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Review

Review on the modern analytical advancements in impurities testing

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Abstract: Impurities in active pharmaceutical ingredients (APIs) and pharmaceutical drug products (DPs) lead to broader antithetical effects related to drug safety, efficacy, and regulatory compliance. This review discusses organic, elemental, and inorganic impurities, and residual solvents and stresses their impact on the quality of APIs and pharmaceutical DPs regarding patient safety. It endorses immensely developed contemporary analytical techniques like High-performance Liquid Chromatography (HPLC), and Gas Chromatography (GC), for organic impurities and discusses their hyphenated Mass Spectrometry (MS) chromatographic methodologies. Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma (ICP) hyphenated with MS, and Optical Emission Spectroscopy (OES) techniques are discussed for mainly improved sensitivity and accuracy in the detection and identification of elemental and inorganic impurities. High-Resolution Mass Spectrometry (HRMS), Supercritical Fluid Chromatography (SFC), and ICP-hyphenated techniques alongside automation are among the emerging technologies that are discussed concerning their impending potential to solve intricacies related to complex drug matrices, challenging regulatory requirements, and new impurity profiles. The review underlines the discussion on harmonized global regulations and affordable access to advanced analytical techniques so that wider adoption is facilitated in the pharmaceutical industry. Imminent prospects are Artificial Intelligence (AI) incorporation, Machine Learning (ML), and green analytical methodologies to overcome the present confinements and to cater to the growing demands of the progressive pharmaceutical sector. This in-depth analysis is intended to help pharmaceutical stakeholders embrace novel impurity management approaches resulting in significantly enhanced drug quality and better healthcare outcomes on a global scale.

Keywords: organic impurities; elemental and inorganic impurities; residual solvents; HRMS; SFC; ICP-MS; ICP-OES; AI; ML; PAT

1. Introduction

The pharmaceutical industry is responsible for delivering quality medicines to fulfill its commitment to patients and society. To ensure the manufacturing of quality medicines, the SISPQ (Safety, Identity, Strength, Purity, and Quality) rule must be followed to demonstrate current Good Manufacturing Practices (cGMP) compliance under the guidance of the Food and Drug Administration (FDA) and European Medical Agency (EMA) [1,2]. The critical quality attributes (CQAs) of APIs and DPs need incessant, unremitting, thorough, and vigilant monitoring during the manufacturing steps to ensure strict compliance. The rigorous control not only ensures quality but also sets up a strong base for controlling impurities so that every step taken closely follows the highest safety and efficacy standards [3].

Impurities in pharmaceuticals may be due to raw materials, intermediates, degradation, and environmental factors. Although usually found at very low levels, impurities further illustrate how some impurities can cause drastic side effects, such as toxicity and allergenicity, and decreased drug stability and performance [4]. Thus, it is not a casual but a compelling scientific and ethical issue linked to the well-being and safety of the general population [5]. Most of these standards are drawn up by regulatory bodies like the FDA, EMA, the International Conference of Harmonization (ICH), and the World Health Organization (WHO). ICH Q3A [6] and Q3B [7] for APIs and DPs impurity control; Q3C [8] for residual solvents; and Q3D [9] for elemental impurities among the drugs follow that those systems require the application of highly advanced analytical techniques for purposes of identifying these impurities with outstandingly high levels of sensitivity and accuracy [10]. To control impurities stringently, it is important to maintain the quality of the product to protect the safety of the patient as well as trust with healthcare providers and regulators [11]. Therefore, robust impurity management is a sine qua non condition so that medicines of high standards regarding safety and efficacy are produced and that drugs are harmless to use and act in the right manner.

To cope with these challenges, comprehensive assessment and recent developments in analytical technologies have indeed been sumptuous. One can analyze impurities within the most intricate matrices using these venerably accurate technologies. Presently, common techniques such as conventional and ultra HPLC, GC, and their hyphenated versions with MS are just some of the colloquially acknowledged standards for the identification of organic impurities, whereas AAS, ICP-MS, and ICP-OES have become critical for the management of elemental and inorganic impurities [12,13]. HRMS and SFC are among the technologies that have impending potential and promise for better accuracy and productivity for impurity profiling and have extensively been focused on during this review to make imminent estimations and future directions. Moreover, the derived integration of AI, ML, and increasing sustainable green analytical practices could answer the contemporary analytical challenges of advancing the pharmaceutical sector [14,15].

In addition, although the current guidelines are valuable in providing guidance and a pathway to impurities analysis [16], regulatory bodies are required to maintain the rationality for global consistency for the testing and control of impurities [17,18], which in turn shall make the world pharmaceutical trade a safer one [19,20]. The international alignment will upgrade international safety standards and prepare a base for easier cross-border pharmaceutical movement, an important feature requisite in the present context.

The review briefly covers the classification and sources of pharmaceutical impurities and makes a comprehensive discussion of the impact, with special reference being drawn to the importance of modern analytical techniques to take control of impurities. The challenges of dynamic regulatory landscapes, drug complexity, and the introduction of new dosage forms as well as new impurities from next-generation formulations such as biologics and nanomedicines are also reviewed. A wide overview of current advancements and future perspectives aimed at this review would lend credence to drug quality and shift pharmaceutical-margined-in-facility-capitalism towards safer and more effective treatments alongside serving as guidance to the

stakeholders in coming up with innovative strategies in assuring quality pharmaceuticals, eventually leading up to the enhancement of global healthcare.

2. Types of impurities in pharmaceuticals

Impurities are mainly classified into the following categories.

