Chapter

Determination of Impurities in Pharmaceuticals: Why and How?

Kung-Tien Liu and Chien-Hsin Chen

Abstract

The presence of impurities, particularly the API-related impurities, i.e., degradation-related impurities (DRIs) and interaction-related impurities (IRIs), may affect the quality, safety, and efficacy of drug products. Since the regulatory requirements and management strategies are required to be established and complied, sources of impurities shall be carefully classified prior to take subsequent steps such as development of analytical methods and acceptance criteria. Current international regulatory requirements for the management of impurities in pharmaceuticals were reviewed. Procedures for the identification of DPIs in pharmaceuticals, i.e., ethyl cysteinate dimer, (R)-N-methyl-3-(2-bromophenoxy) -3-phenylpropanamine, sestamibi, etc., using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) were studied. Scheme for the establishment of analytical methods and acceptance criteria of process-related impurities (PRIs) and DRIs in accordance with the requirements of International Council for Harmonization (ICH) and algorithm to perform the identification of DPIs by using LC-MS/MS has been proposed. Practice of kinetic study to distinguish PRIs and DRIs, determination of the potential core fragments coupled with a predicted list of relevant transformations for conducting MS/MS scans, applications of stable isotope distribution patterns or natural abundances, practice of mass balance, etc., have been well demonstrated to justify the reliabilities of identification results.

Keywords: pharmaceutical products, impurities, regulatory requirements, analytical strategy, structural identification, validation, verification, LC-MS/MS, kinetic study, stable isotope distribution patterns

1. Introduction

As defined by the United States Pharmacopeial (USP), impurity is "any component of a drug substance that is not the chemical entity defined as the drug substance and in addition, for a drug product, any component that is not a formulation ingredient" [1].

Impurities in drug substance (i.e., active pharmaceutical ingredient, API) or drug product can arise due to synthetic/manufacturing processes, degradation, storage conditions, container, excipients, or contamination. They can be identified or unidentified, volatile or nonvolatile, organic or inorganic species [1–3].

Since different regulatory requirements and management strategies are required to be established and complied, sources of impurities shall be carefully classified

prior to take subsequent steps; for instance, to distinguish an impurity which is simply derived from API alone or actually derived from interaction products of API-excipient, excipient-excipient, or API-residual impurities existing in excipients [4–6].

Despite an increase in the research of impurities, a number of problems are still arisen in the development of identification technologies for degradation products and pathways. The first aim of this research is to address a brief review of the current major international regulatory requirements regarding the management of impurities in pharmaceutical products. Then secondly, a general scheme to establish an analytical method and acceptance criteria of degradation-related impurities (DRIs) and process-related impurities (PRIs) can be proposed, accordingly. Finally, our research will focus on developing a practicable algorithm to perform the identification of DPIs by using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS). Meanwhile, verification method for the justification of reliabilities regarding identification results will be assessed.

1.1 Classification of impurities

According to the definitions of International Council for Harmonization (ICH), Food and Drug Administration (FDA), and USP, impurities are classified into DRIs, PRIs, residual solvents, and heavy metals as shown in **Figure 1** [1, 2, 7].

Two types of impurities might be API-related. The first type of API-related impurities is generated by degradation of API itself under specific storage conditions, e.g., oxidation, dehydration, carbon dioxide removal, etc. The other type is occurred due to the interaction between API and excipients, container, or residual impurities in excipients, reagents, or solvents [8, 9]. API-related impurities are potentially genotoxic, mutagenic, and carcinogenic risk due to their structure-activity relationship (SRA) [10, 11].

