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Review Article

Advanced Analytical Techniques for Identification and Quantification of Impurities in Active Pharmaceutical Ingredients - Review Article

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ABSTRACT

Imporities in Active Pharmaceutical Ingredients (APIs) may arise from starting material, synthetic intermediates, reagents, catalysts, basing agents, production steps, and storage or degradation conditions. Their presence can affect drug safety, efficacy, and quality requiring appropriate analytical methods for detection, identification, quantification, and control. Impurities have different polarities, volatilities, concentrations, molecular weights, elemental compositions, and chemical structures, and it is impossible to characterise all the impurities by a single analytical technique. This edition reviews some of the major APIs impurity profiling analytical tools including high-performance liquid chromatography (HPLC), ultrahigh performance liquid chromatography (UHPLC), LC–MS, gas chromatography/mass spectrometry (GC/MS), nuclear magnetic resonance (NMR), FTIR or infrared (IR) spectroscopy, and inductively coupled plasma mass spectrometry (ICP–MS). Analytical method validation, contribution of ICH guidelines in the impurity control, and complementary approaches are also discussed. Although used alone and in routine quantitation, chromatography is generally less informative than mass spectrometry analysis of the ionised molecule or spectroscopic methods constituent or elemental information. In general, a single technique is less robust than an orthogonal approach with validated techniques and good reference standards.

INTRODUCTION

The active pharmaceutical ingredient (API) is the portion of any drug that is pharmaceutically active. During synthesis, purification, formulation, packaging, and storage minor quantities of undesirable materials can be created or introduced. These are generally referred

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to as an impurity. Chemical impurities may be significant or trivial not only by percentage levels but also depending on their nature, toxicity, pharmacological activity, and exposures even at trace levels. Therefore, impurity profiling plays an important role in pharmaceutical research, production, quality analysis, and regulatory evaluation [1, 2]. Pharmaceutical impurity profiling reviews also propose not a general method of impurity analysis but that the selection of the analysis method of the impurity should be targeted according to the source and type of the impurity [3,4].

Impurities can be introduced from starting materials, reagents, catalysts, solvents, equipment, cross-contamination, or from degradation of the API. For example, a method developed to analyse the drug ingredient may not be selective enough to distinguish trace impurities. In choosing a method, consideration should therefore be given to the anticipated nature of the impurity, the anticipated level of the impurity, the sample matrix, and the purpose of the test. In modern impurity analysis, separation of the impurities, usually chromatographic, is required for quantitation and characterisation of the relevant analytes by spectrometric or spectroscopic detection [4–6].

The objective of this review is to describe the state-of-the-art of analytical techniques in impurity detection and quantification, to compare their application and their advantages and disadvantages, and to highlight major validation and regulatory issues.

1.1 Review methods

This narrative review is developed from several peer-reviewed reviews and key ICH guidelines on pharmaceutical impurity profiling, hyphenated analytical techniques, and forced degradation studies. Criteria for selection of the sources engaged

keywords searched in the indexed scientific literature and regulatory websites (ICH, EMA, US FDA) including terms such as "impurity profiling," "API impurities," "HPLC," "LC–MS," "GC–MS," "NMR," "ICP-MS," and "method validation." This information focused on material discussing the rationale of the method, with its pros and cons concerning analysis, and on the regulatory situation. The analysis was elaborated [1, 3−7] by considering the main approaches to the analysis and fallacies of the approaches on the basis of sources of interpretation regulations (ICH materials).

Since there is no formal screening flow diagram or meta-analysis, this article serves as a focused narrative review rather than a systematic review. As a result, it should not be considered a comprehensive list of all published techniques available in the literature.

2. Impurities in APIs: Types and origins

2.1 Organic Impurities

These contaminants may be starting materials, intermediates and reagents/byproducts, or derivatives and degradation products of the API. Process-related impurities can be generated from incomplete reactions, side reactions, or inadequate purification. Products formed by hydrolysis, oxidation, photolysis & heat stress during production or storage. These compounds are usually separated from the API by chromatographic methods, and spectroscopic techniques such as NMR and LC–MS can be employed for structural elucidation [1, 3].