2.1. Organic impurities

Organic impurities are most concerning since they are generated from the manufacturing of drug substances or the interactions of drug substances and excipients in various environmental factors in drug product form [6,7,21]. They are generally classified into three major categories: 1) process-related impurities; 2) API-related impurities; and 3) degradation products (time-related) [22–24] as shown in **Figure 1**. To ensure safety, efficacy, and regulatory compliance, it is essential to comprehend these impurities' control, effects, and sources. Impurities related to the process are undesired impurities present as traces in the API caused by incomplete reactions, side reactions, or unreacted starting materials [25]. Some familiar sources include unreacted raw materials, synthetic by-products, residual reagents, and catalysts present post-synthesis [26]. If these impurities are not managed well, they could make the drug inappropriate for its intended use while also likely jeopardizing the patient's life.

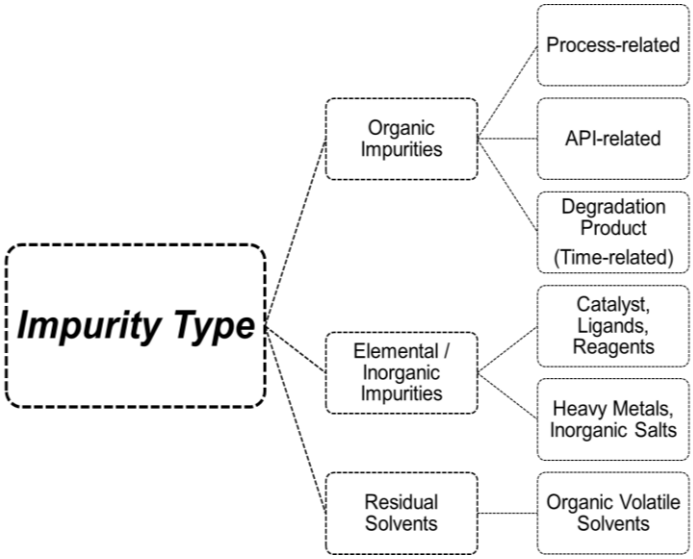


Figure 1. Impurity types and their possible routes.

The API degradation in the product form can occur under exposure to environmental stressors like heat, light, moisture, or oxygen, leading to the production of degradation products, which may impede the safety and efficacy of the drug [10,27]. For instance, heat may cause increased catalytic decomposition reactions or accelerated inter-reactivity between the API and excipients [28]. UV/visible light may photochemically convert the actinic form of several drugs, such as antibiotics and statins, leading to possible impotence, reduced activity, or enhanced toxicity of the API molecule [29]; however, not every API molecule is light-sensitive. Exposure to moisture, especially for those APIs having esteric and amidic functional groups, can undergo hydrolysis and cleave the actual molecule. Some sulfur-containing APIs, like

thiols, can also readily undergo hydrolysis, producing sulfoxides and disulfides [30,31]. Molecules containing chiral centers can undergo chiral rearrangement and racemization to orient themselves to produce different stereoisomers with varied pharmacological or toxicological functionalities upon prolonged storage [32,33]. Furthermore, some excipients—such as glycols that produce peroxides—can interact with sensitive API molecules to produce excipient-derived impurities [34,35]. To tackle this, judicious selection and a thorough literature review are necessary during the formulation development stage [36]. Currently, nitrosamine impurities have also become a prevalent concern, drawing intense scrutiny from regulatory and public health authorities. Commonly found nitrosamines include the highly potent carcinogens N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) [37]. These impurities are produced from secondary or tertiary amines and guanidine bases of the API molecules that are prone to be nitrosated under acidic conditions in the presence of nitrosating agents like nitrites [38]. The sources of impurities are contaminated raw materials, cross-contaminations during manufacturing, and decomposition reactions. Therefore, it is only through strict compliance with guidelines like ICH M7 that risks can be minimized [39].

Albeit these degradation products may lead to toxicity and subsequently reduce therapeutic drug efficacy, the process-related impurities can also be harmful in altering the intended pharmacological activity of the administered drug for the patients [40]. This emphasizes the necessity of well-implemented control strategies such as advanced analytical methods for early detection of impurities, comprehensive stability studies for identification of degradation pathways and their influence on the product, and determination of stringent storage conditions to mitigate the effects of environmental factors on drugs in a way to preemptively respond to the jeopardy posed by these impurities.

2.2. Elemental and inorganic impurities

Elemental and inorganic impurities are two key classifications that affect the safety, efficiency, and quality of medicines. Elemental impurities are trace metals that originate from raw materials, catalysts, or manufacturing processes, whereas inorganic impurities encompass a broader range of non-metallic substances, including residual reagents, salts, and reaction by-products [41]. **Table 1** mentions the discernable distinguishment, impact, and analysis techniques of elemental and inorganic impurities.

Table 1. Discernable distinguishment between elemental and inorganic impurities.

Aspect	Elemental impurities	Inorganic impurities
Description	Trace metal elements	Non-metallic substances and leftovers (salts, acids)
Sources	Metals in catalysts, equipment	Reagents, raw materials
Examples	Arsenic, cadmium, lead, mercury, nickel, palladium, platinum, rhodium	Chlorides, nitrates, sulfates
Impact on safety	Toxicity concerns	Quality and stability issues
Analytical technique	ICP-MS, ICP-OES, AAS	Ion chromatography, gravimetry, titration

Both of these types of impurities are critical to be controlled, mainly when the products have to conform to specific regulatory thresholds and be safe for the patients [42]. Regulatory thresholds and guidelines like ICH Q3D underscore this fact, prescribing caution in pharmaceutical manufacturing [9]. Inorganic impurities stem from the raw materials, the operations and processes themselves, or impurities that come from the environment, such as dirty water, attrition from equipment, or packaging materials; among these are leftover acids (e.g., HCl), bases (e.g., NaOH), and salts (e.g., KBr) [43]. Although less toxic compared to heavy metals, these impurities may be a nuisance regarding the stability and solubility of a drug if all of them are not entirely removed. Therefore, purification is needed and would be congruent with pharmaceutical quality standards [21,44].

