structures of API and contaminants and the varying concentrations of each. The authors presented the AQbD approach, which uses DoE and probabilistic DS computing in this setting. A thorough comprehension of procedures and threat assessment is made easier with the AQbD technique.

2.4. Strategies for the Identification, Control, and Determination of Genotoxic Impurities in Drug Substances

Ali (2019) [15] stated that "mutations, chromosomal damage, and/or chromosomal rearrangements are all ways in which genotoxic impurities (GTIs) may cause cancer in humans. Both the International Council for Harmonization (ICH) and the European Medicines Agency (EMEA) publish recommendations outlining the maximum allowable amounts of pollutants in drug substances and medicinal products. To mitigate the negative effects of GTIs, daily dosage limitations must be established". Even though this is preferable from a quality standpoint, it still requires allocating resources to new method creation. Scientists need to find GTIs early on in the process development phase, provide analytical methodologies, and prove the synthetic process controls to combat this. But not all producers of pharmacological substances or APIs have easy access to the required strategies. As a result, researchers have attempted to provide a summary of the methods used to identify, regulate, and quantify GTIs in pharmaceutical ingredients.

According to Kumar (2023) [16] "The Pharmaceutical Research and Manufacturers of America (PhRMA)" set up a procedure for testing, classifying, and certifying GTIs to determine their level of safety. It provided a variety of functional groups (structural alerts or alerting structures) that are known to engage in interactions with DNA. Three distinct classes were established for them. "Purines and pyrimidines, intercalators, PNAs and PNAHs, and aromatic groups like N-hydroxy aryls, N-acylated aminoacyl, aza-aryl N-oxides, aminoacyl, and alkylated aminoacyl" makeup Group 1. "Aldehydes, N-methylols, N- nitrosamines, nitro compounds, carbamates (urethanes), epoxides, aziridines, propi-

olactones, propiosultones, N or S mustards (beta haloethyl), hydrazines, and azo" compounds are all examples of the second group, which consists of alkyl and aryl groups. "Michael-reactive acceptors, alkyl esters of phosphonates or sulphonates, haloalkenes, primary halides, and other heteroaromatic groups make up Group 3. (alkyl and aryl-CH2)".

Liu (2019) [17] analyzed that there are five distinct types of contaminants, according to PhRMA's classification system. Group 1: contaminants with established links to both genotoxicity (mutagenesis) and carcinogenesis. The default choice is to remove these contaminants by adjusting the method since they pose the most danger. If this is not feasible, the TTC idea will have to be implemented. Second-class contaminants are those with confirmed genotoxic (mutagenic) but not yet established carcinogenic properties. Class 3: impurities with warning structures that are unrelated to the API structure and have an undetermined genotoxic (mutagenic) potential; these impurities must be managed according to TTC principles. In this category are contaminants that have functional moieties that have been related to genotoxicity via structural analysis. Lastly, Liu (2019) [17] has Class 4 contaminants, which are API-related warning structures including impurities. These contaminants share a functional moiety with the parent structure that serves as an alarm. Substances without detectable markers of genotoxicity are included in Class 5.

According to Kasina (2021) [18] pharmacists and toxicologists must conduct a toxicology evaluation to discover the presence of GTIs and how they entered the synthetic process, then look for ways to get rid of them and set limits that are in line with safety and regulatory requirements. Methods such as computational toxicological assessment and literature reviews are also suitable for this purpose. Based on their observations of connections between electrophilicity and DNA reactivity in Ames-testing data, Ashby and Tennant first proposed the idea of identifying structural alarms for genotoxic activity. The literature produced publications with many structural warnings. Most people use Windows and use programs like MDL-QSAR, MC4PC, and DEREK because of the lack of certainty around structural warnings, regulatory action should not be taken based merely on the existence of any certain functional group. Each instance of anticipated genotoxicity has to be assessed independently in light of the existing research and the outcomes of genotoxicity tests.