2.2 Residual solvents

Residual solvents are organic volatile chemicals used or produced in the manufacture of active ingredients of medicinal products or excipients or in the preparation of pharmaceutical products. They are controlled by the solute and environmental load provisions. Gas chromatography is particularly useful, typically with flame-ionization detection (GC-FID) or with mass-spectrometric detection (GC-MS), because many residual solvents are somewhat volatile and thus separable by interaction with stationary phase. Headspace sampling can reduce interference from non-volatiles [8].

2.3 Inorganic and chemical residues

Inorganic residues are filter residues, salts, and material processing residues. Salts, filter aids, and residues of processing material. Elemental impurities may originate from catalysts and raw materials, equipment, and plants. Inductively coupled plasma-optical emission spectrometry (ICP-OES) can also be used if the sensitivity requirement is met, or the more sensitive ICP-MS is generally used to measure multiple elements. Risk assessment, i.e. interpretation of the measured

elemental content within the framework of the relevant specification and exposure route, is required; in this respect, adequate sample preparation is required [9].

2.4 Mutagenic contaminants

There are impurities that are mutagenic and need to be carefully assessed and controlled. ICH M7 has provided a guideline for manage and the evaluation of DNA reactive (mutagenic) impurities in medicinal products. The analytical procedure should be appropriately sensitive for the relevant acceptable intake and dose, and the method should be justified from the basis of the chemical nature of the molecule and the required limit [10].

3. MAJOR ANALYTICAL TECHNIQUES

3.1 HPLC (high-performance liquid chromatography)

HPLC separates components based on the distribution of components between a stationary phase and a mobile phase. Dias is non-volatile and thermally labilized; therefore, reversed-phase HPLC is used for APIs and related organic impurities. Detectors may include ultraviolet (UV), photodiode-array (PDA), fluorescence, etc. It allows for quantification (e.g., peak area, if properly calibrated) and (peak) identification (e.g., spectral information or retention time matching a reference standard).

Advantages: Broad applicability and frequent usage; many reliable or classical methods. Disadvantages include possible co-elutions, low UV sensitivity for some chemicals, and lack of structural information with a UV detector only. Relative response factors are required if the detector response of the impurity and API are not comparable. For a stability-indicating method, specificity should be demonstrated if applicable degradation products are to be considered.

3.2 Ultra almost high performance liquid chromatography (UHPLC)

UHPLC uses columns and systems with much smaller particles and runs under much higher pressure compared to traditional HPLC. This, when performed properly, can result in less solvent use as well as quicker and better separation. UHPLC is suitable for high-throughput impurity profiles and can be coupled to mass spectrometry. However, for the co-benefits of fast separation, such as different particle size, column diameters, pressure limit, and dwell volume, method transfer is limited.

3.3 LC-MS (liquid chromatography-mass spetra) and LC-MS 1/2 LC separation mass analysis.

The two most common interfaces are electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). Accurate masses and fragmentation and/or secondary isotopic patterns may help suggest and/or verify chemical formulas and structures. MS/MS can also supply fragment-ion information and can be used for sensitive quantitative determination (target) by selective or multiple reaction monitoring (MS/MS).

LC-MS is beneficial for non-UV-absorbing, low-level contaminants and for structural elucidation. Triple-quadrupole instruments are often used for targeted quantitation, while high-resolution mass spectrometry is more suitable for non-targeted screening. These include differences in ionisation efficiency, adduction, matrix suppression or enhancement often with change in stoichiometry, and lack of adequate reference materials. Mass spectra may not be able to distinguish structural isomers and thus require chromatography, spectroscopy, or standards to provide further evidence. Tandem MS and high-resolution MS have been particularly useful where the unknown impurity must be detected sensitively and meaningfully [4,6]. The confidence level in an assignment reflects the quality of evidence.

3.4 Gas chromatography mass spectrometry GCMS

GCMS is suited to GC-separated volatile and thermally stable chemicals. It may be helpful for volatile process contaminants, residual solvents, and volatilisable compounds amenable to derivatisation. It may also help in identification by comparison with reference retention and mass spectrum. Headspace GC for solvent analysis is less tolerant of non-volatility of API than other methods.