The heavy metals of most concern among these impurities are lead, arsenic, cadmium, and mercury, for the serious health implications with which they are associated [45]. For example, neurological and developmental disorders associated with lead, and cardiovascular and skin diseases caused by arsenic, and carcinogenic as well as the renal toxic effect associated with cadmium and nephrotoxicity for mercury [46]. These elements get infused into the products through raw materials, exposure to the environment, or water that has impurities in it, thereby stressing the requirement for tight controls.

Impurities from the elements from catalysts, such as palladium, platinum, rhodium, and nickel, used in chemical synthesis are elemental impurities that could be harmful to human health [42,47]. For instance, such impurities as palladium and platinum used in hydrogenation reactions may cause hypersensitivity and cumulative toxicity [48,49]. Residual nickel in the product can also give the patient an allergic reaction and carcinogenic effects [50]. Furthermore, direct contact with the metal surface with manufacturing equipment might also cause them to leach metal ions such as iron, chromium, or nickel into pharmaceutical products [51,52]. Environmental exposure at manufacturing sites puts them at risk of contamination by arsenic, lead, or cadmium, which are toxic metals [53]. These impurities, though trace in quantity, prove to be quite crucial when it comes to the safety of these patients [9,52]. Current regulatory frameworks, such as the ICH Q3D, which, by embracing a risk-based approach, have entirely transformed impurity control to emphasize patient safety. The ICH Q3D sets permitted daily exposure (PDE) limits for 24 toxicologically significant elements in oral, parenteral, and inhalation formulations. This guideline emphasizes ultra-modern analytical techniques like ICP-MS and ICP-OES, offering precise and accurate results [9].

2.3. Residual solvents

Residual solvents are the organic chemicals that are left over after the synthesis, purification, or formulation of APIs and the final drug product [8]. These solvents help to conduct chemical reactions, solubilization, and formulation of drugs as carriers or excipients within pharmaceutical products. A risk is posed to patient safety and/or to drug quality when these solvents are left behind in minute amounts within the final product, leading to issues of toxicology associated with organ toxicity, carcinogenicity, or irritation [8,54].

Incomplete removal during manufacturing or a lack of process control may lead to the retention of residual solvents; thus, there is a need for proper evaluation and management. The ICH, among other regulatory bodies, has laid down exhaustive guidelines such as ICH Q3C and USP <467> that categorize solvents based on their toxicity and recommendations of acceptable daily exposure limits [8,55]. For such measurements, advanced analytical techniques need to comply with the safety threshold values recommended and, therefore, necessitate the use of sensitive analytical techniques for precise identification and quantification of residual solvents, particularly Gas Chromatography, preferably with headspace [55]. Residual solvents can enter into drug products in several ways. They are used as reaction media to dissolve reagents—as a reaction medium—to regulate the rate of reaction or in the formation of intermediates. The solvents used for recrystallization or extraction (e.g., ethanol or isopropanol) might leave traces behind if the drying or purification process is not thorough. Some solvents may function as carriers or excipients, especially in liquid or semi-solid formulations [56]. Common residual solvents are characterized by their toxicities as well as the potential environmental impacts associated with them [54,56]. For instance, they are classified as Class 1, Class 2, and Class 3 solvents. Class 1 solvents are highly toxic and tagged as must be warded off. These solvents possess extremely inexorable toxicological risks, especially concerning their carcinogenic properties, and should only be applied when there is an absolute stipulation. Benzene and carbon tetrachloride are examples of Class 1 solvents. Class 2 solvents are less toxic than Class 1; however, they are also tagged to be used limitedly owing to their toxicity concerns. Their common examples are dichloromethane, methanol, and toluene. Class 3 solvents have low toxicity and are generally used during the API and drug product manufacturing steps. Common examples are acetone and ethanol [8,54,55]. Looking at the risk profile of these solvents, they seem to be low-risk; nevertheless, they are monitored for the degree of imbuing drug quality. Ethanol: Widely used and generally safe at allowable limits. Acetone: Low-toxicity solvent; may impact product stability at very high levels [56,57]. For minimization of residual levels of solvents and compliance with stipulated standards of safety, effective controls are rudimentary, which include process optimization, solvent recovery, and rigorous purification. **Figure 1** shows the impurity types and their possible routes.

3. Advancements in modern analytical tools

The advancement in pharmaceutical analysis has been the result of the reliance on faster and more reliable high-throughput tests. The impetus is the need to handle increasingly complex pharmaceutical formulations, the introduction of biosimilars, safety, and quality requirements [58–60]. These methods continue to play their imperative roles in determining impurities in terms of both APIs and final pharmaceutical products. They also check compatibility by testing the quality and stability of the tested compounds to ensure that developed and marketed medications meet the required standards. The face of pharmaceutical analysis has changed with the progress of contemporary analytical tools that are highly specific for the detection and quantification of nearly all types of impurities, be they organic, elemental, inorganic,

or residual solvents [25,61]. HPLC and GC in combination with LC happen to be highly evolved methods of identifying organic impurities and volatile residual solvents in comparison to all other existing methods. While ICP-MS, ICP-OES, and AAS have achieved the status of gold standards for elemental and inorganic impurities.