Reddy (2019) [19] stated that GTI might be used in a synthetic process in a variety of capacities, such as a precursor, intermediate, catalyst, by-product, isomer, or degradant. Some researchers have advocated using a synthetic, computer-generated pathway to depict the vector of entry for GTIs in their research. The reaction of a pharmacological substance's salt counter ion (such as hydrogen halide) with alcohols also produces these. Common counter ions used to create API salts include "methane sulfonic acid (mesylate), benzene sulfonic acid (besylate), and toluene sulfonic acid (tosylate)". In certain cases, GTIs may be produced when these acids react with lingering alcohols. "Imatinib mesylate, amlodipine besylate, and denagliptin tosylate are three examples of sulfonylureas

that may form associations with alkyl methane sulphonates, alkyl benzene sulphonates, and alkyl p-toluene sulphonates, respectively". While camphor sulfonyl chloride may be used to successfully resolve racemic omeprazole magnesium, the inclusion of alcohols in the synthesis process can lead to the medication associating with alkyl camphor sulphonates.

Wang (2022) [20] pointed out that sulfate alkyl esters are also carcinogenic both epoxides and hydroperoxides are genotoxic. Researchers have discovered that isomers of some medication contaminants are genotoxic (e.g. EE isomer of terbinafine impurity). Using genotoxic reagents is just one potential drawback; the formation of signaling degradants is another. The degradation of pharmacological compounds leads to the creation of GTIs, as shown in recent research. Structural indicators in degradants include "aldehydes, unsaturated carbonyls, aromatic amines, hydroxylamine and its related esters, epoxides, and polyaromatic hydrocarbons". Miniyar (2022) [21] examined that two primary sources of degradants may serve as alerts: 1) a parent medicine that already has an alarm built into its structure. Yet again, there are two distinct kinds of this. In the case of oxybuprocaine, a drug that contains a structural alert for aromatic amines, hydrolysis produces "a degradant acid with the same alerting structure for aromatic amines; 2) degradants with a different alerting structure than the parent drug are formed, such as acetaminophen, which contains a structural alert for Nacylated aminoacyl, yields a degradant with a different alerting structure than the parent drug; p-Aminophenol now has a new structural red flag, an aromatic amine. 3) a degradant is formed from a parent medication that previously lacked a structural warning but now does; for example, propofol, which did not have a structural alert, oxidizes to a dimeric degradation product with several conjugated unsaturated carbonyl systems, structural alerts for mutagenicity".

According to Fu (2022) [22] the synthetic process is dynamic and genotoxins may enter several different locations, making it difficult to detect and eliminate them. Accordingly, structural alarms that induce genotoxicity must be found by screening synthetic approaches. The discovery of GTIs would need the exploration of potential replacement synthetic methods that would allow for the regulation of GTIs. If this cannot be done technologically, then the TTC notion of safety restrictions must be implemented. Analytical results with sufficient selectivity and sensitivity are required at these lower values. Additionally, GTIs need ongoing monitoring throughout the drug development process. If the route is changed, then new intermediate compounds must be considered and evaluated. "If the tolerable toxicological limit changes as a consequence of the daily dose decrease, the process and analytical techniques for control at the new level must be assessed. The overall risk and expense during drug substance creation must be balanced via interdisciplinary cooperation with specialists in toxicology, synthetic, and analytical chemistry". Finally, it is important to note that the USFDA acknowledges that "although marketed medicinal products are required to be safe, safety does not mean zero risk" in their final comment on the topic. If the risks are small in comparison to the potential payoffs and the alternatives, then

the product may be considered safe.

2.5. Importance of Impurity Analysis in Pharmaceutical Products

According to Rahman (2006) [23], the pharmaceutical business is expanding rapidly to discover and develop novel pharmaceuticals either from natural materials or synthetic chemically generated pharmacological compounds; nevertheless, "one thing remains constant: the product should be as pure as possible". Purity, therefore, has long been seen as a crucial criterion in guaranteeing medication quality. Keep in mind that no medication has been proven safe and that even a small dose can have fatal results. From the time of Paracelsus (who lived in Basel in the first half of the 16th century) to the time of Ehrlich, there is a clear progression away from the use of natural goods in their whole condition, toward the use of either pure extracts from those products or toward the use of synthetic chemically created compounds (who was awarded a Nobel prize in 1909 for his extraordinary study and breakthrough during the first decade of this century). In the last century, there has been a dramatic increase in interest in the study and assessment of the purity of natural goods due to claims made by researchers that apparent amounts as measured by weight are not virtual quantities after accounting for the alloy of impurities. As a result, it is abundantly obvious that pharmaceutical research during the last century has substantially improved human health and quality of life.

