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Sources of Pharmaceutical Impurities in Formulations: - A Review

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Abstract

Various regulatory authorities such as the International Conference on Harmonization (ICH), the United States Food and Drug administration (FDA), and the Canadian Drug and Health Agency (CDHA) are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredients (APIs). The various sources of impurity in pharmaceutical products are — reagents, heavy metals, ligands, catalysts, other materials like filter aids, charcoal, and the like, degraded end products obtained during \ after manufacturing of bulk drugs from hydrolysis, photolytic cleavage, oxidative degradation, decarboxylation, enantiomeric impurity, and so on. The different pharmacopoeias such as the British Pharmacopoeia, United State Pharmacopoeia, and Indian Pharmacopoeia are slowly incorporating limits to allowable levels of impurities present in APIs or formulations. Various methods are used to isolate and characterize impurities in pharmaceuticals, such as, capillary electrophoresis, electron paramagnetic resonance, gas–liquid chromatography, gravimetric analysis, high performance liquid chromatography, solid-phase extraction methods, liquid–liquid extraction method, Ultraviolet Spectrometry, infrared spectroscopy, supercritical fluid extraction column chromatography, mass spectrometry, Nuclear magnetic resonance (NMR) spectroscopy, and RAMAN spectroscopy. Among all hyphenated techniques, the most exploited techniques for impurity profiling of drugs are Liquid Chromatography (LC)-Mass Spectroscopy (MS), LC-NMR, LC-NMR-MS, GC-MS, and LC-MS. This reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

Keywords: Characterization, chromatography, identification, impurities, NMR, mass spectrometry

INTRODUCTION

Pharmaceutical impurities are the unwanted chemicals that remain with active pharmaceutical ingredients (APIs) or drug product formulations. The impurities observed in drug substances may arise during synthesis, or may be derived from sources such as starting materials, intermediates, reagents, solvents, catalysts, and reaction by-products. During drug product development, impurities may:

- Be formed as a result of the inherent instability of drug substances
- Be due to incompatibility with added excipients, or
- Appear as the result of interactions with packaging materials and container closure systems (CCSs)
- Purification of chemicals is an expensive process, substances should not be purified more than required as it brings about waste of time, material and money.
- Sugarcane, dextrose, inorganic salts 99% purity while many other only are having traces of impurity.
- Pure Relative term Pharmacopoeia of India and BP has been prescribed test for purity.

Various regulatory authorities focus on the control of impurities:

- The International Council for Harmonization (ICH)
- The United States Food and Drug Administration (USFDA)
- The European Medicines Agency (EMA)
- The Canadian Drug and Health Agency
- The Japanese Pharmaceutical and Medical Devices Agency (PMDA)
- The Australian Department of Health and Ageing Therapeutic Goods

In addition, several official compendia, such as the British Pharmacopoeia (BP), the United States Pharmacopeia (USP), the Japanese Pharmacopoeia (JP), the European Pharmacopoeia (EP), and the Pharmacopoeia of the People's Republic of China (ChP) are incorporating limits that restrict the impurity levels present in APIs and drug formulations. The regulations are based on exposure limits, so the level of contaminants must be controlled in the final dosage form, as administered. In practice, this means that drug manufacturers must perform a risk assessment that takes into consideration sources of contamination post-manufacturing—such as packaging, transportation, and CCS, as well as from raw materials and manufacturing processes.

Sources of Impurity in Medicines

According to ICH guidelines, Pharmaceutical impurities are classified into 3 major categories:

1. Organic Impurities
2. Inorganic Impurities (Elemental)

3. Residual Solvent

1. Organic Impurities

Organic impurities can arise in APIs or drug product formulations during the manufacturing process or during the storage of drug substances. They may be known, unknown, volatile, or non-volatile compounds with sources including starting materials, intermediates, unintended by products, and degradation products. Although the end products are always washed with solvents, there is always a chance that the residual unreacted starting materials remain, unless the manufacturers are very careful about the impurities.