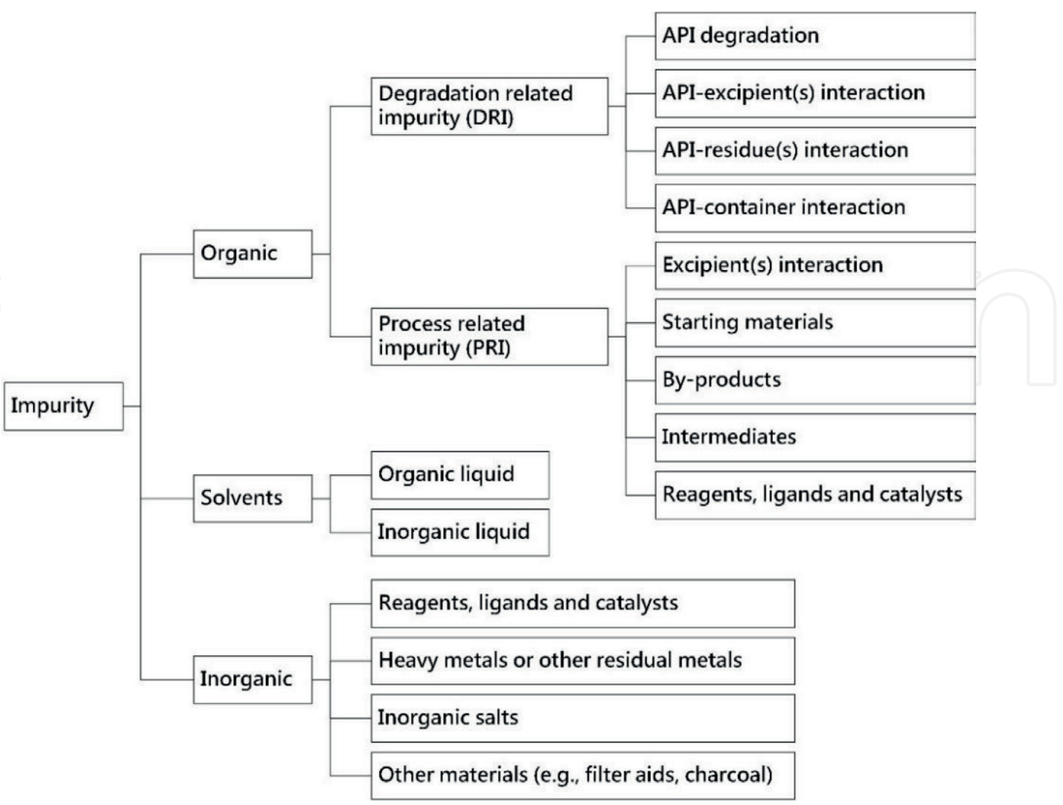


Figure 1.
Classification of impurities [1, 2, 7].

It is well known that excipients or the residual impurities in excipients can be very likely to cause instability of the API and drug product. A lot of impurities in excipients, such as presence of reactive peroxides or high water content in povidone or polyethylene glycols (PEGs), antioxidants in magnesium stearate, aldehydes in lactose, benzaldehyde in benzyl alcohol, formaldehyde in starch, lignin and hemicelluloses in microcrystalline cellulose were illustrated to demonstrate how reactive chemical entities are commonplace in excipients and incompatible to API. Some specific functional groups in API may be susceptible to degradation mechanisms, i.e., hydrolysis, oxidation, polymerization, etc. [4–6, 12–14].

Additionally, extractables and leachables such as initiators/catalysts, storage stabilizers, antioxidants, processing aids, light stabilizers, antistatic agents, colorants, lubricants associated with pharmaceutically relevant materials may also produce uncertain risks to the stability or quality of products [15].

Regardless of the classes of impurities, presence of impurities may have the potential to affect the quality, safety, and efficacy of drug products. Therefore, studies of impurities are one of the most important works in the development of APIs and drug products [1, 16, 17].

1.2 Aims to conduct impurity study

Study of impurities in pharmaceuticals is one of the most highly regarded topics; it is essential, but time consuming and challenging. In terms of regulations and technology, we must keep pace with the times [18, 19]. Comprehensively speaking, aims to develop an impurity study have two major directions as follows: regulatory requirements and scientific/technical demands (**Table 1**).

From the perspective of regulatory requirements, impurities may affect the quality of APIs and DPs and ultimately affect the safety of the patient. Views for the dealing of impurities may differ between biologists, toxicologists, and analytical chemists, and therefore need to be integrated [20]. Potential genotoxic impurities can be determined according to the published literature, results of gene mutation in bacteria, in vitro and in vivo tests of chromosomal damage in mammalian cells or rodent hematopoietic cells, or/and comparative structural analysis to identify chemical functional moieties correlated with mutagenicity [16]. Moreover, daily exposure, duration of exposure on the effects of degradation products and genotoxic impurities, and theoretical clinical dose, whereas potential

Regulatory requirements	Scientific and technical requirements
• Quality and safety of products • Method validation, i.e., specificity • Acceptance criteria determination • Expiry date, retest date, and shelf-life evaluation • Stability and storage conditions study • Threshold limits evaluation, i.e., threshold of toxicological concern (TTC), permitted daily exposure (PDE), etc.	• Synthetic and production processes optimization • Formulation development and optimization • Efficacy improvement • ADME and toxicology study • Manufacturing of reference materials • Stability improvement • DPIs and pathways prediction • Cost consideration

Table 1.
Examples of the aims to conduct impurity studies [20, 23, 25–27].

mutagenic impurities must be controlled to levels less than the threshold of toxicological concern based on lifetime exposure shall be evaluated as a risk consideration [16–18].

Adequate qualification must include genotoxicity and repeat-dose toxicology studies of appropriate duration to support the proposed indication. Moreover, other specific toxicity studies, e.g., embryofetal developmental toxicity study may be appropriate. Genotoxic impurities and degradation products pose an additional risk and should be controlled in accordance with the requirements of ICH M7(R1), unless they are qualified for safety [18, 21].

In addition to the regulatory requirements, internal and external scientific and technical needs are the second perspective to conduct an impurity study. Impurity determination and forced degradation studies are two of the basic requirements as a tool to predict potential DPIs, to develop analytical method, synthetic processes, and formulation, to receive a better understanding of storage conditions, stability of drug product, and to obtain information of degradation products/pathways, as well as to evaluate the specificity (selectivity) of assay method [22–25].

2. Regulatory requirements for the management of impurity

A number of international/local guidelines and guidances for the evaluation and control of impurities in drug substances and drug products have been published [1–3, 7–9, 21, 28–38]. Comparison of the application scopes in line with the impurity categories was drawn as indicated in **Figure 2**.