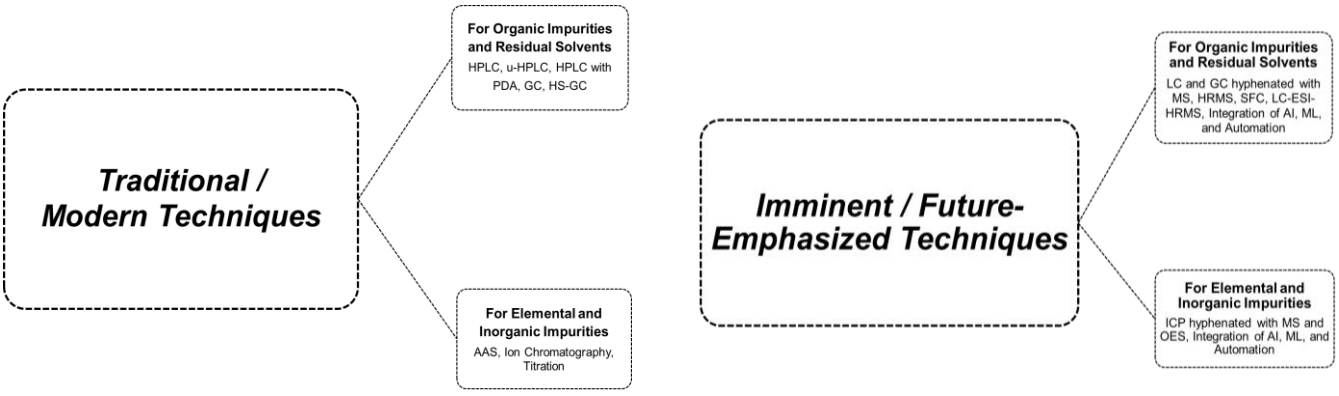
3.1. Traditional chromatographic techniques

Chromatographic techniques are likely the simplest yet most relied upon for virtually any sample preparation when it comes to separating mixtures into their more readily analyzed parts. The components of the sample mixture relate to their miscibility rates and affinities with the mobile and stationary phases. Therefore, microscopic techniques like thin-layer chromatography and column chromatography take advantage of the differential distribution between the solid or liquid stationary phase and typically the gaseous or liquid mobile phase [62]. To isolate individual species in a mixture, separation is done from the mixture by their distribution between two homogeneous phases with different polarity, size, or charge. These are selected to identify and measure impurities or target compounds according to requirements and presumably, boost higher sensitivity and specificity and arm valuable information even at relatively low levels of quantification [63].

The LC-related techniques in pharmaceutical analysis are more colloquial and user-friendly for employment and are associated with usage in pharmaceutical analysis [64]. These techniques entail introducing a sample into a liquid mobile phase under high pressure and having it flow through a column that is filled with a solid stationary phase (i.e., generally silica). Compounds in the sample interact differently with the stationary phase; however, causing their separation to correspond to their polarity, size, or affinity to the stationary phase [63,64]. Therefore, they are versatile and are capable of analyzing almost every kind of compound, ranging from small organic molecules to enormous biomolecules like proteins. They are highly effective for detecting residual solvents, process-related impurities, degradation products, and any other contaminants in the APIs and complex drug formulations [21,65,66]. LC is commonly coupled with an ultraviolet (UV) detector—termed HPLC—and in some cases with a fluorescence detector to provide detailed information on the separated components. This technique is indispensable for quantitative analysis, offering high precision and reproducibility in impurity profiling [4,10,67].

On the other hand, Gas Chromatography is quite famous for analyzing volatile compounds as well as solvents that can vaporize without decomposition. The gaseous mobile phase carries the sample through a column comprised of a solid or liquid adsorbent acting as a stationary phase. The components of the sample—when in contact with the stationary phase—sort themselves out according to their volatility. That is, the lower-boiling compounds travel further through the column more quickly than the higher-boiling ones [68]. Almost all pharmaceutical preparations contain residual solvents. GC with flame ionization or mass spectrometric detection can identify and quantify trace levels of organic contamination with high sensitivity. This property makes it very applicable to the analysis of degradation products and impurities resulting from a manufacturing process or those formed in storage [69]. The

traditional chromatographic techniques for organic impurity and residual solvent analysis are provided in **Figure 2a**.



(a) **(b)**

Figure 2. (a) Traditional/modern techniques for impurity analysis; **(b)** imminent/future-emphasized techniques for impurity analysis.

Nevertheless, while widely used, these traditional chromatographic techniques are often constrained to perform an extremely low level of quantification that requires high specificity, as in the case of chiral and nitrosamine impurities. Additionally, in some cases, extensive method optimization—which could be labor-intensive, time-consuming, and may be less accurate—is required to analyze the impurities effectively. This subsequently urged the need to embrace more advanced and sophisticated chromatographic techniques to efficiently analyze these impurities while ensuring good accuracy.

