Genotoxic and non-genotoxic impurities in pharmaceuticals

Current regulations, status and trend

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ABSTRACT *Drug Substance (DS) process development and Drug Product (DP) formulation development are two major areas of the drug development process. Impurities/degradants can be generated in either of the processes, from DS degradation or DS-excipient interaction. These impurities may be non-genotoxic or genotoxic in nature. Regardless, they are regulated by Food and Drug Administration (FDA)/ International Conference on Harmonization (ICH) guidelines. Routine impurity analysis in pharmaceuticals requires identification at levels of 0.05 percent to 0.2 percent depending on the daily dose. However, genotoxic impurities can be much harder to detect due to their presence at low ppm levels. This review concentrates on the regulations and analytical technologies used to detect and quantitate impurities (genotoxic and non-genotoxic) in pharmaceuticals.*

INTRODUCTION

Drug Substance (DS) development and Drug Product (DP) development are two major areas of pharmaceutical development. Impurities can be observed in either of the process and they can be organic impurities, organic volatile impurities (e.g. residual solvents), or inorganic impurities (e.g. metals, mainly in DS development process).
During the DS development process, impurities can be generated from the synthetic process or as a result of degradation. During DP development, impurities can be generated as either degradation products, as a result of drug-excipient interaction, via external contamination or from packaging components.

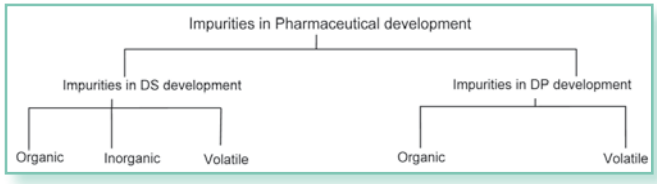

Figure 1. The nature of impurities in pharmaceutical development.

Profiling of impurities in either development process is governed primarily by ICH and FDA guidelines. The quality guidelines provided by ICH, represent one of the most rigorous and thoroughly written manuals for pharmaceutical development. It is very important to understand the regulations provided by FDA and ICH on impurity profiling before discussing analytical methodologies that can be used to profile these impurities. For the sake of clear discussion, the impurities are grouped into two wide areas: non-genotoxic and genotoxic impurities. All the FDA and ICH guidelines discussed below are mainly applicable for non-genotoxic impurities. For almost all the guidelines on impurities, if the word "genotoxic" is not present, the guideline is only applicable to non-genotoxic impurities.

FDA GUIDELINES ON IMPURITIES

US FDA provides several guidelines, which are of importance relative to impurity profiling for drug development of NDA's (New Drug Application) and ANDA's (Abbreviated New Drug Application).
The following two guidelines have particular importance:

a) *Guideline for Industry, NDA: Impurities in Drug Substances* (February 2000): This document provides guidelines on NDA applications and Type II Drug Master Files that support an NDA application. It is important to remember that this guideline does not apply for biological and herbal products.
b) *Guidance for Industry, ANDAs: Impurities in Drug Substances* (February 2005): This document is for generic drug applications and it outlines the information to be submitted in the Chemistry, Manufacturing and Control (CMC) section e.g. reporting, identification and qualification of impurities in drug substances and degradation products in drug products.

Both of these FDA guidelines refer to ICH guidelines Q3A, Q3B and Q3C.

ICH GUIDELINES ON IMPURITIES

The guidance on impurities can be found under *Quality guidance*. There are three main guidances as given below.

a) Impurities in New Drug Substances Q 3A (R2): The objective of this guideline is to recommend acceptable amounts for impurities in a drug substance based on safety concern for the patient. The latest revision (Rev 2) was adopted in October 2006. Revision 2 divides impurities in DS into three separate groups (i) Organic impurities (ii) Inorganic impurities and (iii) Solvents.
According to this document, organic impurities can be starting materials (SM), by-products, intermediates, degradation products, reagents, ligands and catalysts.

Maximum Daily Dose	Reporting Threshold	Identification Threshold	Qualification Threshold
≤ 2g/day	0.05 percent	0.10 percent or 1.0 mg per day intake (whichever is lower)	0.15 percent or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03 percent	0.05 percent	0.05 percent

Table 1. Reporting, identification and qualification thresholds for impurities in DS.