As said by the requirements of ICH Q3A(R2), all types of impurities present in API at a level greater than (>) the identification threshold must conduct studies to characterize their structures, no matter they are shown in any batch manufactured by the proposed commercial process or any degradation product observed in stability studies under recommended storage conditions. Specified identified impurities shall be included in the list of impurities along with specified unidentified impurities that are estimated to be present at a level greater than the identification threshold [2, 7, 33].

Briefly, five major steps for the management of degradation products, no matter they are degradation products of API or reaction products of API with excipient(s) or container closure system, have been requested by the ICH Q3B (R2) and summarized as follows [3]:

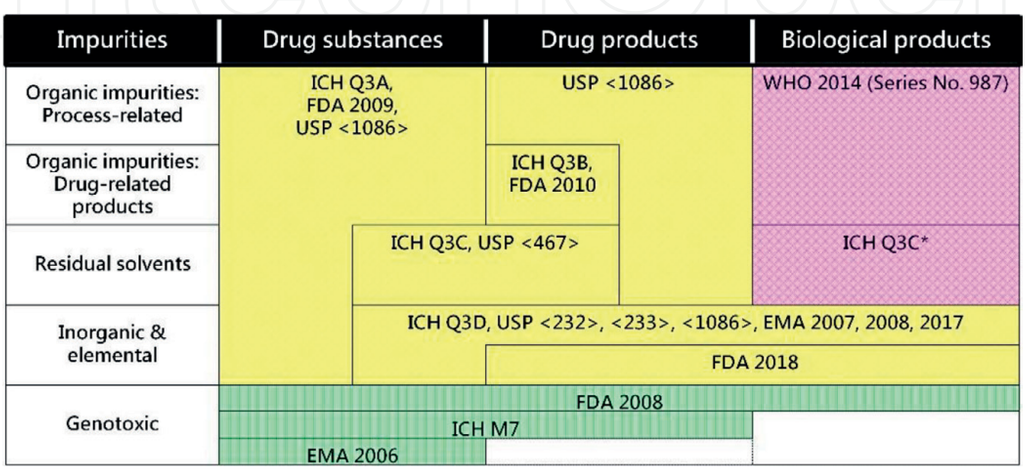


Figure 2.
*Comparison of the application scopes of regulatory guidelines/guidance for the management of impurities in pharmaceutical products [7, 28–34]. *Not clearly stated in the regulation.*

1. Confirm which impurities are degradation products?

2. Monitor and/or specify the amount of all degradation products.

3. Summarize all degradation products during manufacture and stability studies.

4. Elucidate and justify a rational evaluation of possible degradation pathway in the drug product or interaction with excipients or container closure system.

5. Establish specifications of all degradation products, including specified identified, specified unidentified, unspecified degradation product with an acceptance criterion of not more than (≤) identification threshold described in Q3B (R2), and their total amount.

Specificity (selectivity) of the method applied to determine specified and unspecified degradation product shall be validated. This includes subjecting of API or drug products to stress studies of light, heat, humidity, acid and base hydrolysis, and oxidation to evaluate the HPLC separation resolution, mass balance, etc. [3, 22, 24, 25].

Although Q3B (R2) was developed by ICH to provide guidance on impurities in drug products for new drug applications (NDAs), it is also considered to be applicable to the drug products of abbreviated new drug application (ANDAs) [33].

Regulation requirements regarding genotoxic, mutagenic, and carcinogenic impurities have been published and revised by European Medicine Agency (EMA), FDA, and ICH in 2006, 2008, and 2017, respectively, to describe how to perform assessments and controls, including prevention and reduction of impurities [21, 28, 32].

Concept of threshold of toxicological concern (TTC) has been developed to define an acceptable intake for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effects [21]. In general, exposure level of 1.5 μg per person per day (i.e., TTC) for each impurity can be considered as a common acceptable qualification threshold for supporting marketing application. Any impurity found at a level below this threshold generally does not need further safety qualification for genotoxicity and carcinogenicity concerns. The threshold is an estimate of daily exposure expected to result in an upper-bound lifetime risk of cancer of less than 10^{-6} (one in a million), a risk level that is thought to pose negligible safety concerns [21, 32].

Currently, ICH Q3C is the major guideline related to the management of residual solvents in API, excipients, and drug products (**Figure 2**). In general, solvents that are used in the manufacturing procedures are the required parts to determine [8]. Types of solvents are sorted according to their carcinogenic and genotoxic risks as follows [8, 37]:

1. Class 1: solvents obviously confirmed or strongly suspected to cause cancer in humans.

2. Class 2: nongenotoxic and possible carcinogenic risks in animals.

3. Class 3: low-toxic solvents.

Elemental impurities may arise from residual catalysts that were added intentionally in synthesis, or may be present as impurities, e.g., through interactions with processing equipment or container/closure systems or by being present in components of the drug product. Because elemental impurities pose toxicological concerns and do not provide any therapeutic benefit to the patient, their levels in drug

Figure 3.
Comparison for the classification of residues of metal and elemental impurities in pharmaceutical products by requirements of EMA and ICH Q3D [9, 29, 30].

products should be controlled within acceptable limits. Appropriate documentation demonstrating compliance for detailed risk assessment, screenings, and validation data for release methods must be conducted [9, 30, 34].