Ahuja (2003) [24] stated that pharmaceutical contaminants in the 0.01% to 0.1% range are receiving more attention from drug registration authorities. The term "impurities" is used to describe unwanted substances that may be present in active pharmaceutical ingredients (APIs), develop during the formulation process, or be formed as a byproduct of the degradation of APIs or APIs used to make drugs. Pharmaceutical goods' viability and safety might be compromised by even trace levels of these undesirable substances.

Tegeli (2011) [25] analyzed that the impurity profile of a drug substance is affected by many different variables, such as the quality of the raw materials, reagents, and solvents used in the synthesis, the conditions under which the reactions took place, the purification steps taken, and the length of time the drug substance was stored. Even a little shift in those factors might have a significant impact on the impurity distribution. Impurities must be detected in drug material made in development batches, commercial batches, and under stress settings. When contaminants are detected at concentrations of 0.1% or more, or in certain situations 0.2% or more, relative to the prescribed daily dose, their structures should be determined. As indicated structures, the impurities are synthesized. To create a selective analytical procedure for its quantification in drug material and/or products, a reference impurity standard is created.

According to Maggio (2014) [26], API impurities may be broken down into three groups for the sake of regulation: "organic impurities, inorganic impurities, and residual solvents. Organic impurities may come from a variety of sources, including raw materials (often isomeric impurities), synthetic interme-

diates (incomplete reaction or excess reagent used), byproducts, degradation products, reagents, ligands, and catalysts". Although not usually present, these compounds, ligands, and catalysts may be troublesome contaminants in active pharmaceutical ingredients (APIs). Pharmaceutical products may include inorganic pollutants such as "equipment, reagents, catalysts, heavy metals, drying agents, and filter aids. Nonetheless, even though impurities from reagents, ligands, and catalysts are infrequent, they may still pose problems if producers are not vigilant. Water used in the operations and the reactors (if stainless steel reactors are utilized) where acidification or acid hydrolysis occurs are the primary sources of contamination of heavy metals".

Singh (2012) [27] examined that using demineralized water and glass-lined reactors makes it simple to eliminate these heavy metal contaminants. Both the potential for toxicity and environmental damage, as well as the fact that volatile contaminants like residual solvents may give medications off-putting organoleptic qualities, need their detection and analysis. Considering that excipients and sometimes medicinal product manufacturing might generate residual solvents. The International Conference on Harmonization (ICH) has established three categories for residual solvents based on their potential damage to human health. "Group I includes substances including benzene (2 ppm limit) and carbon tetrachloride (4 ppm limit). Category II solvents include methylene chloride (600 ppm), methanol (3000 ppm), pyridine (200 ppm), toluene (890 ppm), N, N-dimethyl formamide (880 ppm), and acetonitrile (410 ppm limit). Category II solvents are widely used in industrial settings. Category III solvents include acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethyl acetate". The tolerance levels of these solvents are greater. Daily doses of 50 mg or fewer are suggested per ICH recommendations.

According to Ramachandra (2017) [28] "With the introduction of ICH, US-FDA, WHO, and European Committee for Directorates recommendations, the need for the construction of a stability-indicating assay became more explicitly demanded". Separation of the drug ingredient from degradation products is a mandatory requirement of these recommendations, which call for forced decomposition experiments to be conducted under a wide range of circumstances including pH, light, oxidation, dry heat, etc. A stability-indicating test is defined as "a validated quantitative analytical procedure that can detect the changes with time in the relevant properties of the drug substance and drug product." Generally, the stability-indicating assay validation process consists of two phases. Prabu (2010) [29] stated that first, the drug material is studied for forced breakdown, and stability indicating test is developed using what is known about the drug's degradation behaviour. At this point, establishing specificity and selectivity is the primary goal of validation, followed by the development of additional criteria like accuracy, precision, linearity, range, robustness, etc. To ascertain the retest or expiration date of a bulk medicine, this established technique is used to analyze stability samples. Second, when the stability indicating the test is used to different matrices, such as formulations, the focus narrows to demonstrating the

assay's applicability in the presence of excipients or other components of the formulation. In this case, just the most important metrics are revalidated, such as specificity, selectivity, accuracy, and precision. In two recommendations, ICH has standardized the necessary conditions. The first provides a concise overview and definition of the validation criteria required for different testing methodologies. The second one goes further by including the necessary experimental data and some statistical interpretation into the first one. These recommendations provide a foundation for regulatory agencies and industries alike throughout the globe, and they bring the necessity of correct validation to the attention of everyone engaged in the process submission.