Reporting Thresholds

Maximum Daily Dose	Threshold
≤ 1 g	0.1 percent
> 1 g	0.05 percent

Identification Thresholds

Maximum Daily Dose	Threshold
< 1 mg	1.0 percent or 5 µg TDI, whichever is lower
1 mg - 10 mg	0.5 percent or 20 µg TDI, whichever is lower
>10 mg - 2 g	0.2 percent or 2 mg TDI, whichever is lower
> 2 g	0.10 percent

Qualification Thresholds

Maximum Daily Dose	Threshold
< 10 mg	1.0 percent or 50 µg TDI, whichever is lower
10 mg - 100 mg	0.5 percent or 200 µg TDI, whichever is lower
>100 mg - 2 g	0.2 percent or 3 mg TDI, whichever is lower
> 2 g	0.15 percent

Table 2. Reporting, identification and qualification thresholds for impurities in DP where TDI= Total Daily Intake.

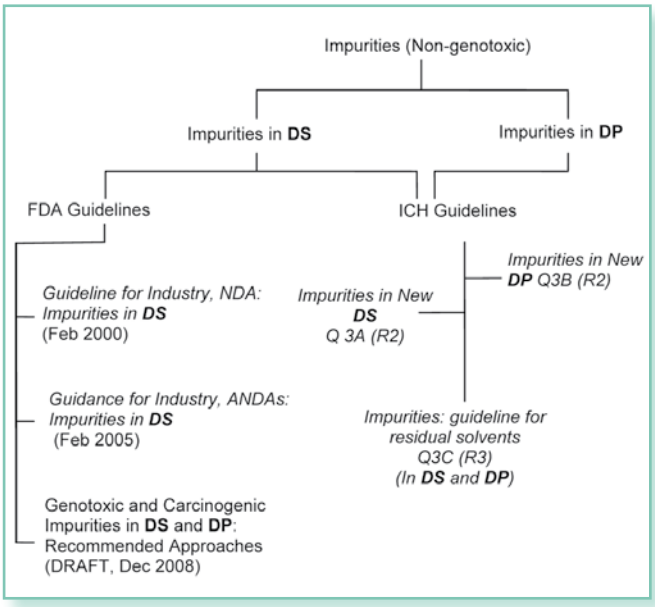

Figure 2. FDA/ICH guidelines as they are related to DS and DP.

The reporting of these impurities can be complicated and a rationale should be included in applications for each specified identified impurity, each specified unidentified impurity and total impurities. Impurities less than one percent must be reported to two decimal places (0.XY percent).

Table 1 shows the current guidance on the level of impurities in DS's:Inorganic impurities can be reagents, ligands, catalysts, heavy metals or other residual metals, inorganic salts, or other materials (e.g. filter aids, charcoal). There is no clear guideline on inorganic impurities in this guidance. It is stated that the acceptance criteria should be based on pharmacopoeial standards or known safety data. However, there is a European Proposal from the Committee for Medicinal Products for Human Use (CHMP) guideline on the specification of inorganic impurities effective from September 2008 (3). The objective of the CHMP guideline is to recommend maximum acceptable concentration limits for the residues of metal catalysts or metal reagents that may be present in pharmaceutical substances or in drug products. Solvents are defined in this guideline as "inorganic or organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a new drug substance". The guidance for organic solvents is discussed in section Q 3C.

b) Impurities in new drug products Q3B (R2): The objective of this guideline is to recommend acceptable amounts for impurities in drug products for the safety of the patient. The latest version (Rev 2) was adopted in June 2006. This document is applicable for impurities in new drug products produced from chemical synthesis only. This guideline addresses only those impurities in new drug products classified as degradation products of the drug substance or reaction products of the drug substance with an excipient and/or immediate container closure system. Impurities present in the DS, need not be monitored in the drug product unless they are a degradation product as well. Any degradation product observed in stability studies conducted at the recommended storage condition should be identified when present at a level greater than (>) the identification thresholds given below. When identification of a degradation product is not feasible, a summary of the laboratory studies demonstrating the unsuccessful efforts to identify it should be included in the registration application.

c) *Impurities: guideline for residual solvents Q3C (R3)*: The latest version Rev 3 is incorporated in Nov 2005. The objective of this guideline is to recommend acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. Solvents are subdivided into three groups: Class I, class II and class III. Class I solvents have high toxicity and should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment. Class II solvents should be limited in order to protect patients from potential adverse effects. Less toxic Class III solvents should be used where practical. The list of each class of solvents is given in this guidance with thorough examples of the calculations.