Recommended maximum acceptable concentration limits for the residues of metal catalysts or metal reagents that may be present in pharmaceutical products were issued earlier by EMA [29, 30]. Another classification of impurities, i.e., elemental impurities that the pharmaceutical industry needs to comply with is defined recently in ICH Q3D [9]. Comparison for these classifications of residues of metal or elemental impurities in pharmaceutical products defined by EMA and ICH was indicated as shown in **Figure 3**. Several significant difference of elemental safety concerns between EMA and ICH, such as Cr, As, Cd, Hg, Pb, etc., can be found.

3. Strategies to establish analytical methods and acceptance criteria of PRIs and DRIs

This chapter will be followed by a discussion of procedure to establish an analytical method and acceptance criteria of DRIs and PRIs.

Steps for the determination of potential degradation products, including a science-based risk assessment, can been addressed as below [11, 25]:

1. Stress studies of API.

2. Accelerated stability studies or kinetically equivalent shorter term stability studies.

3. Validation/verification by long-term stability studies of both the drug substance and formulated drug product.

An integrated scheme in accordance with the requirements of ICH for the establishment of analytical methods and acceptance criteria of PRIs and DRIs is proposed as demonstrated in **Figure 4** [2, 3, 17, 22, 39, 40].

In general, when an unknown peak was found, no matter it was found in a stress or stability studies of API or drug product, the first step is to distinguish the classification of unknown impurity belongs to. Different regulatory requirements of the

management for different kinds of impurities, i.e., PRIs and DRIs are required to apply. For instance, requirements of ICH Q3B(R2) and Q1A(R2) request that impurities present in API need not be monitored or specified in the drug product unless they are also degradation products. Due to the probability of degradation during storage period and are likely to influence quality, safety, and/or efficacy, degradation impurities must be included into the plan of stability studies [39]. Meanwhile, degradation impurities can ultimately determine the expiration, retest, or shelf-life periods of API and drug products, by evaluating the intersection of extrapolation-upper confidence limit and upper acceptance criterion of degradation product(s) [40]. Reporting threshold, identification threshold, and qualification threshold in the case of maximum daily dose ≤2 g/day of APIs administrated are illustrated in **Figure 4** [17].

Structure of impurities present in API at a level greater than (>) the identification threshold needs to be elucidated. An identified impurity content can be either