GC-MS cannot analyse non-volatile, thermally labile substances that do not derivatise. Sample treatment and introduction parameters must be chosen to avoid discrimination or degradation. Matrix interference vs.

fractionation; recovery and accuracy; calibration of quantitation.

3.5 Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H and ^{13}C chemical environments in nuclear magnetic resonance spectroscopy (NMR). Experiments, both one- and two-dimensional, exist to solve structures. NMR is particularly useful if an impurity has been isolated or the structure from MS analysis is ambiguous. A validated technique may use qNMR with an appropriate external or internal reference.

Advantages of NMR over other techniques include the potential for obtaining structural information without the need for ionisation. However, it has the drawbacks of requiring large amounts of sample, spectral interpretation, the high cost of the equipment, and being less sensitive than some MS-based methods. Consequently, it is frequently used in conjunction with and not as a substitute for conventional chromatographic impurity analysis.

3.6 FTIR and Raman spectroscopy

FTIR and Raman spectroscopy afford vibrational information on molecules and are useful in identifying functional groups, characterising raw materials, and examining isolated contaminants. Trace impurity analysis depends on concentration, spectral overlap, and availability of suitable reference spectra, depending on the technique. An impurity present at significantly higher concentrations than the API can make them less than useful for yielding values for low-level contaminants in complex samples (absent separation or careful sampling).

3.7 ICP-OES & ICP-MS

ICP-OES is capable of measuring spectral emissions, given a low enough detectability, of species elements, and ICP-MS can sample likely elemental measurements, including gas, atoms, and ions output from inductively coupled plasma. These methods are more concerned with elemental impurities than organic degradation products. Such humanisation is required for accurate results with control over contamination, internal standards, and recovery checks as well as digesting samples and spectral or mass interferences [9].

4. IDENTIFICATION VERSUS QUANTIFICATION

Identification asks, "What is an impurity?" Quantification asks, "How much impurity?" These goals are related, but not interchangeable. Retention time alone often would not be sufficient for chemical identification, but it does provide chromatographic peak separation and an estimate of relative abundance. Comparison with a valid standard, similar UV spectrum, precise mass, MS/MS fragmentation, NMR, or other evidence may aid identification. Confidence should be stated if possible with the data available. Quantification requires calibration and a good measurement model. Depending on the method and analyte, validated relative response factors, standard addition, internal-standard calibration, or external-standard calibration may be used. If there is no impurity standard, then it may be a guess or an initial estimate from the API result. Such assumptions should be recorded and should be improved or substantiated if additional relevant information is available. Tentative ID or estimated concentration should be differentiated from a confirmed result.

5. COMPARISON AND COMPLEMENTARY USE OF METHODS

Table 1 compares the main analytical techniques discussed in this review.

Table 1: Comparison of Analytical Techniques Used in API Impurity Analysis

Technique	Best-suited applications	Main strength	Key limitation
HPLC/UPLC-UV/PDA	Routine related-substance testing; non-volatile organics	Robust separation and quantification	Limited structural specificity; co-elution risk
LC–MS/MS	Trace organic impurities; targeted confirmation	Sensitivity and fragmentation data	Matrix effects; standards and method development
GC–MS	Residual solvents and volatile impurities	Separation plus mass spectral information	Requires volatility/thermal stability or derivatization

Technique	Best-suited applications	Main strength	Key limitation
NMR/qNMR	Structure elucidation; content determination	Rich structural information	Lower sensitivity; sample quantity and cost
FTIR/Raman	Functional groups and material characterization	Rapid molecular fingerprinting	Mixture overlap and limited trace sensitivity
ICP–MS/ICP–OES	Elemental impurities	Multi-element determination	Does not identify organic impurities; contamination control

Orthogonal methods will depend on different physiochemical principles. If enough material is available, a new impurity peak can be isolated by HPLC, and its exact mass and fragmentation determined by high resolution LC-MS before NMR, for example. Headspace GC may be used to check residual solvents, and elemental concerns by ICP-MS or ICP-OES. The availability of instrumentation should not dictate what method is used; again, the analytical question to be answered and the risk of impurities present should dictate the method.