GENOTOXIC IMPURITIES

Genotoxic impurities are chemicals that harm an organism by damaging its genetic material (DNA), these materials could cause somatic mutation or be carcinogenic (4). To the best knowledge of the authors of this paper, ICH has not provided any specific guidance on threshold or limits for genotoxic materials. However there exists a "safety" document revision of the ICH S2 Guidelines dated March 2008: "Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals" (S2A) and "Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals" (S2B). These guidelines are mainly for testing of pharmaceuticals for genetic toxicity. The purpose of the ICH S2 A and B revision is to achieve the following goals:

1) Reduce the numbers of animals used in routine testing by improving the current procedures (limitation in the number of animals used as positive controls) and clarifying the follow-up testing in case of positive findings.
2) Avoid or more adequately manage/interpret any irrelevant findings in order to reduce barriers in early drug development by improving risk assessment for carcinogenic effects that have their basis in changes in the genetic material.
3) Update and improve internationally agreed upon standards for follow-up testing and interpretation of positive results, especially from in vitro assays, in the standard genetic toxicology battery.

There are a few other documents available from sources including:

1) European Proposal from the Committee for Medicinal Products for Human Use (CHMP)
2) Pharmaceutical Research and Manufacturers of America (PhRMA) group
3) Guidance from FDA (Draft December 2008)

As per CHMP, genotoxic impurities can be distinguished into two classes (5):

a) Genotoxic compounds with sufficient (experimental) evidence for a threshold related mechanism: For these compounds, exposure levels can be calculated according to the procedure outlined for class II solvents in the ICH Q 3C guidance.
b) Genotoxic compounds without sufficient (experimental) evidence for threshold related mechanism: For these compounds, the threshold levels should be guided by the ALARP principle (As low as reasonably practicable). In this regard, The Threshold of Toxicological Concern (TTC) is defined and it allows a maximum intake of 1.5 µg /day of any one genotoxic impurity over a patient's life time. The concentration limit in ppm of a genotoxic impurity in a drug substance derived from the TTC, can be calculated based on the expected daily dose to the patient using the following equation:

Concentration limit (ppm) = TTC [µg/day]/dose (g/day]

The TTC concept should not be applied to carcinogens where adequate toxicity data (long-term studies) are available and allow for a compound-specific risk assessment.

There is a high correlation (~90 percent) between structural alerts to DNA reactivity. Genotoxic chemicals that act as an electrophile covalently bind to nitrogen and oxygen atoms in DNA. Well-documented examples of electrophilic structural alerts include carbonium ions, nitrenium ions, epoxides, oxonium ions, aldehydes, Michael acceptors (alpha-beta unsaturated ketones), peroxides, free radicals and acylating reagents. Different degrees of predictivity apply to each alert e.g. alpha-beta unsaturated ketone toxicity is highly dependent on the precise structure. In the white paper, published by PhRMA group, impurities were classified into five groups with respect to the genotoxic potential (6).

- class 1: "Impurities known to be genotoxic and carcinogenic", they are known to be animal carcinogens with reliable data for genotoxic mechanism and also known to be human carcinogens.
- class 2: "Impurities known to be genotoxic (mutagenic), but with unknown carcinogenic potential", they have demonstrated mutagenicity in conventional genotoxic tests, but have unknown carcinogenic potential.
- class 3: "Alerting structure, unrelated to the structure of the API and of unknown genotoxic potential", they are based on the generic rules of functional moieties that can be linked to genotoxicity based on structure, however they have not been tested as isolated compounds.
- class 4: "Alerting structure related to the API", they are impurities that contain an alerting functional moiety that is shared with the parent structure.
- class 5: "No alerting structure or sufficient evidence for absence of genotoxicity" they are covered by existing ICH Q3 A, B, and C guidelines.

