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Recent trends in the impurity profile of pharmaceuticals

Kavita Pilaniya,
Harish K. Chandrawanshi[1],
Urmila Pilaniya[1],
Pooja Manchandani[2],
Pratishtha Jain[3], Nitin Singh[3]

Department of Pharmaceutics, Shivdan Singh Institute of Technology and Management, Aligarh, U.P., [1]V.N.S. Institute of Pharmacy, Bhopal, [2]SGSITS, Indore, [3]Oriental College of Pharmacy, Bhopal, M.P., India

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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.

Key words: Characterization, chromatography, identification, impurities, NMR, mass spectrometry

INTRODUCTION

The impurities in drug products can be attributed not only to the drug substance or inert ingredients used for formulating a drug product; but they can also be brought into the drug product through the formulation process or by contact with packaging of the various impurities that can be found in drug products.

"Any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product." (ICH Q6A: Specifications).[1] It is important to give greater consideration to these detrimental impurities. In general, most of these impurities are small molecules. This is especially true in solid dosage forms where the limited mobility restricts the reactivity of larger molecules. For most drugs, the reactive species consist of water (which can hydrolyze some drugs or effect the dosage form performance), small electrophiles (e.g., aldehyde and carboxylic acid derivatives), peroxides (which can oxide some drugs), and metals (which can catalyze oxidation and other drug degradation pathways). Additionally, some impurites can cause toxicological problems. The presence of these unwanted chemicals, even in small amounts, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now receiving critical attention from regulatory authorities. The different pharmacopoeias such as BP (British pharmacopoeias), USP (United States pharmacopoeias), IP (Indian pharmacopoeias), and so on, are slowly incorporating limits to the allowable levels of impurities present in active pharmaceutical ingredients (APIs) or formulations. The large number of compounds under investigation in drug discovery presents a significant analytical challenge for the detection, quantitation, and characterization of the compounds alone.[2] Here, in Figure 1, we have summarized all classes of impurities.

Address for correspondence
Dr. Harish K Chandrawanshi, Department of Pharmaceutics, Saraswati Nagar, Vidisha - 464 001, M.P., India.
E-mail: hchandrawanshi@gmail.com

SOURCES OF IMPURITY IN MEDICINES

According to the International Conference on Harmonization (ICH) guidelines, impurities associated with APIs are classified in different ways.

Organic Impurities

Organic impurities are the most common impurities found in every API unless proper care is taken in every step involved, throughout the multi-step synthesis. 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. In a paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for one manufacturer or be an intermediate for others [Figure 2].

Oxidative degradation

Hydrocortisone, methotrexate, adinazolam, hydroxyl group directly bonded to an aromatic ring (e.g., phenol derivatives such as catecholamines and morphine), conjugated dienes, heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (e.g., flavones) are all susceptible to oxidative degradation.

Decarboxylation

Some dissolved carboxylic acids, such as p-aminosalicylic acid, lose carbon dioxide from the carboxyl group when heated, in the case of photoreaction of rufloxacin.[3]

Hydrolysis

Hydrolysis is a common phenomenon for the ester type of drugs, especially in liquid dosage forms. Examples include benzyl penicillin, barbitol, chloramphenicol, chlordiazepoxide, lincomycin, ethyl paraben,[4] and cefpodoxime proxetil.[5] Moreover, the hydrolysis scheme of benzocain has been depicted in Figure 3.

PHOTOLYTIC CLEAVAGE

Pharmaceutical products are exposed to light while being manufactured as a solid or solution, and then they are packaged. Most compounds will degrade as solutions when exposed to high energy UV exposure (Ergometrine,[6] Nifedipine,[7] riboflavin, and phenothiazines are very labile to photo-oxidation.). Fluoroquinolones antibiotics are also found to be susceptible to photolytic cleavage.[8] In ciprofloxacin eye drops, the photocleavage reaction produces the ethylenediamine analog of ciprofloxacin.[9]

Enantiomeric Impurities

The single enantiomeric form of a chiral drug is now considered as an improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index, with a more favorable adverse reaction profile.