Bari (2007) [30] examined that validation work requires an in-depth understanding of the parameters' context and the consequences of changing them. Linearity assessments need five concentration levels, accuracy evaluations require nine determinations across three concentration levels, and precision evaluations require six determinations at the 100% level. The ICH guideline offers a wide variety of options for determining the lowest concentration that can be reliably measured. They often depend on the scatter (variability) of analytical data in the low concentration range or the analysis of blanks. By utilizing the blank method, the computed value is increased by a factor of 3.3 for the detection limit and a factor of 10 for the quantitation limit. The estimated result might reflect either the blank's signal, blank standard deviation, or blank intercept (corresponding to an extrapolated blank). Nonetheless, these limitations are essential for the disclosure of pollutants and the dissemination of analytical methodologies.

2.6. Role of Impurity Profiling Methods Using Modern Analytical Techniques

Ramachandra (2017) [28] stated that nowadays, the pharmaceutical industry is an essential part of the global economy. The establishment of this commerce has already had and may continue to have, a profound effect on people's daily lives. Research and development (R & D) for novel medication compounds is a major area of investment for the pharmaceutical industry. It takes over $1 billion and 10 - 15 years to create a new medicinal compound. Around 280 therapeutic novel molecular entities were authorized by the FDA in the decade from 2010 to 2020, making the oversight of these medications' quality, safety, and efficacy a top priority for regulators. The last decade has seen a rise in the use of analytics to aid in the drug development process because of this, advances in technology are always being made to meet these needs. These days, pharmaceutical analysis is used to provide reliable data for a variety of purposes, including medication safety research, development, and monitoring, as well as post-market regulatory compliance.

Venkatesan (2014) [31] analyzed that there may be no more pressing issue than the need to create an analytical technique for a newly released medicinal ingredient or formulation. Titrimetric, spectrophotometric, qualitative, and activity procedures are only a few of the many analytical methods utilized to verify

the quality standards of the drug. Artificial, medical specialty, pharmaceutical, and clinical analysis all rely heavily on the analytical input provided by pharmaceutical research today. This allows for the creation of incredibly cost-effective medication treatments. In particular, there would not be an official way to measure things like impurity levels, degradation products, or medication dosages if they were ever accumulated, and no suitable analytical methods for doing so. These contaminants are largely to blame for the poor quality of drugs on the market today. In the process of creating a new medicine, identifying and characterizing any possible contaminants may be essential. Spectroscopic tests (such as NMR, IR, and MS) should be performed to characterize the structure of the actual contaminant or degradation product present among the medication constituent.

Holm (2016) [32] stated that impurities should be scientifically even at the 0.1% level or greater. Given the pharmaceutical industry's ever-increasing need for refined analytical methods, this review's subject matter was chosen to cater to those needs. Current analytical methods such as "UPLC, LC-MS, LC-Q-TOF, GCMS, HPTLC, and LC-NMR" were cited among many others. "Impurity sources, kinds, management strategies, identification, regulatory factors, degradation products, and stability-indicating assay methods (SIAMs)" were also discussed.

According to Lemasson (2015) [33] at the turn of the twentieth century, there was no system in place to ensure the quality and safety of the medicines being produced and sold. The best medicines available at the time were of questionable safety and efficacy and sold for exorbitant prices. In the early 1960s, for instance, the term teratogen was established, and it quickly became a pejorative term. Infants born to mothers who drank throughout their childbearing years were severely malformed. Further research confirmed that the s-enantiomer of teratogen has agent activity but cannot provide the desired sedation effect. According to Shah (2012) [34] as a result, the FD & C was updated to stipulate that all new medications must provide evidence of their safety in advance, in addition to a slew of "other and several other" safeguards. Analgesic pills provide the simplest illustration of the need for the development of impurity detection procedures. Titrimetric analysis using American, British, and Indian samples was used to determine its quality in the 1970s. The book includes a color check to help in the identification of free hydroxy acid, a byproduct of degradation. It was not just hydroxy acid (H_3O^+) that was found to be contaminating bulk medicinal compounds after HPLC's widespread use in the pharmaceutical industry. Allergies are a direct result of the reaction between these contaminants and the amino functionalities of supermolecules. Pharmaceutical analysis, and notably impurity detection, necessitates the use of sophisticated instruments. The action procedures, which include HPLC, UPLC, and LC-MS, have become the most prominent kind of analytical method.