FDA's approach on genotoxic impurities was published by McGovern et al. (7) before the official draft guideline published in December 2008. According to the publication (7), every effort should be made to prevent the formation of genotoxic impurities during DS synthesis or DP manufacture and to reduce them by purification steps. When identified at levels below the ICH qualification limit, impurities can be evaluated by structure activity relationship (SAR) for genotoxicity. The evaluation of genotoxicity includes a review of published literature and application of appropriate software e.g. MCASE (Multi Computer Automated Structure Evaluation), DEREK (Deductive Estimation of Risk from Existing Knowledge). Impurities with an identified structural alert, need to undergo genotoxic testing.

The official FDA draft guideline entitled "Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches" was published in December 2008 (1). The intention of the draft guideline is to be an adjunct to the ICH guidelines Q3 A, B and C. CHMP proposed guideline (2006) on genotoxic impurities (5) is mentioned in this FDA document. Recommendations from the CHMP document are acceptable for exposure threshold limits for clinical development and marketing applications. FDA recommended approaches for initial assessment of genotoxic potential is similar to that mentioned in the literature above (7). The recommended approach for handling genotoxic and carcinogenic impurities is either prevention or reduction of those impurities. For a marketing application, any impurity lower than 1.5 µg/day does not need further safety qualification. A threshold of 0.15 µg/day was recommended for a selected class of compounds. This document also provides guidance on genotoxic impurities during the clinical development process. The recommended impurity threshold for a clinical study lasting up to 12 months was 0.15 µg/day for safety reasons since subjects are usually healthy. For trials greater than 1-year duration, the recommended threshold value was 1.5 µg/day since subjects are more certain to derive benefits. The recommended approaches based on the development stage are given below:

Clinical Development Stage	Recommended Approach
IND	• Evaluate identified impurities for genotoxic and carcinogenic risk via SAR assessment • Conduct assay for the presence of anticipated genotoxic and carcinogenic impurities • If impurity with genotoxic and carcinogenic potential is identified: − Modify synthetic pathway to eliminate the impurity, if possible OR − Conduct genotoxicity assays to characterize the genotoxic potential if not already known AND / OR − Set specification to that associated with a potential daily impurity exposure supported by compound-specific risk assessment or relevant qualification threshold (Table 1)
Marketing application (NDA, BLA, or ANDA)	• Evaluate identified impurities for genotoxic and carcinogenic risk via SAR assessment • If impurity with genotoxic and carcinogenic potential is identified: − Conduct genotoxicity assays to characterize the genotoxic potential if not already known AND / OR − Set specification to that associated with a potential daily impurity exposure supported by compound-specific risk assessment or 1.5 µg per day threshold

Table 3. Recommended approach for genotoxic impurities based on the development stage.

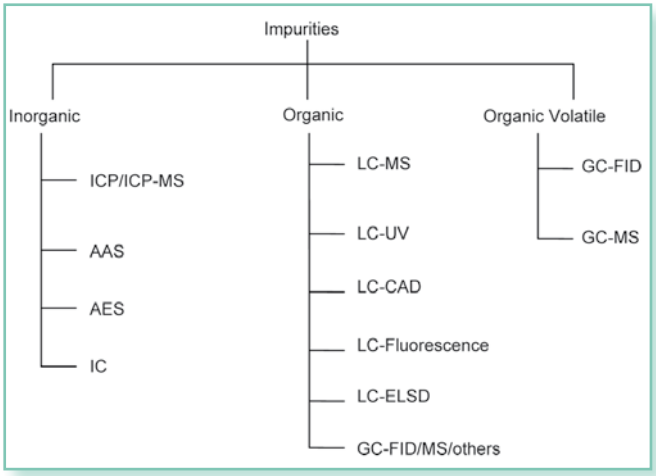

Figure 3. Common analytical techniques used for impurity analysis according to their origin.

ANALYTICAL METHODOLOGIES FOR IMPURITY ANALYSIS

As discussed above, impurities found in the DS or DP can be sub grouped into Figure 3.