3.2. Emerging chromatographic techniques

While more and more elaborate analytical tools in the field of impurity analysis—like LC-MS, LC-MS/MS, GC-MS, and GC-MS/MS—are now used alongside cutting-edge analytical instruments, the actual fusion of a good liquid chromatograph or gas to a mass spectrometer possessing the newfangled and heightened sensitivity and specificity achievable translates into unparalleled fine record impurity profiling even down to a trace level [70,71] These hyphenated techniques are especially accentuated while analyzing nitrosamine impurities that need nano-level detection. Furthermore, HRMS and LC in tandem have recently attracted much interest in impurity determination. To illustrate, the Liquid Chromatography-Electron Spray Ionization-High-Resolution Mass Spectrometry (LC-ESI-HRMS) method for nitrosamine impurity detection in the metformin molecule and its product was recently adopted by the FDA, proving the preference for HRMS over traditional MS techniques [72]. Unlike conventional MS, HRMS offers nonpareil mass accuracy and resolution for precise identification and trace-level quantification, especially for those impurities having indistinguishable mass-to-charge ratios [73]. The sensitivity of operation has further been enhanced by the ESI source, which renders it great for LC operation and hence highly advantageous for pharmaceutical complex matrices [74]. This combination enhances the detection sensitivity and provides better insight into the complex matrices under investigation, thereby setting new standards for analytical

performance in pharmaceutical research and quality control. Although HRMS offers extraordinary precision in analyzing unknown impurities, advanced professional expertise combined with complex operational functionality acts as major hurdles to restricting its ease of use and accessibility [75]. However, most of the compendial methods in USP, BP, and international pharmacopeia still merely rely on conventional chromatographic techniques, examples being thin-layer chromatography (TLC), HPLC, HPLC with PDA detector, and in some instances GC, which inevitably calls for more emphasis on the utilization of the modern hyphenated techniques to meticulously enhance the sensitive approach with which these impurities must be analyzed.

More recently, chromatography has entered its super-analog with the use of Supercritical Fluid Chromatography (SFC) along with a solid or liquid stationary phase and supercritical carbon dioxide (CO_2) as the mobile phase [76]. The separation of both polar and non-polar compounds is made more effective by supercritical carbon dioxide, and this is what gives SFC its immense value for pharmaceutical analysis. When considering that impurities often tend to be chiral compounds of the parent molecules, chiral resolution has become a crucial aspect of pharmaceutical analysis. The most efficient and modern alternative to classical chiral column chromatography in this respect is SFC. Due to its usage of supercritical CO_2 as the mobile phase, it substantially increased separation efficiency compared to liquid-based systems, had shorter total analysis times, and improved resolution, which is why it has become the most favored method for chiral separation in recent years [77,78]. This is particularly well illustrated by the example of omeprazole analysis. As we know, omeprazole is a chiral drug; the enantiomers demonstrate diverse pharmacologic activities. Only the S-enantiomer is therapeutically active (i.e., as esomeprazole). With SFC, it would be possible to very quickly and accurately separate omeprazole enantiomers so therapeutically active ingredients can be produced with minimal on-column waste of less effective or even potentially harmful isomers [79]. The emerging chromatographic techniques for organic impurities and residual solvent analysis are provided in **Figure 2b**.

Besides being fast and accurate, SFC is also expedient and beneficial in lowering solvent consumption, directly in line with the industry's impelling move toward greener analytical practices. The considerable transformation and reputation it has been gaining in pharmaceutical quality control reflect an analysis of chiral drug advancement regarding safety, efficacy, and regulatory compliance. Despite that, its direct application, other than the analysis of chiral impurities, is not that straightforward. For instance, poor retention of highly non-polar compounds (i.e., cholesterol, testosterone derivates, fatty acids, and lipophilic vitamins) [80] and solubility challenges and optimization of co-solvent composition are major issues.

3.3. Traditional spectroscopic techniques

Spectroscopic techniques have been important in analyzing elemental and inorganic impurities, except nuclear magnetic resonance (NMR), which is used in atomic environments to provide structural elucidation of unknown and unidentified organic impurities [81]. Other spectroscopic techniques, such as AAS, are used for the

identification and determination of trace elements, which are impurities, including heavy metals. AAS is preferred for selective analysis of trace metals, is easy to use, and is relatively inexpensive for single-element analysis [82,83].

AAS is a widely applied technique for ascertaining elemental impurities in pharmaceutical samples, more specifically metallic impurities. AAS is based on the fundamental fact that atoms in the vapor phase absorb light at certain wavelengths. It involves aspirating a sample (usually a solution) into a flame or graphite furnace for atomization. The specific wavelength of light is entered into the sample, and then after atomization by flame or furnace, the intensity of the light absorbed by these atoms is measured. And this absorption is directly proportional to the concentration of the respective element in the sample [84]. It is commonly applied to both quality control and environmental testing to comply with safety standards and regulatory requisites. It is also very useful for common analyses since, though somewhat less sensitive compared to ICP-MS and ICP-OES, this comes at much less cost and is easier to handle [85]. The basic types of techniques include flame AAS, which atomizes the sample in a flame, and graphite furnace AAS, which increases sensitivity through atomization within a graphite tube under controlled conditions [86]. AAS is employed in the analysis of raw materials and excipients to verify that undesirable elemental impurities hazardous to health are not introduced into the formulation. The traditional techniques for elemental and inorganic impurity analysis are provided in **Figure 2a**.

However, AAS limits in its ability to analyze more than single elements simultaneously, making it inefficient for multi-element analysis. Additionally, it does not have the sensitivity required to detect trace-level impurities (i.e., ppb or ppt). Therefore, analytical techniques that can provide both enhanced sensitivity and the ability to analyze multiple elements at an instance are needed.

