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HOW DO YOU DETECT MUTAGENIC IMPURITIES AT PART-PER-BILLION LEVELS?

WHITE PAPER

The Challenge of Detecting Mutagenic Impurities

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Current guidelines, including ICH M7,[1] provide an overview of assessing and evaluating limits of pharmaceutical impurities suspected or classified as mutagenic impurities. These impurities may be associated with known added agents, environmental factors or degradation products from pharmaceutical compositions. Proposed limits for genotoxic impurities reside well below common impurities discussed in ICH Q3A[2] guidance and require analytical techniques capable of detecting and measuring ppm to ppb levels. This presentation provides an overview of analytical technologies for detecting mutagenic impurities.

INTRODUCTION

Investigational new drug development requires a demonstration of safety and efficacy. Over the last two decades the safety requirements for CMC have become more clearly defined. Specifically, evaluation of impurities of actives and drug products in relation to container closures, as well as manufacturing, are covered in the guidelines including ICH, regulatory agencies, and USP. The introduction of guidelines for trace metals and mutagenic impurities suggest rigorous control of impurities. The ICH M7 guidance outlines limiting carcinogenic risk by assessing possible mutagenic impurities in new drug substance and products. The primary challenge associated in measuring mutagenic impurities (MI) is often the need for low to very low-level detection limits.

Early industry articles and draft guidance often used terminology such a “genotoxic” and “carcinogenic” impurities, however the 2015 issued M7 guidance refers to mutagenicity. The significant difference is that a genotoxin may not be a mutagen and a mutagen is defined as follows:

Anything that causes a mutation (a change in the DNA of a cell). DNA changes caused by mutagens may harm cells and cause certain diseases, such as cancer. Examples of mutagens include radioactive substances, x-rays, ultraviolet radiation, and certain chemical.[3]

ASSESSING LEVELS OF MIs

Non-mutagenic impurities are typically evaluated in drug substances at levels above 0.05% weight/weight or relative peak area using standard detection techniques (ICH Q3A). Suggested threshold levels of MIs are determined by daily intake and dose duration. These limit MIs to less than 1.5 µg per day for a concentration of less than 10 ppm. Therefore, a detection technique of 70-fold lower may be needed, as profiled in Table 1. One way to view the introduction of MIs is to categorize from three primary sources with the detection complexities differing

Table 1: Comparing Q3A and M7 levels.

Standard Impurities			Mutagenic Impurities				
Table			Dose Duration				
Daily Dose [mg]	Q3A ID Threshold	Daily Intake [µg]	≤ 1 Mo.	> 1-12 Months	>1-10 Years	> 10 Tears to LT	TTC (µg) ICH M7
50	0.10%	50	120	20	10	1.5	
100	0.10%	100					
250	0.10%	250					
500	0.10%	500					
2,001	0.05%	1000.5					

It is clear from Table 1 and a little math, the Mutagenic Impurities to be quantitated may require much higher sensitivity than for standard Q3A impurities at the 0.05% level and at 30% TTC.

based on the source of the MI.

Thus, it is clear from Table 1 and a little math, the MIs to be quantitated may require much higher sensitivity than for standard Q3A impurities at the 0.05% level and at 30% TTC (Threshold of Toxicological Concern).

This paper discusses two of the three sources of mutagenic impurities: things that are added and things that may form in the matrix. Environmental MIs, also known as leachables, are not covered here, as these are typically analyzed in independently defined programs.

MIs THAT ARE ADDED

Finding "things that are added" is less complicated than "things that may form." Both require an initial assessment. For example, knowing that an acid chloride was added at step 3 of a 5-step synthesis, a sample is available, and detection characteristics can be extrapolated, suggests a straightforward process of detecting the MI. In addition, available toxicological data simplifies the assessment.