The analytical methodologies applied to most inorganic impurities are ICP and ICP-MS, AAS, AES, and IC. For organic volatile impurities, GC is a very well known technique. Of all these, the profiling of organic impurities can be most challenging. If a genotoxic material is expected to be present in the DS or DP, quantitative analytical methodology is needed. In this article, we will concentrate on the analytical profiling of genotoxic materials since there are numerous literature references available for quantitative and qualitative analysis of non-genotoxic impurities. There are also excellent reviews available on the topic of impurity analysis (8-10). For quantitative analysis, LC-UV and LC-MS (or MS/MS) are very widely used. For impurities where a suitable chromophore is not present, a derivatization can be done to make it UV active. For qualitative analysis like structure elucidation, LC-MS with MS(n) or LC-NMR are very powerful techniques (11, 12). With the easy availability of high resolution or exact mass instrumentation, MS is often used for preliminary identification work. Online deuterium exchange methodologies (13) can be used to distinguish between similar structures and isobaric masses. For the separation of enantiomeric impurities, chiral chromatography is employed. For the complete identification of an unknown impurity, semi-preparatory scale isolation or synthesis work is often carried out to get orthogonal information (14). Solid phase extraction, column chromatography and solvent extraction can be useful in cases.

Genotoxic impurities, however, pose a special challenge because of their lower detection limits. In most cases, the amount of impurity to be reported is in the range of 1 to 20 ppm. For a non-genotoxic impurity, if the reporting limit is 0.1 percent, the equivalent ppm level is 1000 ppm. That means to get to 1 ppm level, (often needed for the genotoxic impurities), the sensitivity increase required would be 1000 times from the regular impurity methods. So other techniques, especially LC-MS, GC-FID, GC-MS and LC-Fluorescence are very valuable tools in the analysis of genotoxic compounds. Current examples of genotoxic impurity analysis include LCMS derivatization for trace level alkyl sulfonate analysis (15). The sensitivity of that method was determined to be of a low ppm level (1-2 ppm). A similar review on the analysis of organo halide alkylating agents, was reported by Elder et al. (16). GC with various detection methods was found to be very suitable for most of the alkyl halide analyses at the trace level. A simple isocratic RP HPLC method for the analysis of alkyl benzene sulfonate was reported by Raman et al. (17). However, the usability of that method is questionable if the impurities are present at smaller amounts in the presence of a DS. Different approaches for quantitation on a case-by-case basis using various detection techniques such as UV, ELSD,

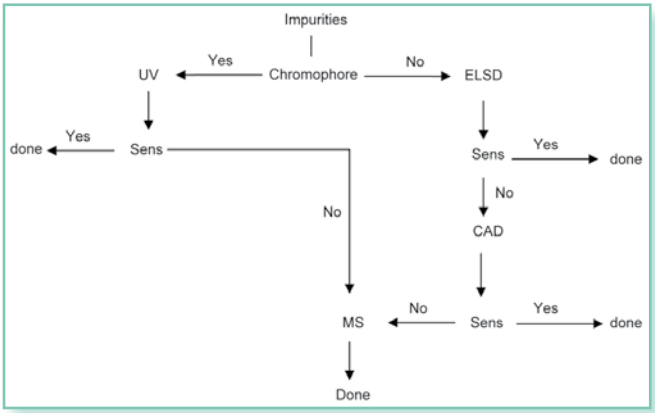

Figure 4. Decision tree for analytical tools, which may be used for profiling of genotoxic impurities (17), where Sens is sensitivity.

CAD and MS were discussed by Yuabova et al. (18). They have also described a tree for evaluating different analytical techniques for the quantitative analysis of genotoxic compounds. The tree is given in Figure 4.

CONCLUDING REMARKS

Impurity analysis in DS and in DP is still very challenging, especially with increased regulatory concern around genotoxic impurities. Whereas in most cases, LC-UV is able to quantify impurities which are non-genotoxic, LC-MS or other techniques may be necessary for the quantitation of genotoxic impurities. It is not the lack of chromophores but the lower detection limit at ppm or ppb level that is responsible for choosing other detection techniques over UV. The analysis of genotoxic impurities in DP can be more challenging than with DS, simply because of the matrix issue and much lower sensitivity limit needed while working with DP. There needs to be a more defined regulatory approach on dealing with genotoxic impurities, especially when removing them is not possible.

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