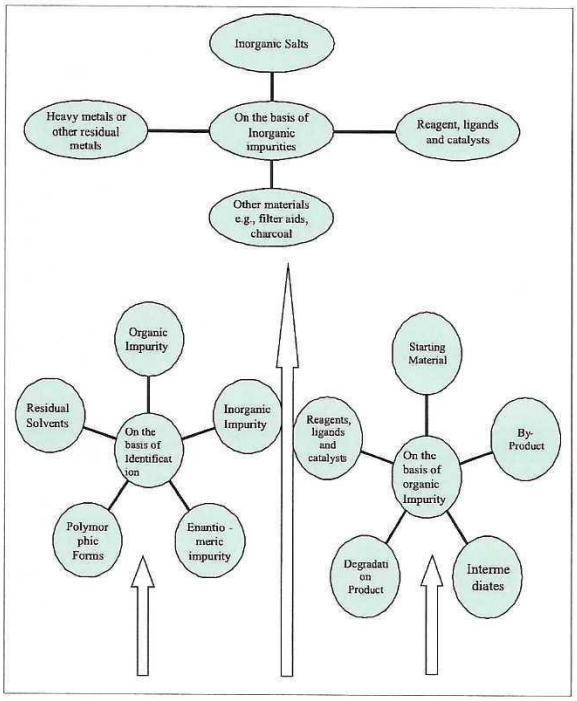

Figure 1: Flow chart depicting various kinds of impurities

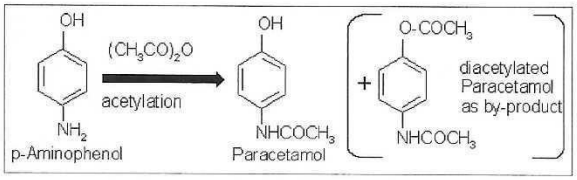

Figure 2: Production of paracetamol from intermediate, p-Aminophenol

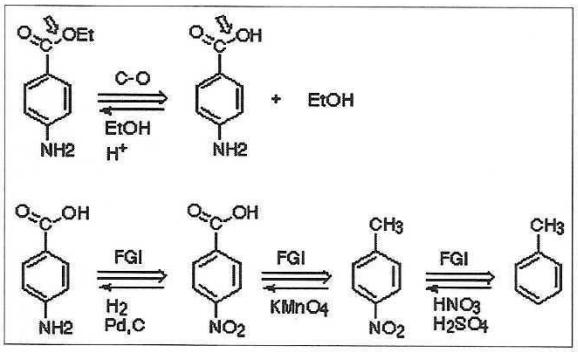

Figure 3: Ester hydrolysis of benzocaine

However, the pharmacokinetic profiles of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of a single isomer in this regard. For the manufacturers of a single enantiomeric drug (eutomer), the undesirable stereoisomers

in drug control are considered in the same manner as other organic impurities.[10]

Inorganic Impurities

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., filter 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.

In-Process Production Impurities

Crystallization related impurities

Impurity can be any substance other than the material being crystallized. Therefore, even the solvent from which the crystals are grown can be considered as an impurity. When impurities are added specifically to produce a desired morphological effect they are referred to as additives. The presence of impurities or additives in a crystallization system can have a radical effect on crystal growth, nucleation, and agglomeration, asn also on the uptake of foreign ions in the crystal structure.[11]

Stereochemistry related impurities

It is of paramount importance to look for stereochemistry related compounds; that is, those compounds that have a similar chemical structure, but different spatial orientation. These compounds can be considered as impurities in the APIs. The single enantiomeric form of a chiral drug is now considered as an improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index, with a more favorable adverse reaction profile, for example, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, other examples are levofloxacin (S-ofloxacin), esomeprazole (S-omeprazole), and lavalbuterol (R-albuterol).[10]

Solvents remain after processing

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs.[12] Depending on the possible risk to human health, residual solvents are divided into three classes [Table 1].