2. Inorganic Impurities (Elemental)

Elemental impurities are unwanted elements that occur in drug formulations. They can arise from active pharmaceutical ingredients, raw materials, synthetic additives, excipients, catalysts, and production processes and equipment used during manufacturing. The levels of the four most toxic elemental impurities: As, Cd, Hg, and Pb, must be measured in all drug products.

Inorganic impurities may also be derived from the manufacturing processes used for bulk drugs. They are normally known and identified, and include the following:

Reagents, ligands, and catalysts: The chances of having these impurities are rare: however, in some processes, these could create a problem unless the manufacturers take proper care during production.

Heavy metals: The main sources of heavy metals are the water used in the processes and the reactors (if stainless steel reactors are used), where acidification or acid hydrolysis takes place. These impurities of heavy metals can easily be avoided using demineralized water and glass-lined reactors.

Other materials: (e.g., lter aids, charcoal etc.) The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs manufacturing plants and in many cases, activated carbon is also used. The regular monitoring of fibers and black particles in the bulk drugs is essential to avoid these contaminations.

The elemental impurity methods allow for separation of the chemical forms of these elements, for example, using HPLC coupled to ICP-MS, to ensure that the more toxic forms of the elements meet the PDE limit, even if the total level exceeds the PDE.

Table 1. The permitted daily exposure (PDE) limits for elemental impurities in drug products, according to their route of administration. Elements shaded in yellow should be considered in product risk assessment. All elements listed should be included in risk assessment if naturally present, or if intentionally or unintentionally added.

ICH/USP Class	Element	Oral PDE (µg/day)	Parenteral PDE (µg/day)	Inhalational PDE (µg/day)
Class 1	Cadmium (Cd)	5	2	2
	Lead (Pb)	5	5	5
	Arsenic (As) (inorganic)	15	15	2
	Mercury (Hg) (inorganic)	30	3	1
Class 2A	Cobalt (Co)	50	5	3
	Vanadium (V)	100	10	1
	Nickel (Ni)	200	20	5
Class 2B	Thallium (Tl)	8	8	8
	Gold (Au)	100	100	1
	Palladium (Pd)	100	10	1
	Iridium (Ir)	100	10	1
	Osmium (Os)	100	10	1
	Rhodium (Rh)	100	10	1
	Ruthenium (Ru)	100	10	1
	Selenium (Se)	150	80	130
	Silver (Ag)	150	10	7
	Platinum (Pt)	100	10	1
	Lithium (Li)	550	250	25
	Antimony (Sb)	1,200	90	20

Class 3	Barium (Ba)	1,400	700	300
	Molybdenum (Mo)	3,000	1500	10
	Copper (Cu)	3,000	300	30
	Tin (Sn)	6,000	600	60
	Chromium (Cr)	11,000	1,100	3

3. Residual Solvents

Residual solvents are the volatile organic chemicals used during the manufacturing process, or generated during drug production. Several organic solvents used in the synthesis of pharmaceutical products are known to have toxic or environmentally hazardous properties, and their complete removal can be very difficult. The final purification step in most pharmaceutical drug substance processes involves a crystallization step that can lead to the entrapment of solvent, which can act as a residual impurity, or cause potential degradation of the drug. Residual solvent levels are controlled by the ICH, USP, and EP.

Residual solvents are categorized into three classes with their limits in pharmaceutical products set by ICH guidelines Q3C. Class 1 solvents, including benzene, carbon tetrachloride, 1,1-dichloroethane, 1,2-dichloroethylene, and 1,1,1-trichloroethane, should be avoided. Class 2 solvents, such as methanol, pyridine, toluene, N,N-dimethylformamide, and acetonitrile have permitted daily exposure limits (PDEs). Table 2 presents a few examples of common organic solvents that are found as volatile impurities and have their limits set by ICH guidelines. Class 3 solvents, such as acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethyl acetate should be limited by GMP or other quality-based requirements.

Table 2. ICH limits for a selected list of common organic solvents found as volatile impurities.