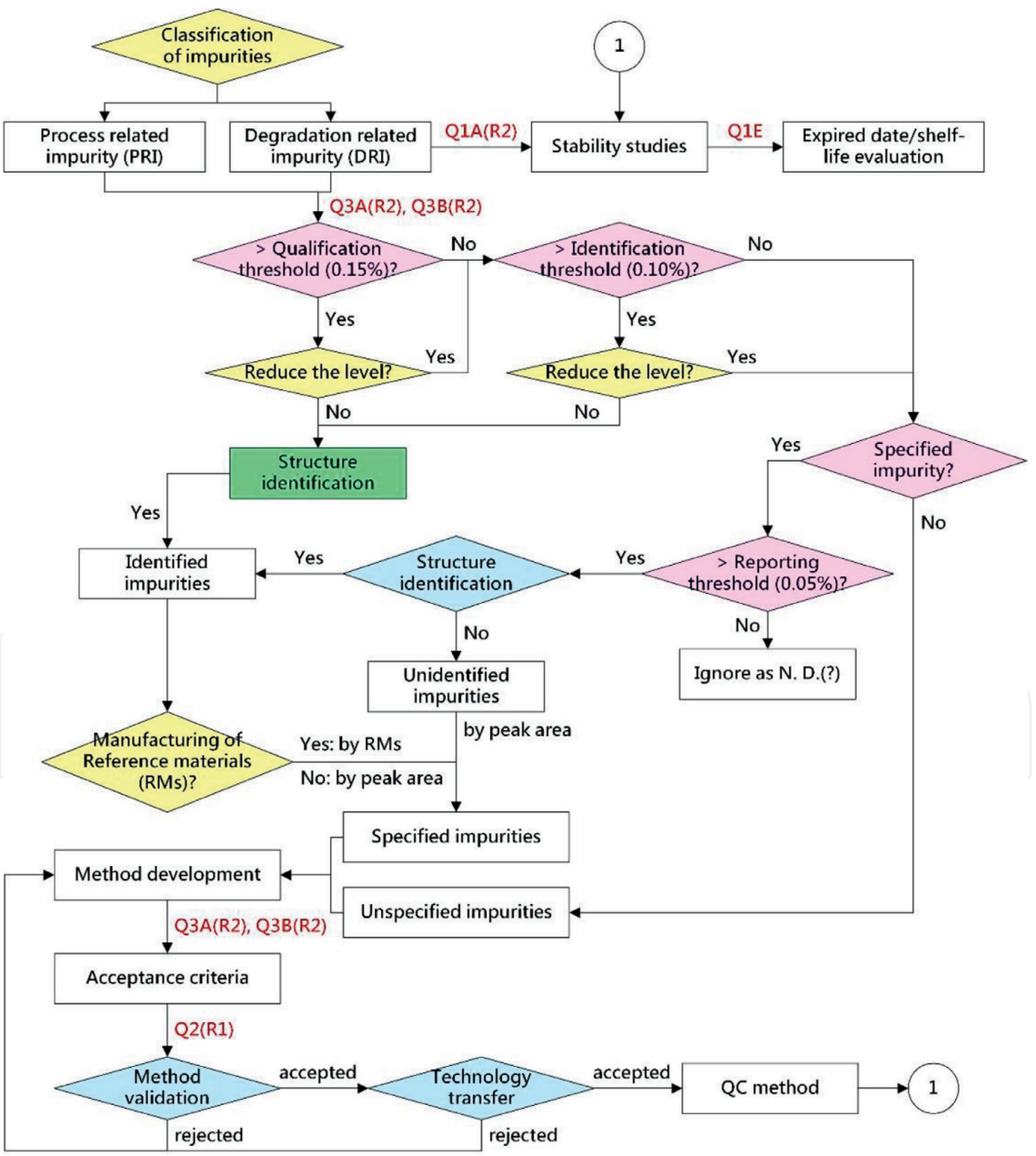


Figure 4.
Scheme to establish analytical methods and acceptance criteria of process-related impurities (PRIs) and degradation-related impurities (DRIs) according to the requirements of ICH guidelines [2, 3, 17, 22, 39, 40].

determined by interpolation with calibration curve of reference material or calculated using the peak area of the main component, i.e., API. In contrast, unidentified impurity content can only be determined using the peak area of API, no matter they are specified or unspecified impurities. Impurities with specific acceptance criteria are referred to as specific impurities, including identified and unidentified impurities [2].

Before conducting method validation, all of the impurities shall be verified by spiked or known addition to demonstrate they do exist under the "real" storage conditions such as accelerated or long-term storage conditions. Otherwise, it may not be necessary to examine specifically for certain degradation products if they are not formed under the "real" storage conditions [11, 25, 39].

The method for technology transfer to QC laboratory, i.e., receiving unit (RU) must be a well-validated and stability-indicating method. A method fails to pass the criteria of validation or technology transfer, investigation to clarify the root cause(s) and revalidation shall be initiated and conducted by the originating unit (OU) and approved by quality unit (QU).

4. Identification and validation of DRIs

4.1 Practice of kinetic study to distinguish PRIs and DRIs

Algorithms for the identification and verification of DRIs are proposed as indicated in **Figure 5**. Degradation reaction kinetics can be represented by a linear regression curve on an arithmetic or logarithmic scale [39]. Meanwhile, nature of degradation relationship is determined by the reaction kinetic constants and can be accordingly used to distinguish whether an impurity is DRI or PRI compound (**Figure 5**).

One example regarding how to distinguish PRIs and DRIs by kinetic study was illustrated as demonstrated in **Figure 6**. Analysis by HPLC revealed that some impurities were existed in one of our products. Kinetic study helps us to distinguish the type of impurities.

Plots of the impurity formation concentration ([A] or Ln[A]) versus time can obtain rate constant, i.e., the slope of a reaction in straight line as arithmetic (i.e., k_0) or logarithmic (i.e., k_1) scale. Furthermore, correlation coefficient (r) of linear regression analysis indicates a perfect positive correlation (r = 1) or conversely, there is no relationship between the two variables (r = 0).

The slopes and correlation coefficients of Pk#5, Pk#6, and Pk#7 indicated that they were not degradation-related products of API. But conversely, kinetic curves showed that Pk#1–4 and Pk#8 were degradation products. These results were also consistent with the findings of molecular weight results shown in LC-MS/MS (data not shown).

4.2 Unknown impurity structure elucidation using LC-MS/MS

As shown in **Figure 5**, the first step for structure elucidation is running full Q1 scans in both positive ion mode and negative ion mode to locate the m/z of parent peak. In this step, sample solution is typically introduced directly into mass spectrometer (MS) at a flow rate of 10 μL/min using a syringe pump. However, since dimer or oligomer may also be one of the potential impurities, range of Q1 scan shall be as wide as possible, e.g., to mass number of 1000–1200 at least.