6. METHOD DEVELOPMENT AND VALIDATION

The first step in developing such a method is the thorough understanding of API along with its synthesis (possibly impurities associated), the physicochemical characteristics, and the necessary specification. Forced degradation studies may determine potential degradation products to verify if a method is stability-indicating [7,14]. Chromatographic parameters (stationary phase, mobile-phase composition, pH, gradient, temperature, and detector settings) are optimised to obtain a satisfactory separation and response. Sample preparations should be designed to minimise analyte loss, contamination, and transformation. These aspects of the life cycle approach are in line with ICH Q14, which encourages science- and risk-based development and control of procedures [12].

Analytical Procedure validation criteria for identification, assay, and purity/impurity are described in ICH Q2 (R2) [11,13]. Validation characteristics for the purpose can be linearity, range, accuracy, precision, specificity/selectivity, robustness, detection limit, and quantitation limit. Not all characteristics are evaluated the same for all operations, so the validation plan should be suitable for the analytical goal and risk. Here ICH Q14 complements and expands on it and contains science- and risk-based development of analytical methods and lifecycle considerations [12].

The quantitation limit for impurities of the approach should be the level that must be reported or controlled. Accuracy can be established by spiking and recovery tests, and precision can be established by repeatability and intermediate precision where applicable. Specificity should account for interference by the API, known contaminants, degradation products, excipients (as appropriate), and reagents used in sample preparation. Ongoing checks to ensure fitness for purpose include monitoring of performance and system suitability.

7. REGULATORY AND QUALITY CONSIDERATIONS

ICH Q3A (R2) deals with the reporting and identification/qualification and control of organic impurities in new drug substances resulting from chemical synthesis [1]. Other related guidelines in this framework are ICH M7 (mutagenic impurities), ICH Q3D (elemental impurities), and ICH Q3C (residual solvents) [8–10]. These guidelines have different scopes (Table 2) and should be consulted with consideration of the impurity type and context of the product. The acceptance criteria are not normalised per se but depend on the relevant guide line, the maximum daily dose, the identified impurity, treatment duration, and safety information.

Table 2: ICH Guidelines Relevant to Impurity Control and Analytical Procedures

Guideline	Subject	Relevance to analytical work
ICH Q3A(R2)	Impurities in new drug substances	Reporting, identification, and qualification of organic impurities [1]
ICH Q3C(R8)	Residual solvents	Basis for controlling and testing residual solvents, commonly by headspace GC [8]

Guideline	Subject	Relevance to analytical work
ICH Q3D(R2)	Elemental impurities	Risk-based control of elemental impurities; ICP-based testing [9]
ICH M7(R2)	Mutagenic (DNA-reactive) impurities	Assessment and control; drives sensitivity needs of trace methods [10]
ICH Q2(R2)	Validation of analytical procedures	Validation characteristics for impurity and assay methods [11,13]
ICH Q14	Analytical procedure development	Science- and risk-based development and lifecycle management [12]

The analysis results should be subjected to a quality system. Studies should address whether an impurity is process related, deterioration, the level changes over time, whether the proposed method can separate the impurity from relevant interferents, etc. Identification/qualification decisions should be supported by scientific and legal evidence; a molecule is not demonstrated safe simply by an instrument output. Where an impurity is known to have a toxicological effect, the analytical results should be interpreted in the light of the appropriate risk assessment.

8. PRACTICAL WORKFLOW FOR IMPURITY INVESTIGATION

There are many options to develop a practical investigation. 1. List possible sources of contamination from raw materials, catalysts, solvents, packaging, stability data, and synthetic process. Second, determine if there is an analytical target of elemental impurity, non-volatile organic impurities, potentially mutagenic, or volatile residue. Third, choose a detector and separation type for it. Headspace GC works for many residual solvents; LC-MS is useful if mass or fragmentation evidence is needed; HPLC/UPLC with UV or PDA detection is often the first choice for related organic chemicals; and ICP-based techniques are for elemental analysis [1,4,8-10].