3.4. Emerging spectroscopic techniques

To tackle the constraints of AA, which offers limited sensitivity and can analyze only a single element at a time, the necessity for more enhanced sensitivity and multiple-element analysis tools became imperative. ICP-MS is an advanced and highly sensitive analytical methodology employed for the measurement of elemental impurities, including trace metals, in pharmaceutical products. It unites excellent mass spectrometric performance with the capability to produce an ionized plasma that, in many cases, allows the identification of elements at ultra-trace concentration levels (frequently in the ppt range) [82]. ICP-MS is operated by injecting a sample into the superhot plasma, where an ionized vapor form is created. These ions are transported to the mass spectrometer, where they undergo resolution according to m/z (mass-to-charge ratio) [87]. This makes possible the most minute parts-per-million measurement of metals and heavy components in the sample. Determination and quantification of metallic elemental impurities in pharmaceuticals are some of the most critical applications of the ICP-MS technique. These include toxic heavy metals like lead, arsenic, cadmium, and mercury, along with other harmful metals [88]. These could easily be a result of raw materials, catalysts, or even the kinds of processes used in manufacturing. Statutory bodies like the FDA [89] and EMA [90] have set stringent limits on these impurities, which makes ICP-MS analysis the ultimate technique for

ensuring that APIs and DPs conform to such safety standards. ICP-MS offers sensitivity above many other analytical approaches (such as the conventional AA technique), ease of operation, speed, and multi-elemental capability, making it a nearly ideal tool for the compliance of pharmacopeia standards and analytical requirements. However, due to its high sensitivity, it also requires extensive sample preparation and may be susceptible to matrix effects, which can produce induced suppression and spectral overlaps to jeopardize the accuracy of the measurements [91]. The emerging techniques for elemental and inorganic impurity analysis are provided in **Figure 2b**.

ICP-OES, or ICP Atomic Emission Spectroscopy, is yet another highly sophisticated analytical technique that pharmaceutical samples can use to analyze trace metals and elemental impurities. Much like the former—ICP-MS—ICP-OES uses a high-temperature plasma to ionize the sample but does not distinguish ions according to the mass/charge ratio; it rather measures the light emitted by the atoms and ions returning to lower energy states. The sample is aspirated into an ICP, where the heat generated excites the atoms and ions to emit light at specific wavelengths corresponding to the elements present. Thus, measurements of the intensity of this light at those particular wavelengths can establish the concentration of each element in the sample [92]. This technique finds outstanding application in the determination of almost all kinds of elements, from alkali and alkaline earth to transition and heavy metals.

Other applications in which ICP-OES is often used include its application in the elemental profiling of drugs and pharmaceuticals, thereby ensuring that the various drug products and raw materials conform to the prescribed safety standards for such metals as calcium, magnesium, zinc, and copper. Several other applications address the monitoring of levels of impurities, specifically lead, cadmium, and mercury, among others [82], which is very critical from the point of view of statutory compliance [89,90]. It is widely perceived as not as sensitive as ICP-MS but a reliable and efficient technique for numerous applications; faster analyses with a multi-elemental capability are some of the features. It also requires meticulous consideration of wavelength selection due to the possible overlapping of elements with emitted light and is limitedly considered in detecting ultra-trace levels of toxic elements. **Table 2** is provided to compare the pros and cons of traditional vs. emerging spectroscopic techniques for better understanding.

Table 2. Pros and cons of ICP hyphenated with MS and OES and AAS.

Description	Pros	Cons
ICP-MS	Ultra-sensitive; capable of detecting elements at ppt levels. Broad elemental coverage, including most heavy metals. Rapid multi-element analysis in a single run.	High cost of equipment and maintenance. Susceptible to matrix effects and may require extensive sample preparation.
ICP-OES	Fast analysis times for routine multi-elemental determinations.	Lower sensitivity compared to ICP/MS (ppm, ppb). Light emission may overlap for some elements and may require careful wavelength selection. Limited in detecting ultra-trace levels of toxic elements.
AAS	Simple to use and lower cost compared to ICP techniques. Good for targeted analysis of specific metals (e.g., lead, cadmium). Flexible configurations: flame AAS for routine testing and graphite furnace AAS for higher sensitivity.	Single-element analysis is not suitable for multi-element detection without additional time. Lower sensitivity than ICP-MS and even ICP-OES. Longer analysis time if multiple elements need to be measured.

4. Contemporary impurities analysis challenges

The impurity analysis in the pharmaceutical industry has greatly been cutting-edge due to the evident increased investment in highly enhanced analytical technologies and escalated regulatory attention. Herein, it has continued to be challenging to detect, quantify, and control impurities accurately for several related reasons. Challenges result from the complexity of drug formulations and ever-increasing regulatory requirements along with the high cost and difficulty of accessing advanced analytical technologies [93]. Thus, future research and development should be directed towards additional improvements in analytical techniques, more robust regulatory frameworks, and making it easier to reach modern technologies for all stakeholders in the pharmaceutical sector.

4.1. Complex drug matrices

One of the biggest challenges impurity analysis faces is the increased intricacy of modern drug formulations, particularly for biologics, and combination drugs [94]. Biologics, including monoclonal antibodies, gene therapies, and protein-based drugs, often contain very complex, heterogeneous matrices that make impurity analysis even harder than it already is [18]. Impurities are difficult to detect and quantify due to their interferential behaviors with impurities from different sources, such as proteins, lipids, and sugars used as excipients in biological products. The other sources of impurities in the biologics are generally from various stages of production, including but not limited to cell cultures, purification processes, and storage conditions [24].

Combination products, which unite drug delivery devices with pharmaceuticals, involve additional intricacy. These products may relate to the employment of variant formulations or materials that may cause possible contamination or even interaction between the drug and device parts [95]. For this reason, impurity analysis of these formulations calls for such advanced analytical techniques that can differentiate between the active pharmaceutical ingredient, excipients, and potential contaminants. Furthermore, the introduction of biosimilars is also a notable advancement, and impurity determination in these is also becoming a major task [96]. As the trend is moving towards more personalized medicine, it can be expected that the complexity of drug products alongside their formulations will also increase, and hence there would be a need for continuous evolution of analytical technologies for identification and control of impurities in such sophisticated drug formulations.