Volatile organic impurity	Limit (ppm)	PDE (mg/day)
Acetonitrile	410	4.1
1,4-Dioxane	380	3.8
Chloroform	60	0.6
Methylene chloride	600	6.0
Pyridine	200	2.0
1,1,2-Trichloroethane	80	0.8

USP <467> 2009 General Chapter contains a more comprehensive method for residual solvent analysis, similar to the ICH guidelines developed in 1997. This document presents a limit test for Class 1 and Class 2 solvents, while Class 2C solvents are usually determined by no headspace methods due to their higher boiling points. The limits of detection (LODs) recommended for Class 3 solvents are up to 5,000 ppm. When the levels of residual solvents exceed USP or ICH limits, quantitation is required.

ICH GUIDELINES

We have summarized various classes based on impurities according to the ICH guideline in Table 3.

Table 3

Classification of Q guideline on the basis of impurities

Section	Impurities	Sub-section
Q3A(R2)	Impurities in new drug substances	Q3A(R)
Q3B(R2)	Impurities in new drug products	Q3B(R)
Q3C(R4)	Impurities: Guideline for residual solvents	Q3C
	Impurities: Guideline for residual solvents (Maintenance) PDE for tetrahydrofuran (in Q3C(R3))	Q3C(M)
	PDE for N-methylpyrrolidone (in Q3C(R3))	Q3C(M)

ICH Limits for Impurities

According to the ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. According to the ICH, the maximum daily dose qualification threshold to be considered is as follows; <2 g / day, 0.1 % or 1 mg per day intake (whichever is lower) >2 g / day, 0.05%.

i) Organic Impurities

Each specific identified impurity

- Each specific unidentified impurity at or above 0.1%
- Any unspecific impurity, with a limit of not more than 0.1%
- Total impurities

ii) Residual solvents

iii) Inorganic impurities

Isolation Methods

It is often necessary to isolate impurities. However, if instrumental methods are used, isolation of impurities is avoided, as it directly characterizes the impurities. Generally, chromatographic and non-chromatographic techniques are used for the isolation of impurities prior to its characterization. The term 'chromatographic reactor' refers to the use of an analytical-scale column, serving both as a flow-through reactor and a separation medium for the reactant(s) and product(s). High-performance liquid chromatography (HPLC) and the chromatographic reactor approach, with solution-phase hydrolysis kinetics can be used for an aprepitant (EmendTM) prodrug and fosaprepitant dimeglumine.[18] In loratidine, the impurity found was ofloratidine; [19] other examples include celecoxib[20] and amikacin.[21]

The structure of impurities — unknown degradation products in drug substances and drug products — must, according to the current FDA and EMEA guidelines, elucidate if they exceed a level of greater than 0.1%. Analytical Services provide the latest analytical techniques for structure elucidation (e.g., high-field NMR, LC-MSMS, GC-MS, and MALDI-TOF) as well as for preparative isolation of unknown impurities (e.g., semi and fully preparative HPLC). Software tools for the prediction of spectra support the study of our experts.

Solid-Phase Extraction Methods

Solid phase extraction (SPE) is an increasingly useful sample preparation technique. With SPE, many of the problems associated with liquid – liquid extraction can be prevented, such as incomplete phase separation, less-than-quantitative recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents. SPE is more efficient than liquid – liquid extraction, yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and laboratory time are reduced. SPE is used very often to prepare liquid samples and extract semi- volatile or nonvolatile analytes, and can also be used with solids that are pre-extracted into solvents. SPE products are excellent for sample extraction, concentration, and cleanup. They are available in a wide variety of chemistries, adsorbents, and sizes. Selecting the most suitable product for each application and sample is important.

Liquid – Liquid Extraction Methods

Liquid – liquid extraction, also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. Liquid – liquid extraction is a basic technique in chemical laboratories, where it is performed using a separating funnel. This type of process is commonly performed after a chemical reaction as part of the workup.