Lemasson (2016) [35] stated that separating, identifying, and quantifying the individual components of a mixture is the goal of high-performance liquid

chromatography, a method developed from high-pressure liquid chromatography in analytical chemistry. High-performance liquid chromatography (HPLC) has surpassed all other analytical techniques since it is non-destructive and may be utilized with thermally sensitive compounds (unlike GC). Several column packings (stationary phase) and detection strategies allow for a wide range of separation selectivities. "Reverse phase liquid chromatography (RPLC) is becoming more popular due to its broad selectivity, reproducibility, compatibility with pharmaceutical materials, and suitability for MS detection because of their dependability and the ability to check the purity of the chromatographic peaks, reversed phase (C18, C8, etc.) columns, and UV and PDA detectors are often employed in HPLC analysis".

Alexander (2012) [36] stated that unidirectional pumping liquid chromatography (UPLC) is a cutting-edge method that provides a fresh perspective on the field of chromatography. Ultra-performance liquid chromatography (UPLC) is characterized by improvements in three key areas: speed, resolution, and sensitivity. Since the 1970s, HPLC Methods have been replaced by Ultra-Performance liquid chromatography (UPLC). UPLC's analytical efficacy is comparable to that of HPLC, however, it operates at significantly greater pressures. particles in HPLC columns typically range in size from 2.5 microns to 5 microns. Whereas porous particles less than 2 microns in size were the basis for the design of UPLC columns. In contrast to particles in an HPLC column, which may achieve high flow rates at lower pressures (6000 psi), they need far greater pressures (15,000 psi). Smaller particles have a shorter distance to travel before they are analyzed by the stationary phase, so their use is more productive. When compared to HPLC detection, UPLC detection is theoretically two to three times more sensitive. Improvements in instrumentation and column technology have allowed UPLC to increase its speed, resolution, and sensitivity. As mass spectrometry is a major motive in the modern pharmaceutical sector, UPLC makes them perfectly suited to use with mass spectrometry. Framework for the estimation of impurities in API's is depicted in **Figure 1**.

3. Discussions and Findings

Rapid growth in the pharmaceutical industry is driven by the pursuit of new therapeutic agents, which may be derived from natural sources or chemically synthesized pharmacological compounds. Therefore, purity has long been acknowledged as a fundamental criterion in assuring medication quality. You should remember that no medication is completely safe and that even a low dose can have fatal effects. From the time of Paracelsus, who lived in Basel in the first half of the 16th century, to the time of Ehrlich, there was a clear shift away from using natural goods in their whole state and toward using either pure extracts from those products or chemically made compounds (who was awarded a Nobel prize in 1909 for his extraordinary study and breakthrough during the first decade of this century).