Synthetic intermediates and by-products

Impurities in pharmaceutical compounds or a new chemical entity (NCE) can originate during the synthetic process, from raw materials, intermediates, and / or by-products. Impurity profiling of tablets by GC-MS and MDMA (3, 4-Methylene dioxy methamphetamine) samples produced impurities in the intermediates via the reductive amination route.[13]

Impurities generated during storage

A number of impurities can originate during storage or shipment of drug products. It is essential to carry out stability studies to predict, evaluate, and ensure drug product safety.[14]

Metal impurities

Metal acts as an impurity in the APIs and excepients. Metals can be divided into three classes, as mentioned in Table 2.[15]

Leachables / Extractables

Regulatory, safety, and scientific considerations in evaluating extractables and leachables is important, along with strategy studies, for analytical identification, quantification, and monitoring.[16]

ICH Guidelines

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

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

Table 1: Classification of solvents on the basis of their limit in parts per million (ppm)

Category	Name of the solvent/limits	Unit/specification
Class I	Benzene (2 ppm), carbon tetrachloride (4 ppm), methylene chloride (600 ppm), methanol (3000 ppm, pyridine (200 ppm), toluene (890 ppm)	More than this should be avoided
Class II	N, Ndimethylformamide (880 ppm), acetonitrile (410 ppm)	More than this should be avoided
Class III	Acetic acid, ethanol, acetone (50 mg)	Have permitted daily exposure of 50 mg or less per day, as per the ICH guidelines

Table 2: Classification of metals on the basis of their safety concern

Category	Examples
Class I (metals of significant safety concern)	Ir (iridinium), Pt (platinum), Rh (rhubedium), Mo (molibidnum), V (vanadium), Cr (chromium), and Ni (nickel)
Class II (metals with low safety concern)	Cu (copper) and Mn (manganese)
Class III (metals with minimal safety concern)	Fe (iron) and Zn (zinc)

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[17]

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,

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)

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.

Column Chromatography

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms to kilograms. The classical preparative chromatography column is a glass tube with a diameter of 50 mm and a height of 50 cm to 1 m with a tap at the bottom. Two methods are generally used to prepare a column; the dry method and the wet method. The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute one at a time. During the entire chromatography process the eluent is collected in a series of fractions. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds, for example, by analytical chromatography, UV absorption or fluorescence. Colored compounds (or fluorescent compounds, with the aid of an UV lamp) can be seen through the glass wall as moving bands.

Flash Chromatography

Distillation, re-crystallization, and extraction are all important techniques for the purification of organic compounds. However, the technique used most commonly in modern organic research is 'flash' chromatography. In traditional column chromatography the sample to be purified is placed on top of a column containing some solid support, often silica gel. The rest of the column is then filled with a solvent (or a mixture of solvents), which then runs through the solid support under the force of gravity. The various components to be separated travel through the column at different rates and are then collected separately as they emerge from the bottom of the column. Unfortunately, the rate at which the solvent percolates through the column is slow. In flash chromatography, however, air pressure is used to speed up the flow of the solvent, dramatically decreasing the time needed to purify the sample.[22]

Thin Layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide or cellulose. This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. As different analytes ascend the TLC plate at different rates, separation is achieved.

Thin layer chromatography finds many applications to determine the components that are contained in plants. It is also used for monitoring organic reactions and analyzing ceramides and fatty acids; for the detection of pesticides or insecticides in food and water; for analyzing the dye composition of fibers in forensics and identifying compounds present in a given substance, and for assaying the radiochemical purity of radiopharmaceuticals [Figure 4]. A number of enhancements can be made to the original method, to automate the different steps, to increase the resolution achieved with TLC, and to allow more accurate quantization. This method is referred to as HPTLC or 'high performance TLC'.

Gas Chromatography

Gas–liquid chromatography (GLC) or simply gas chromatography (GC), is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.[23]

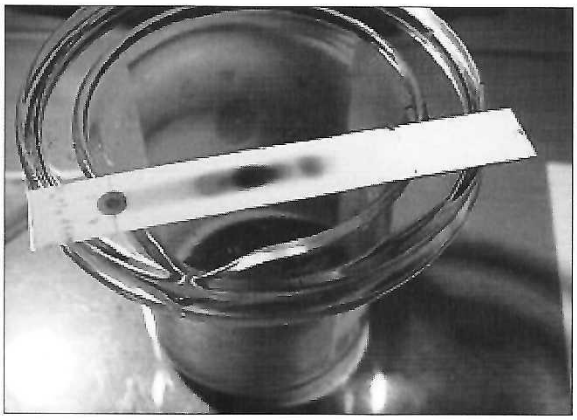

Figure 4: Separation of different chemical constituents by TLC

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