Accelerated Solvent Extraction Methods

Accelerated Solvent Extraction (ASE) is a better technique for the extraction of solid and semisolid sample matrices, using common solvents, at elevated temperatures and pressures. ASE systems are available in the entry level ASE 150 system and the fully automated ASE 350. Extractions that normally take hours can be done in minutes using ASE with pH hardened pathways, using Dionium™ components. Compared to techniques such as Soxhlet and sonication, ASE generates results in a fraction of the time. The many steps involved in sample preparation can now be automated with the ASE flow-through technology.

Filtration and clean-up of solid samples can be achieved as part of the solvent extraction process in a single step. ASE offers a lower cost per sample than other techniques, reducing solvent consumption by up to 90%.

Supercritical Fluid Extraction

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix), using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g., decaffeination) or collect a desired product (e.g., essential oils).

Carbon dioxide (CO2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO2 are above the critical temperature of 31°C and critical pressure of 72 bar. Addition of modifiers may slightly alter this.

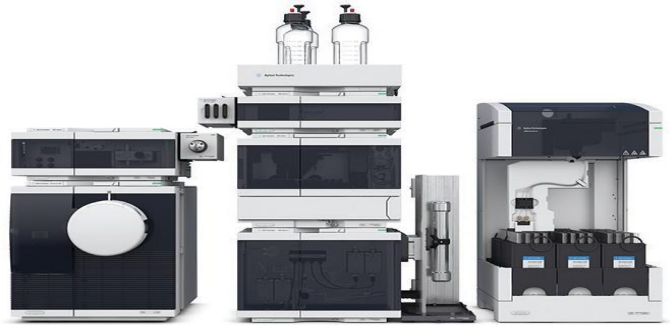
Analytical Technologies for Impurity Profiling in Pharmaceutical Development

Overview

To better detect, identify, quantify, and characterize the impurities present in drug substances and products, pharmaceutical scientists rely on robust analytical tools with high sensitivity and specificity. Major analytical tools for impurity analysis include spectroscopy, chromatography, mass spectrometry, and various combinations of these, that is, tandem techniques. The appropriate technique is selected based on the nature of the impurity and the level of information required from the analysis. There are various complex analytical problems in pharmaceutical development that require the use of more than one analytical technique for their solution. Analytical techniques such as LC/UV, LC/MS, GC/MS, CE/MS, and LC/UV provide the orthogonal detection and complementary information that can address these challenges in a time-efficient manner. As a result, they play a vital role in impurity profiling of pharmaceuticals from identification to the final structure elucidation of unknown impurities.

Preparative Liquid Chromatography (LC)

This is a major challenge in pharmaceutical laboratories. Preparative LC helps isolate impurities (usually from impurity-enriched analytes, such as the solution remaining from the crystallization of APIs) in sufficient quantities to carry out structural analysis, using techniques such as FTIR, NMR, LC/MS, or GC/MS.



Agilent 1260 Infinity II Preparative LC/MSD System

Several impurity analysis methods found in pharmaceutical quality control (QC) laboratories use high-performance liquid chromatography (HPLC) coupled with UV detection (HPLC/UV methods). UV detection helps identify impurities or degradants in drug substances based on absorption maxima. This technique is one of the most important and versatile analytical methods available for impurity profiling due to its selectivity (that is, the ability to quantitatively determine
many of the individual components present in a sample using a single analytical procedure), especially for routine analysis where standards are available. Stationary phase systems are available that operate in several modes, such as ion pairing, increased hydrophobic interactions, and variable pH, allowing a variety of samples to be analyzed concurrently based upon their unique properties. High resolution is particularly helpful when using LC/UV analysis for impurity detection, because all impurities can be identified with less chance of error.

Agilent Infinity Lab LC Series instruments and columns

Liquid Chromatography and Mass Spectrometry (LC/MS)

LC/MS is a highly sensitive and specific analytical tool that is routinely used in pharmaceutical development to detect, identify, and quantify product impurities. A detection limit of a few hundred ppm is readily achievable, ensuring the identification of impurities present at concentrations greater than 0.1 %. Mass spectrometry-based methods generally provide additional sensitivity and specificity compared to techniques such as UV alone. While single quadrupole mass spectrometers are well suited to the confirmation of known impurities and the preliminary structural assessment of unknown impurities, highly sensitive Q-TOF mass spectrometers provide high-resolution accurate mass information that enables the unambiguous identification of unknown trace impurities. This makes them particularly useful for genotoxic impurity analysis. MS-based methods are often selected for the impurity profiling of APIs during process development. UV-based methods are generally used to test for genotoxic impurities in QC laboratories at manufacturing sites.