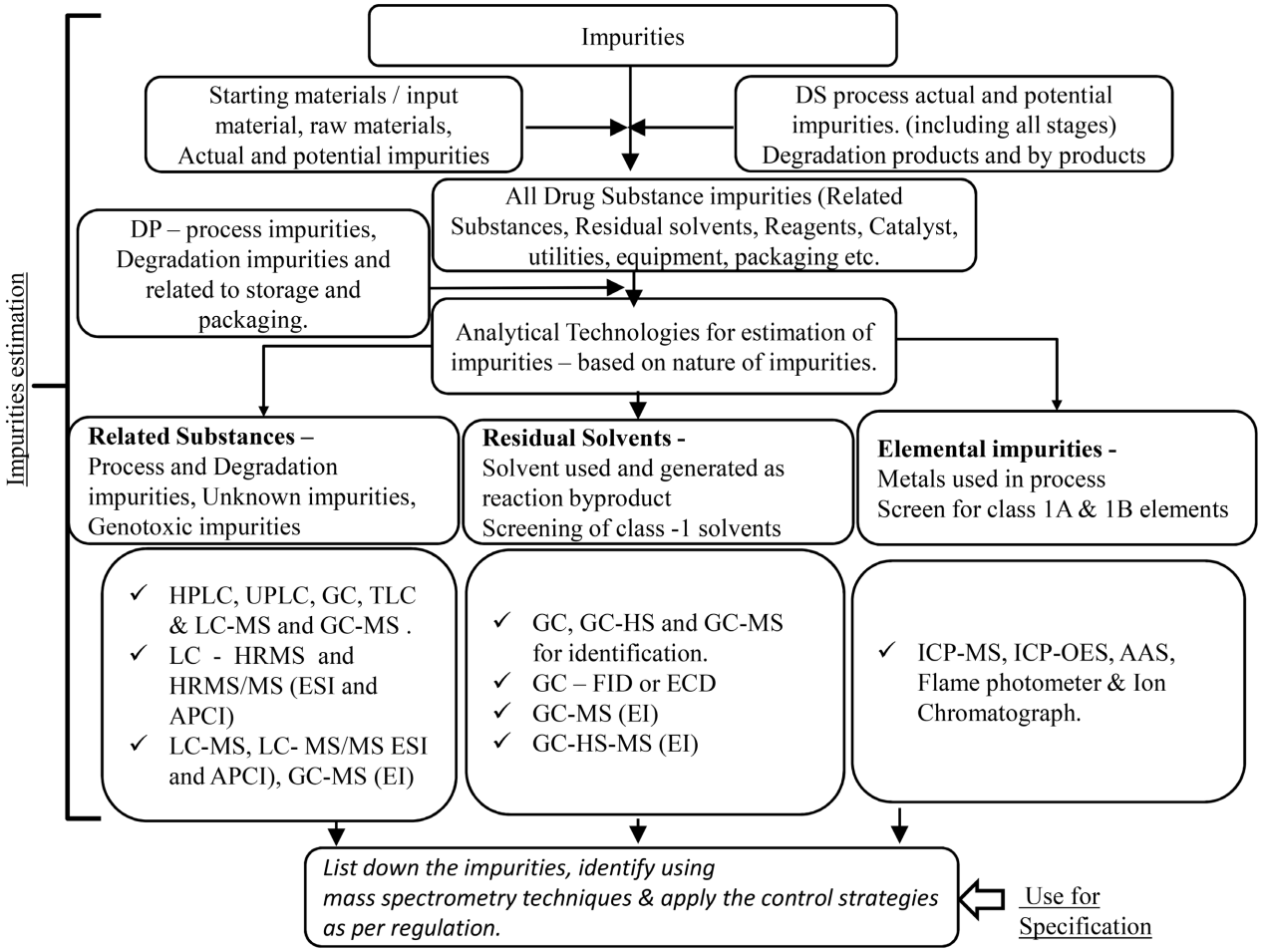

Figure 1. Framework for impurities estimation for active pharmaceutical ingredients.

Analytical methods can be improved by first establishing the identities, purities, physical properties, efficacies, bioavailability, and stability of drugs. Analytical method development and validation can be seen as the process of proving that analytical processes are adequate for a given purpose; in this case, evaluating pharmaceuticals, and more specifically the active pharmaceutical component (API). Through these procedures, particular aspects of the compounds are examined and compared to standards of acceptability. Therefore, the most precise assay processes must be evaluated and chosen during the development of analytical methods to determine a drug's composition.

Successful drug development programs require the creation and verification of analytical methods. Researchers have identified at least three major reasons why the creation of new analytical techniques is essential for any pharmaceutical company to create new drug candidates. Companies that are developing new compounds should invest heavily in the study and creation of suitable analytical methods, as the quality of medicine is crucial to the success of a pharmaceutical development program. Second, submissions for clinical trials and marketing approval are often rejected unless supporting evidence of analytical technique va-

lidation can be provided to regulatory agencies around the world (Raman, 2011) [2]. Early phase clinical trials (first in human/Phase 1 studies) involve administering the IMP to humans for the first time; as a result, it is essential to ensure patient safety and, potentially, show promising effectiveness in the novel medicines via development and manufacturing quality.