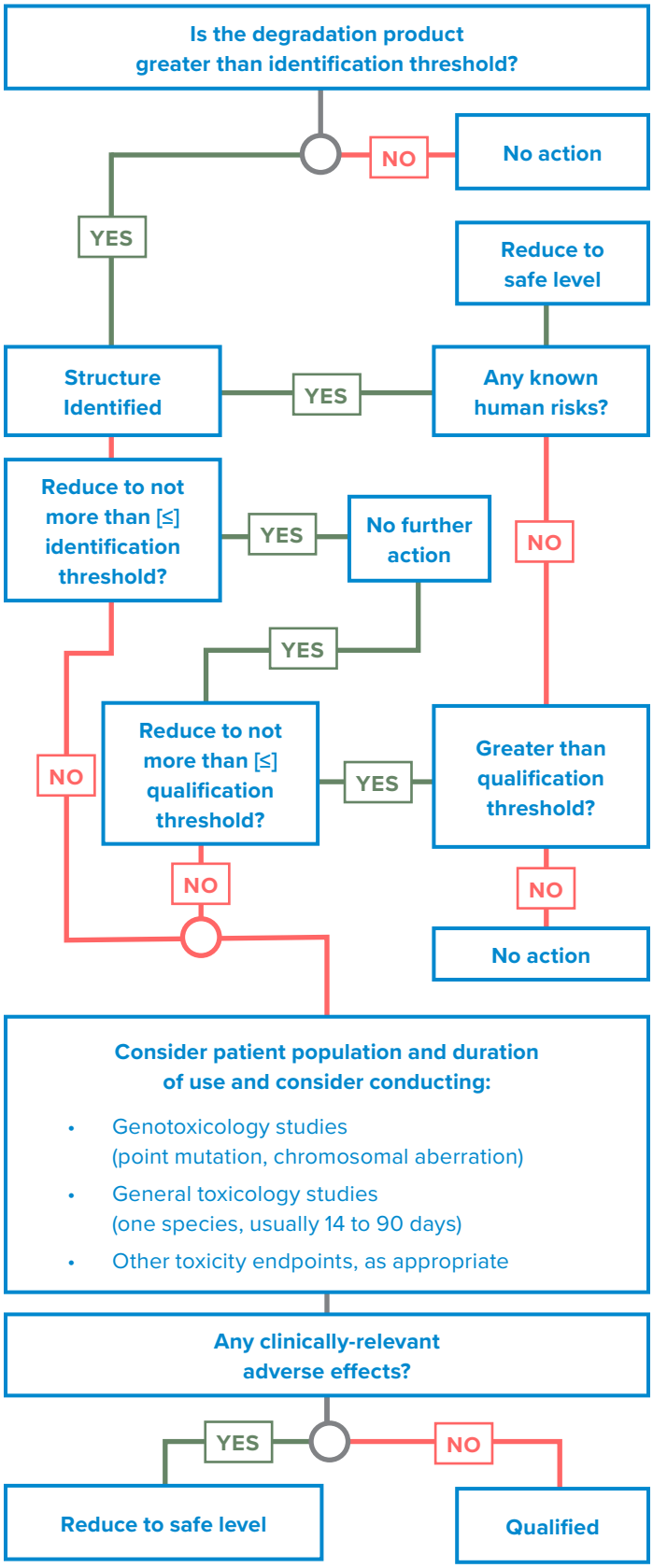
When the assessment requires evaluating the final drug substance or intermediate for the presence of the added MI, a separation technique and detection technique is evaluated. This raises some questions to consider:

- **Does my current analytical methodology detect the MI, and if yes, what is the detection limit?**
- **What is the desired detection or quantification limit based on TTC?**
- **Is the compound volatile?**
- **What is the expected ionization characteristic of the MI and its applicability to MS?**
- **How reactive is the MI and should derivatization be considered?**

In general, added MIs typically are of higher chemical reactivity and this should be considered during method development to assess stability of these reactive species when API is spiked into samples as part of accuracy. For example, alkyl halide MIs are known to react with amines and have been observed in GC headspace analysis to affect accuracy in recovery studies.

MIs AS DEGRADATION PRODUCTS OR FORMED FROM MATRIX OR PROCESS

More complex than "MIs That are Added" is the discovery of degradation products that alert for mutagenicity. If the Q3A(R2) process for impurity qualification or other information finds a degradation product with toxicological concerns, such as defined in Figure 1, additional efforts may be required. We find a subtle gap in both the Q3A(R2) decision tree and the note in the decision tree diagram stating, "Lower thresholds can be appropriate if the degradation product is unusually toxic." This addresses toxic degradation products but at the same time does not suggest the need for identification. The decision tree suggests an option to reduce the degradation product to less than the identification threshold, thus no further action is needed. However, the note's



Note: Lower thresholds can be appropriate if the degradation product is unusually toxic.

Figure 1: A Q3B(R2) decision tree for the identification and qualification of a degradation product

Detector	Scale
UV	1
Diode Array-DAD	7
CAD-Charged Aerosol	1.5
Light scattering (ELSD)	7
Refractive Index (10)	10
Electrochemical	0.1
Conductivity	2
Fluorescence	0.001
MS	1
MS Trap	0.0001

Table 2: General Sensitivity Overview-HPLC Detectors

GC Detection Type	Compounds	Approximate Detection Limits[1]
FID	Carbon compounds	0.1 ppm
ECD	Halogen, NO3,	0.1 bbp
FPD	S, P	10 ppb
TCD	Most	10 ppm
FTD	Nitrogen Organics	0.1- 1 bbp
	(phosphorous)	
MS/SIM (EI)	Most	100 ppt
MS (EI) SCAN	Most	10 ppb

[1]*Compound and sample concentration dependent*
Source: *http://www.shimadzu.com/an/gcms/support/faq/sensitivity.html*

Table 3: GC Detector Sensitivity[4]

mention of the unusual toxicity of the degradation product implies prior identification. That is, how does one assess toxicity of an unidentified degradation product? M7 is a better source for assessing mutagenic impurities than Q3A R2.