Carefully compare the difference of mass-to-charge (Δm/z) numbers between experimental and nominal values of parent (molecular) peak as well as their stable isotope distribution patterns and natural abundances. Previous study for the

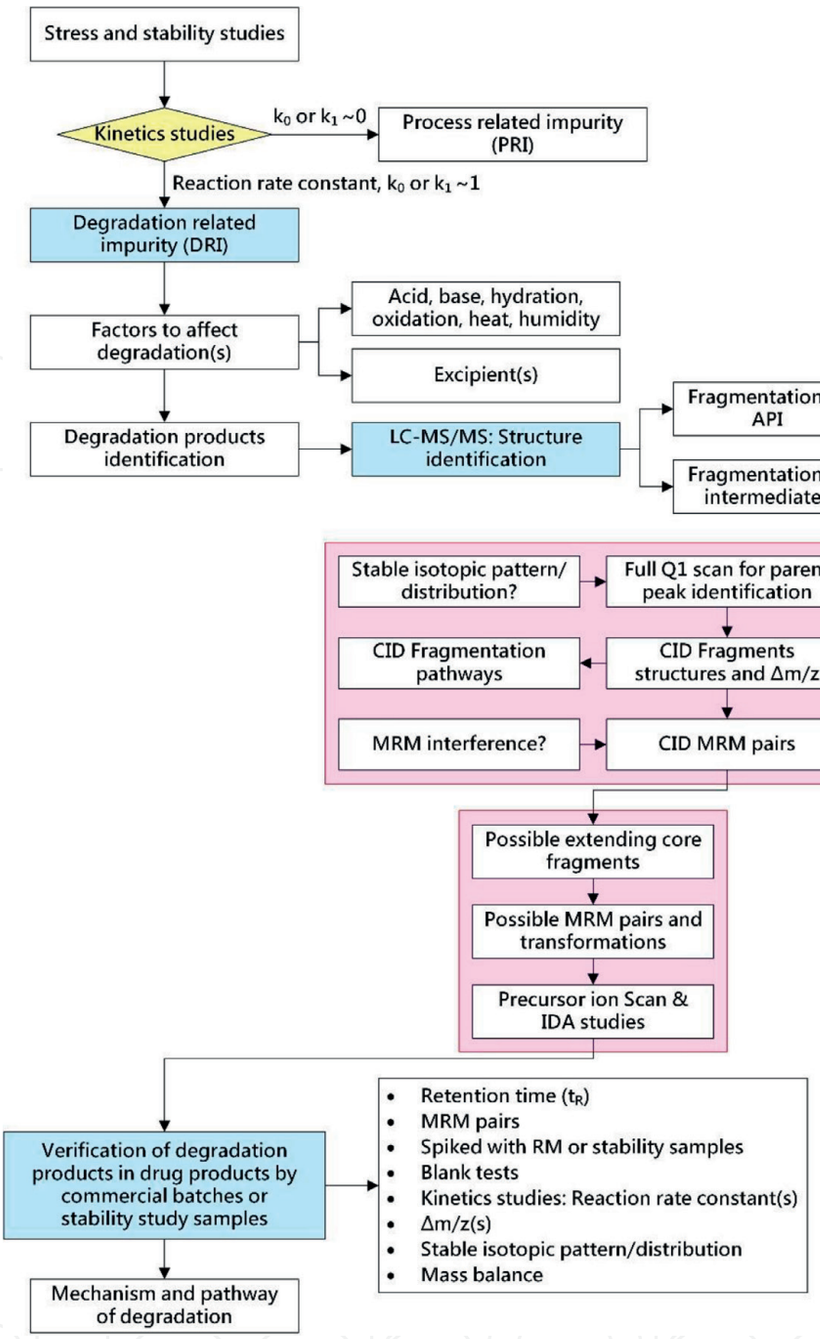


Figure 5.
Algorithms for the identification and verification of API-related degradation impurities (DRIs).

elucidation of degradation pathways of ethyl cysteinate dimer (ECD), a significant Δm/z value of −2 in Q1 scan between experimental result (m/z = 323.60) and nominal result (m/z = 325.46) of parent peak was found and indicating that an intramolecular disulfide (S-S) product, i.e., $[ECD_{S\text{-}S}+H]^+$, was the prominent form of ECD (not $[ECD+H]^+$) in aqueous solution before labeling of radioisotope, i.e., technetium-99m for i.v. injection (**Figure 7**) [25].

Repeat the product ion scans, precursor ion scans, and neutral loss scans of API to establish its collision-activated dissociation (CID) fragmentation database, including the optimal CID energies of each fragment and multiple reaction monitoring (MRM) pairs. Propose the promising structures of CID fragments and fragmentation pathways of API, accordingly. Provide the comparison of Δm/z results between experimental and nominal values for each peak, which is related to the fragmentation to verify the reliability of proposed fragments and fragmentation pathways [24, 25].