4. Conclusion and Recommendations

Rapid growth in the pharmaceutical industry is driven by the pursuit of new therapeutic agents, which may be derived from natural sources or chemically synthesized pharmacological compounds. Therefore, purity/absence of impurities has long been acknowledged as a fundamental criterion in assuring medication quality. You should remember that no medication is completely safe and that even a low dose can have fatal effects. Researchers claim that apparent amounts as measured by weight are not virtual quantities after accounting for the alloy of impurities have sparked a surge of interest in the study and assessment of the purity of natural goods in the last century and because of this, it is beyond dispute that medical advancements made in the last century as a result of pharmaceutical research have greatly boosted people's health and standard of living.

Drug registration agencies are paying closer attention to trace amounts of contaminants in pharmaceuticals, specifically those in the 0.01% to 0.1% range. Impurities in pharmaceuticals are unintended compounds that either originate in the APIs themselves, appear as a byproduct of the formulation process, or are created as a byproduct of the degradation of APIs or APIs used to create medications. The efficacy and safety of pharmaceutical products could be jeopardized by even minute amounts of these unwelcome substances. Therefore, the drug registration authority has proposed the following methods for detection and measurement. Many factors influence the impurity profile of a drug substance, including the purity of the starting materials, the reaction conditions, the purification procedures, and the amount of time the drug substance was stored.

Good Manufacturing Practices (GMP) regulations emphasize quality control in the pharmaceutical industry. To maintain quality and GMP compliance, this idea must be present throughout the entire lifecycle of a pharmaceutical product. All aspects of the laboratory, including its environment, equipment, procedures, and staff, must be managed. This includes supplies, reagents, references, data, and deliverables of any kind. Now, however, the importance of risk management in pharmaceutical quality assurance is widely acknowledged. "An effective quality risk management approach can further ensure the high quality of the drug (medicinal) product to the patient by providing a proactive means to identify and control potential quality issues during development and manufacturing," states ICH Q9.

The first step in developing a separative method is selecting suitable parameters. The focus of the following explanation will be liquid chromatography due

to its relative simplicity; however, the principles discussed are not unique to this technique. The unique difficulties of chromatographic factor screening have prevented the widespread use of DoE. One of the issues is that early studies of key components like the stationary phase and solvent tend to be qualitative. The sheer number of extra factors necessitating statistical modelling makes it impossible to develop sparse and efficient experimental designs because the chromatographic interactions of continuous components (pH, etc.) may vary greatly for different stationary phases, and it is difficult for simple models to account for all column-to-factor interactions.

The pharmaceutical market has become an integral part of the international economy. The establishment of this trade has had and may continue to have far-reaching effects on people's daily lives. The pharmaceutical industry devotes significant resources to research and development (R & D) of novel medication compounds. Developing a new pharmaceutical compound costs over $1 billion and takes between 10 and 15 years. To ensure the quality, safety, and efficacy of the approximately 280 therapeutic novel molecular entities approved by the FDA in the decade from 2010 to 2020, monitoring these medications is a top priority for authorities. Analytics' role as a tool in the pharmaceutical industry has grown significantly over the past decade. As a result, new technologies are constantly being developed to provide for these requirements. To ensure post-market regulatory compliance and aid in the research, development, and monitoring of drug safety, pharmaceutical analysis is increasingly used to generate trustworthy data.

The development of an analytical method for a novel pharmaceutical ingredient or formulation is one of the challenging tasks. Analytical procedures such as titration, spectroscopy, qualitative testing, and activity assays are used to ensure the drug meets all of the required quality standards. The analytical input provided by pharmaceutical research is currently used extensively in the artificial, medical specialty, pharmaceutical, and clinical analysis fields. This paves the way for the development of highly cost-effective pharmaceutical solutions. In particular, there would be no official means of measuring impurity levels, degradation products, or medication dosages if they were ever accumulated, and no appropriate analytical methods for doing so. Drugs on the market today are of low quality due in large part to these contaminants. Finding and describing any contaminants that could be present during drug development is a crucial step. The structure of the actual contaminant or degradation product present among the medication constituent should be characterized through spectroscopic tests (including NMR, IR, and MS).

Conflicts of Interest

The authors declare no conflicts of interest.

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