Consider an example of a worst-case scenario:

- **The M7-like assessment identifies a possible degradation product of concern in API or the corresponding drug product contains two actives and many excipients.**

Further studies may be considered such as purposeful stressing of drug substance to identify the presence of the alerting structure followed by in silico analysis, and a bacterial assay. Additional questions to ask in addition to the above in "MIs that are added",

- **Is isolation and/or synthesis of the degradation product required to confirm absolute structure, provide analytical reference material, and provide material for in vivo studies?**
- **Should this degradation product be monitored or evaluated in one's complex drug product such as part of long term stability studies?**

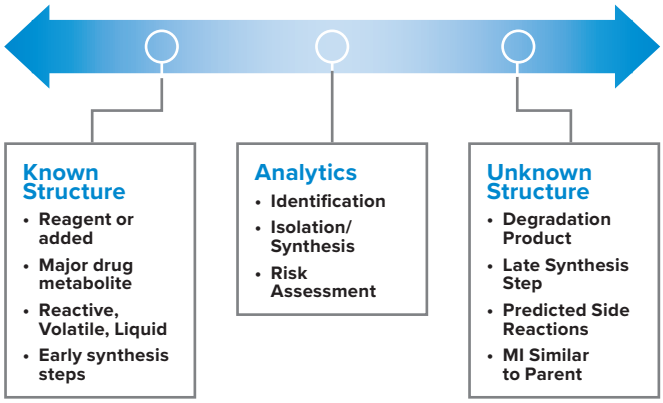
One situation that may arise is the presence of an in silico MI alerting functional group that is contained in the primary structure such as a substituted aniline. Clearly any proposed or known degradation product containing the aniline substructure would give an in silico alert. It is generally accepted if the parent molecule is shown not be mutagenic, then similar degradation products would follow this pattern. However, at least a risk assessment would be recommended. ICH suggests M7 is not applicable for advanced cancer drugs.

DETECTION TECHNIQUES

When we encounter the need to quantitate low level impurities, some options for detection prove more suitable than others. Table 2 profiles the general sensitivity of listed detectors where UV is arbitrarily assigned a value of 1 and the scale represents the relationship to other detectors. Thus, an electrochemical detector has a value of 0.1 or in general 10X more sensitive than UV. Note that these general sensitivities are very compound dependent.

Clearly mass spectrometry detection, as shown in Table 3, has superior sensitivity and the added advantage of identification potential. For example, a trap MS with single ion monitoring capability with instruments such as a Q Executive® Orbitrap allows for low level quantitation in a complex matrix and is very useful in both screening and/or monitoring MIs.

When assessing and possibly quantitating MIs, it is important to have input from synthesis, toxicology, analytical, and manufacturing experts to apply a compound specific strategy with continual evaluation through drug development.



SUMMARY

- **Three sources of MIs may arise from:**
  1. Things that are added *(includes in-process impurities)*
  2. Environmental Contamination
  3. Degradation products

- **Low level detection capabilities are often required for mutagenic impurity profiling**
- **Many techniques and detector options are available**
- **Newer MS technology is a useful tool for ID and quantitation**
- **The complexity of identifying and quantitating MIs is related to whether the MI is a known or an unknown entity, its compound properties, and the required level of detection.**

ABOUT THE AUTHOR

Harley Everett Wilcox brings 25 years of experience in drug research and development to EAG Laboratories. Prior to joining our team, he served in various technical and management positions with both large and startup pharmaceutical companies, including working as director of manufacturing responsible for CMC regulatory support and CRO/CMO outsourcing. He served as a CMC project leader for Anzemet® NDA, and has supported numerous IND's, CTA's, and CTX's as well as an ANDA. Having begun his career as an organic chemist, Wilcox's expertise includes analytical methods development and validation, isolation and identification of impurities in drug products, and invitro metabolic characterization supporting pre-clinical research; he also has assisted many small and virtual pharmaceutical companies with regulatory aspects of early CMC development programs, and has participated in collaborations, contracts, and intellectual property management in support of business development objectives. Wilcox is recipient of the Marion Laboratories presidential award for developing a high yield reclamation process for the active ingredient of a commercial formulation.

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