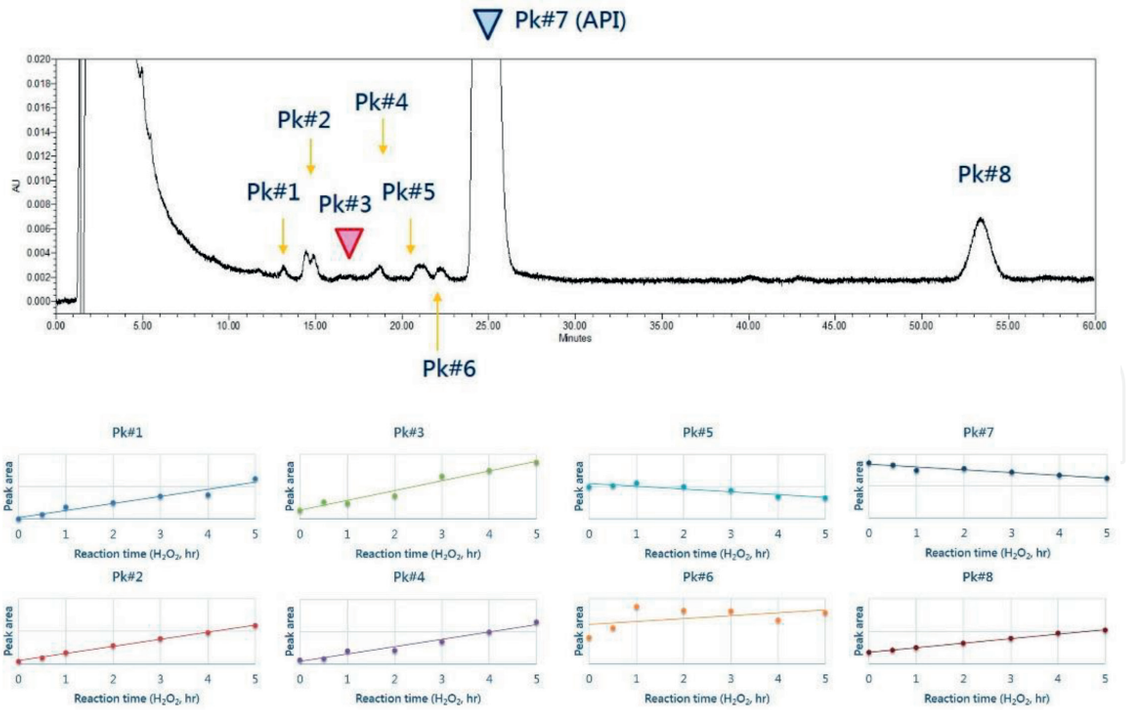


Figure 6.
Kinetic study of impurities formation by conducting stress studies to distinguish DRIs and PRIs.

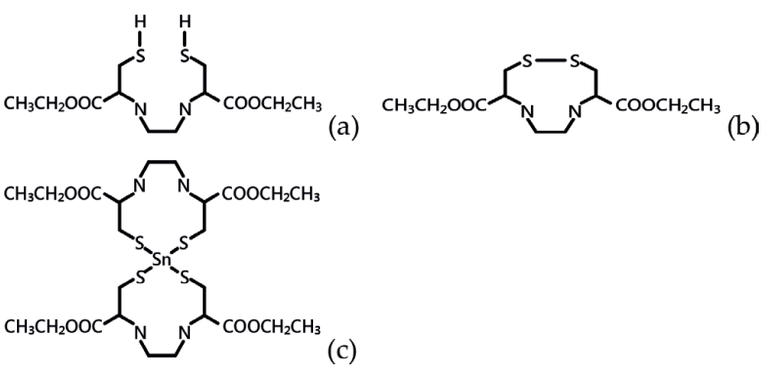


Figure 7.
Structures of (a) ethyl cysteinate dimer (ECD), (b) intramolecular disulfide (S-S) product of ECD, i.e., ECD_{S-S}, and (c) intermolecular dimer of ECD and reducing agent ($SnCl_2$), i.e., $Sn(ECD)_2$ (DP#4) [25].

Linear relationship within dynamic ranges for the quantitation of MRM pairs, i.e., correlation coefficients (r = 1) between precursor ions and product ions is another indication to verify high stability and reproducibility of fragmentation in CID conditions of tandem MS [24, 25].

Before using the MRM pairs for impurity scanning, interference of fragments generated from background, matrix, or contaminants such as plasticizers present in the solvents and mobile phase must be verified. Plasticizers, e.g., di(2-ethylhexyl) phthalate (DEHP) are one of the most common contaminants in organic solvents, including acetonitrile and alcohol [41].

Repeat the same procedures mentioned above in **Figure 5** to obtain a comprehensive information of fragments for any available intermediates and degradation products which are received from synthetic division, from contract manufacturing organization (CMO), from a stress study, or stability study sample conducted by the R&D team.

Steps for the determination of impurities related to degradation of API are illustrated as follows:

1. Step 1: According to the CID fragments of API, intermediates, or/and degradation products, a list of potential core fragments, which may be related to the unknown component(s) is proposed.

2. Step 2: Predict a set of potential/extending MRM pairs in line with the list obtained in step 1 and then coupled it with the relevant (bio-) transformations under the storage conditions of APIs/drug products for conducting MS/MS scans.

3. Step 3: Conduct the precursor ion scans together with function of information-dependent acquisition (IDA), where CID is automatically performed on the two highest intensity MS peaks to find the possible precursor ions containing core fragments established in step 2.

4. Step 4: Perform the reliability assessment by analysis commercial batches or long-term/accelerated stability samples to verify the identification results of step 3.

One preliminary study was illustrated as shown in **Figure 8** can be used to detail the algorithms of **Figure 5**. Core fragment of m/z 243 was found in the MS/MS study of API. In the meantime, four potential extending core fragments, i.e., m/z 183, m/z 185, m/z 197, and m/z 199 were obtained by the MS/MS studies of intermediate and degradation product (Step 1).