Agilent 6100
Series Single Quadrupole MS

Agilent 6500 Series Q-TOF

Agilent 6400 Series Triple Quadrupole MS

Gas Chromatography (GC)

In combination with flame ionization detection (FID), GC is the standard choice for the analysis of volatile organic impurities, such as residual solvents. The gas chromatography headspace method is used worldwide for residual solvent analysis in quality control laboratories because it closely follows ICH Q3C guidelines. Sample preparation and introduction is through a static headspace, which facilitates the selective introduction of volatile solvents without contamination by mostly non volatile drug substances or drug products. Therefore, the use of an FID detector helps preferentially identify and quantify residual solvents. The combination of gas chromatography and mass spectroscopy (GC/MS) can be used for confirmation and identification purposes, highlighting the flexibility of this technology.

Agilent 7890A combined with an Agilent 5975C GC/MS system with an Agilent 7697A Headspace sampler

Capillary Electrophoresis (CE)

The determination of drug-related impurities is one application of CE in pharmaceutical analysis because it achieves high separation efficiencies compared to other chromatographic techniques. CE can be used when HPLC techniques are not able to adequately measure impurities, especially very polar compounds. A reporting threshold of 0.05 % is widely accepted as a minimum requirement for a related impurities determination method, and this can be achieved using CE. In addition, CE is very useful for the separation of closely related compounds such as diastereomers and enantiomers. An example of the value of CE in impurities analysis is demonstrated using heparin (a polymeric anticoagulant). In this case, standard LC failed to distinguish drug lots associated with adverse events, while CE was easily able to identify an unknown impurity (Figure 2). As a result, the use of CE helped to solve this analytical challenge.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is very useful for identifying and confirming the structure of an impurity or degradant because every compound has a unique spectral fingerprint.

Especially useful in analyzing organic compounds, FTIR spectra are determined by molecular structure and the functional groups present. Conversely, functional group analysis can help identify the structure and measure the concentration of the compound under investigation. Changes in structure can be correlated by comparing the FTIR library spectrum of a pure reference material to that of the impurity or degradant.

The Agilent Cary 630 FTIR provides a powerful combination of precision and compliance, making it one of the best FTIR systems for both qualitative and quantitative analysis in pharmaceutical laboratories

Agilent Cary 630 FTIR

Impurities commonly found in Medicinal preparations:

1. Activity depressing impurities.
2. Due to colouring or flavouring substances, e.g., Sodium Salicylate.
3. Humidity.
4. Decrease shelf life.
5. Physical and chemical properties.
6. Impurities due to which substances become incompatible.

Application of isolation and characterization of impurities

Numerous applications have been sought in the areas of drug designing and monitoring. Quality, stability, and safety of pharmaceutical compounds, whether produced synthetically, extracted from natural products or produced by recombinant methods.
The applications include :-

- Alkaloids
- Amines
- Amino acids
- Analgesics
- Anti bacterials
- Anti Convulsants
- Anti depressants
- Tranquilizers
- Anti neoplastic agents
- Local anesthetics
- Macromolecules
- Steroids and so on.

Conclusion

Isolation and characterization of impurities is mandatory for acquiring and evaluating data that establishes biological safety, which reveals the need and scope for impurity profiling of drugs in pharmaceutical research. To isolate and quantify the impurities, various instrumental analytical techniques have been used routinely. Moreover, the recognition and regulatory contemplation of organic impurities is an extremely complex problem owing to numerous sources ranging from microbial contamination to degradation products of APIs apart from traces of intermediates. Although, ICH has an out lighted course of action with regard to impurities, but still much more needs to be done. Hence, there is a strapping need to have unified specifications / standards with regard to impurities.

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