4. Evaluate the specificity of the signal. Confirm that the peak/response is not an API or co-eluting component, reagent, degradation product, or sample-preparation artefact. Fifth, in the case of continued structural ambiguity, identify the impurity by the best possible comprehensive basis of evidence, for example accurate mass plus MS/MS fragments plus comparison with a reference standard plus NMR. Sixth, in the absence of an authentic impurity standard, characterise the uncertainty, response variables, and recovery and state any assumptions made and qualify the quantitative process for its intended use [4,11,12].

This exercise emphasises an important distinction, that identification is not qualification and detection is not identification. A device may give a repeatable signal without defining the molecular makeup or whether or not it is toxicologically acceptable. Regulatory decisions should be based on analytical evidence, an appropriate impurity standard, and a safety evaluation, where applicable [1,8-11].

8.1 Critical review of the status quo

The literature is biased toward a complementary rather than competitive view of analytical platforms. Chromatography can resolve mixtures, but perhaps not with sufficient structural specificity from a detector. MS can give information on molecular mass and fragmentation, but ionisation effects can bias the quantitative response and the difficulty in differentiating isomers. NMR can answer structural questions but can be time-consuming and resource-intensive. ICP-MS is sensitive for elemental measurement but not for organic molecular contaminants. For these reasons, orthogonal evidence is often more convincing than method selection by high sensitivity [3,4,6].

Another conundrum that is frequently encountered is measurement of an impurity with no reference standard. Screening may be in form of estimated impurity from API response (which may be biased by different detector response); however, such estimations should be provided as estimates qualified by other research or response elements that justify the response. Therefore, when presented as numbers, they should be qualified and supplemented with other studies and better-justified response elements. Appropriate sensitivity for trace or mutagenic contaminants should be based on

allowable intake and dose [10,11] and not arbitrarily chosen.

9. CHALLENGES AND FUTURE PERSPECTIVES

Big problems are impurities/low-level contaminants, isomers, lack of reference standards, matrix effects, and transfer of techniques between laboratories/instrument platforms. High-resolution MS, enhanced chromatography, automated data processing, and multivariate analysis could all enhance information per sample. However, prediction by automated or computational means must be experimentally confirmed to avoid misassignment. Green analytical chemistry aims at smaller sample sizes and lower solvent consumption and at more efficient work flows without sacrificing performance.

Hence, future impurity control will increasingly rely on combining data from lifecycle monitoring, high-resolution analytics, process knowledge, stability studies, and risk-based specifications. The best approach is not the most sophisticated tool but a combination of methods and controls that provides sufficient, convincing evidence for the choice made.

CONCLUSION

Impurity profiling and quantification are critical for guaranteeing the quality and safety profile of APIs. HPLC and ultrahigh-performance LC (UHPLC) are still key methods for routine separations and quantitation, while LC–MS/MS and GC–MS add additional selectivity and identification information for relevant organic and volatile impurities. ICP methods are most suitable for elemental impurities, additional vibrational information by FTIR and Raman, and structural information by NMR. All methods have their limitations, and the use of orthogonal methods can add to the confidence in the impurity assignments and measurements. A method should be designed with an impurity control strategy, using science-based method development, fitness-for-purpose validation, appropriate use of suitable standards, and ICH guidelines.

ABBREVIATIONS

API, active pharmaceutical ingredient; APCI, atmospheric-pressure chemical ionization; ESI, electrospray ionization; FTIR, Fourier-transform infrared; GC–FID, gas chromatography–flame ionization detection; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; ICH, International Council for Harmonisation; ICP–MS, inductively coupled plasma–mass spectrometry; ICP–OES, inductively coupled plasma–optical emission spectrometry; LC–MS, liquid chromatography–mass spectrometry; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; PDA, photodiode array; qNMR, quantitative NMR; UHPLC, ultra-high-performance liquid chromatography; UV, ultraviolet.

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