4.2. Regulatory/compliance requirements

Another thought-provoking fact that impinges on the pharmaceutical industry is the ever-changing regulatory demands in impurity analysis. The EMA, FDA, ICH, and WHO impurity limits of APIs and DPs are also appropriately defined in impurities concerning the safety issues, advancements in science, and technological evolution for an update on a regular basis. Though such advancing benchmarks help ensure the competence and safety of pharmaceuticals, they need validation of methods with updated testing procedures to be compliant. As an example, the recently implemented changes in the ICH Q3D guideline regarding elemental impurities require that the pharmaceutical industry use a risk-based approach for the control of metals and other

elements present in the DPs. Equally similar, the new legislation on nitrosamine impurities and degradation products covers the area where impurities have to be checked and monitored rigorously, most of the time at very low levels [97]. Such ever-developing regulation needs ongoing investment in R&D toward updating these impurity-testing methodologies to ensure pharmaceutical products continue to comply with the latest rules.

One recurring issue the pharmaceutical industry has to deal with is related to global harmonization regarding impurity testing and regulatory expectations. The disparity in standards that diverse regulatory authorities maintain over impurity limits and testing methods often translates into incongruities that further complicate the approval and distribution processes of medicines across international markets. These incongruities are not only burdensome for manufacturers but also hold the potential to prevent life-saving drugs from reaching patients across the globe [98]. It would be a monumental step forward if impurity testing were consolidated to establish universally acknowledged standards that would cut through regulatory measures currently in place, lessen tedious duplicative testing, and promote better working among regulatory bodies. Notably, it would benefit in bringing safe, efficacious, and good-quality medicines much more efficiently to people across the globe with universally acknowledged standards. This type of harmonization can also be a driver of innovation in impurity analysis techniques and instill confidence in the pharmaceutical supply chain while benefiting both the industry and its consumers. This contemplation manifestly reveals that if pharmaceutical community professionals attempt to solve this matter, it will be plausible to establish a structure in which universal standards for impurity testing are in harmony as well as set very stringent safety benchmarks, thereby expediting a heightened and more symmetrical global healthcare system.

4.3. Accessibility and cost

Advanced analytical techniques such as HRMS, multi-dimensional NMR, and ultra-high-performance liquid chromatography (UHPLC) can be applied for organic impurities and analysis, and ICP-MS, and ICP-OES for elemental and inorganic impurities analysis due to their excellent sensitivity and specificity [72,77,82,88]. However—quite the reverse—the costs associated with these measurements are significantly more exorbitant than conventional techniques such as HPLC, GC, and AAS because all these advanced analytical tools require heavy investments by way of equipment, maintenance, qualification, and personnel training. Access to such high technologies may be limited in small-scale pharmaceutical companies, contract research organizations, or academic labs; moreover, without in-depth impurity testing capabilities, they cannot meet regulatory requirements. Take, for instance, HRMS and multi-dimensional NMR; these would require experts both in the operation and in data interpretation. It adds further to the cost of analysis. The small-scale manufacturers or those working on novel drug formulations might not have the resources to adopt such costly testing procedures to detect compromised quality control measures on their part or delay the development of new therapies.

To meet these challenges, there is an increasing demand for more economical and easily available solutions. A possible route could be toward developing easily portable

analytical devices capable of impurity testing with less equipment and overhead. Alternatively, simplified or miniaturized versions of complex and intricate techniques that retain much of the analytical power could be brought into the picture, but at a fraction of their cost. Further, the improvements that have been realized in data analytics and Machine Learning make it possible to come up with predictive models for impurity identification, which can do away with the extensive testing that would again find many takers among small-scale manufacturers who need harmonized procedures.

4.4. New technologies integration

The outlook for impurities analysis in the pharmaceutical industry undoubtedly includes the adoption of new technologies and methodologies using AI, ML, and automation. These tools would greatly enhance the way the impurities are being analyzed since massive data sets can be evaluated with the optimization of testing methodologies, and trends can be divulged for improved understanding. For instance, newly detected impurities that were previously not reported and capable of going unidentified through routine analyses might be detected by complex and enhanced chromatograms or spectroscopic data processed by AI algorithms [99]. As a result, AI-based predictive models would be able to forecast the impurity profile, especially in drug products, leading to more focused and efficient methods of analysis development. Besides, automation could also surge efficiency and subside anthropological stumbles in impurity analysis as well. Integrations of automated sample preparations, automated data collections, and analyses would perhaps cut down testing time and turnaround time on results while making them more reproducible. Focusing on the imminent future would make it easier to analyze impurities with less cost for drug manufacturers, especially the ones having scarcer resources.

4.5. Emergent/nascent impurities and new drug categories

Developing new drug classes and formulations in the course of pharmaceutical research also brings new impurities that are problematic to analyze. For example, biologics and gene therapies have new impurity profiles that may not be amenable to analysis using traditional analytical techniques [100]. In part, this arises from the impurities associated with more complex delivery systems; for example, nanoparticles and liposomal formulations, among others [18,94]. With the emergence of new impurities, much of their characterization is weak, which more likely demands new analytical methods and even new regulations from the regulatory bodies to address them effectively.

The increasing complexity of drug products also brings about challenges in impurity profiling. For instance, impurities may comprise host cell proteins as well as product-related variants and aggregation products that cannot be analyzed using standard strategies, especially for a biological product. The regulatory framework around these new classes of drugs might be less evolved than established medicines; thus, it requires constant cooperation between pharma industry professionals, regulators, and academia to highlight and address emerging impurity concerns

effectively. **Figure 2a**,**b** represent the traditional/modern and imminent/future-emphasized techniques for the impurity analysis.

5. Future perspectives

Impurity testing in the pharmaceutical industry is set to witness a much-needed overhaul in its imminent future, with trends toward increased automation, AI, and ML. Improved impurity detection methods, along with the ease and economy of reaching impurities, will be some of the promises, among many, to be delivered as drug formulations and delivery systems change and the industry continuously reshapes its analytical toolkit to address the impurity profiles of new drug classes and with the introduction of biosimilars. Pursuant to the heightened complexity embodied in advanced pharmaceuticals that range from biologics to nanomedicines, specificity needs to be channeled into the development of very special test methodologies capable of perceiving trace impurities that have an impact on the safety and efficacy of the drug. Several technologies, including process analytical technology (PAT) with real-time monitoring, advanced HRMS, LC hyphenated with sophisticated technology alongside MS, SFC, ICP hyphenated with MS and OES, and other non-destructive techniques such as Raman and NIR spectroscopy, are expected to play a pioneering role along with the conventional analytical tools such as HPLC, GC, and AAS being imperatively involved in the subsequence.

Moreover, it is important that impurity testing standards and all regulations evolve together with this advancement. Therefore, as new drug categories are introduced and impurity profiles become more diversified, such guidelines must be updated to ensure that international standards and regulations on pharmaceutical quality and patient safety are maintained. The problem lies in being able to harmonize a range of such updates from differently stringent markets toward quicker approvals and wider access to safe, effective medications all over the world.

Despite the enormous strides impurity analysis has made, significant challenges remain to be met. The complexity of drug matrices, increasing regulatory pressures, and the very high cost of modern analytical technologies all contribute to the problems. However, hurdles should be read as a call to action for innovating, partnering, and adding more workflow-oriented and user-friendly analytical tools by breaking barriers within and outside the pharmaceutical industry to improve and continue the enhancement of better healthcare outcomes for patients across the globe while sustaining and making the ecosystem of pharmaceuticals greener and more efficient. Ultimately, by advancing together over such barriers, the return will further benefit not just patients but also healthcare systems the world over. **Table 3** presents the future prospects and challenges that need to be tackled in the field of impurity analysis.

Table 3. Future prospects and challenges in the field of impurity analysis.

Challenge	Prospective Remedy
Drug matrices intricacy and improved detection of impurities	To adopt advanced analytical techniques such as PAT, HRMS, NIR spectroscopy, and ICP-MS for the analysis of complex formulations for impurities with accuracy. To integrate AI, ML, and automation to improve impurity testing by using specialized equipment.
Increasing regulatory demands	Harmonization of impurity testing guidelines throughout the world will help in standardizing processes for approval to bring safe drugs to the market in a shorter time.
High cost of state-of-the-art analytical techniques	New innovative, performance-based, low-cost technologies to drive impurity testing with more accessibility to more pharmaceutical companies within the affordable bracket.
Specialized testing for new drug classes (i.e., biologics, biosimilars and nanomedicines)	Innovative test methods focusing specifically on impurities in biologics, biosimilars and nanomedicines toward safety and efficacy testing of these advanced drugs.
Sluggish regulatory adjustment to new impurities	To make regulatory frameworks dynamic, updated, and more responsive as impurity profiles become more diversified in or with time to cater for the new impurity testing concerns.
Limited global availability of modern impurity testing methodologies	To partner with regulatory and tech developers to ensure global acceptance and implementation of advanced test methods.
Assessment of the environmental impact of analytical procedures	To adopt cleaner and greener analytical techniques for impurity testing during pharmaceutical analysis for enhanced sustainability practices.

6. Conclusion

The critical necessity of modern and advanced impurity analysis for the safety, efficacy, and quality of pharmaceuticals has well been brought out in this review. It has been emphasized how much organic, inorganic, and residual solvent impurities can affect drug performance and patient safety. New analytical advances such as High-Resolution Mass Spectrometry (HRMS), Supercritical Fluid Chromatography (SFC), Inductively Coupled Plasma (ICP) with its hyphenated versions like ICP-MS, and ICP-OES techniques, and automated systems are the answer to the contemporary challenges related to intricate drug matrices, stringent and harmonized regulatory requirements, and emergent/nascent impurity profiles. The review also stressed balanced international rules and the adoption of inexpensive and technically sound analytic technologies for better accessibility to the pharmaceutical industry. Impending technologies such as AI and ML are said to totally revolutionize impurity analysis by enhancing detectability, operational efficiency, and cost-effectiveness. However, this thrilling opportunity is bedeviled by the high cost of technology, the intricacy of the new formulations for drugs, and a lack of global alignment on regulations. The future directions are the incorporation of advanced technologies, especially AI, ML, and automation, for enhanced impurity detection, reduced analysis time, and improved reproducibility and expansion to real-time monitoring tools like PAT. These tools would involve the development of specialized methods for new drug categories: biologics, biosimilars, and nanomedicines. The need for low-cost, portable, and simplified analytical devices for better accessibility and application would ease up the impurity analysis, particularly for small-scale manufacturers. In addition, adherence to green analytical chemistry principles as one of the main thrusts in the impurity testing frameworks is crucial due to enhanced sustainability measures and abiding by the compliance of sustainability. It is necessary to address all the challenges related to the analytical capabilities to analyze impurities in contemporary and future

times so that the pharmaceutical industry would be able to provide safe and efficacious medicines, contributing to better healthcare outcomes on a global scale.

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