A total of five potential core fragments, coupled with the experience accumulated by degradation products that may be produced by similar chemical structures and prediction of relevant (bio-) transformations reactions under storage conditions, such as oxidation (+O, +2O), dehydration ($-H_2O$, $-2(H_2O)$), remove of carbon dioxide, and remove of acetic acid, a set of MRM pairs for scanning is established (Step 2).

Conduct the precursor ion scans by coupled with the IDA function for automatic performing collision on the two highest intensity MS peaks in the targeting regions of HPLC (Step 3). (Note: IDA is a build-in function of API 4000 QTrap (AB Sciex) for conducting an automatic collision on the highest intensity peak(s) scan.)

4.3 Verification of degradation products (step 4)

In addition to the methods mentioned above, i.e., kinetic study and difference of mass-to-charge ($\Delta m/z$) between experimental and nominal results, three other evaluation methods to verify the reliability of the identification results are available: including verification by real samples, by stable isotope distribution patterns, and by mass balance.

1. Verification by real samples

Investigation results of unknown degradation product(s) must be verified by the "real samples", i.e., commercial batches or long-term/accelerated stability studies samples. Verification of reliability is achieved by comparison the difference of retention time (t_R), MRM pairs, and stable isotope distribution patterns between real samples and stress study samples. If it is available, purified or enrichment sample of impurity can be spiked into a real sample for further verification.

2. Verification by stable isotope distribution patterns or natural abundances

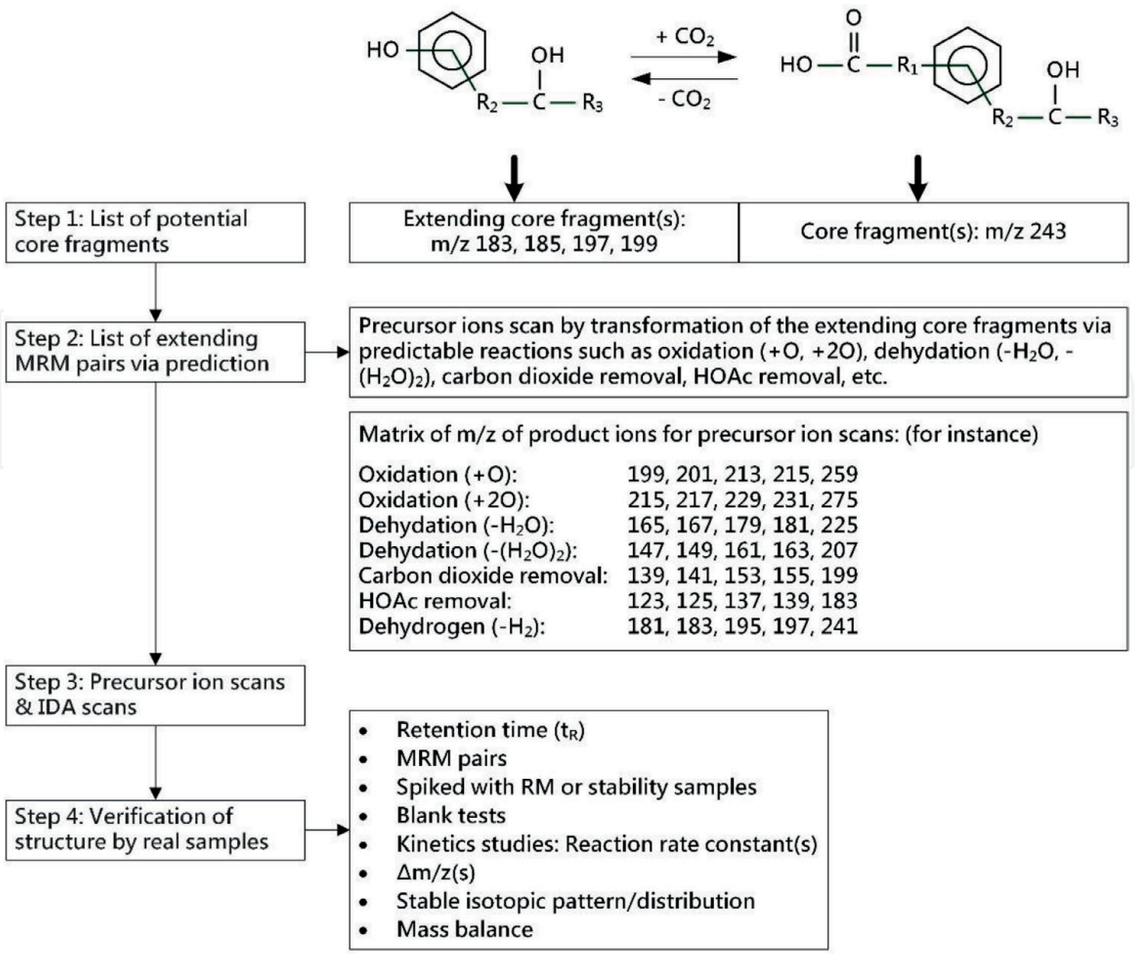


Figure 8.
Step for the establishment of potential extending core fragments, conduct of product ions screening with transformation/IDA function, and validation/verification.

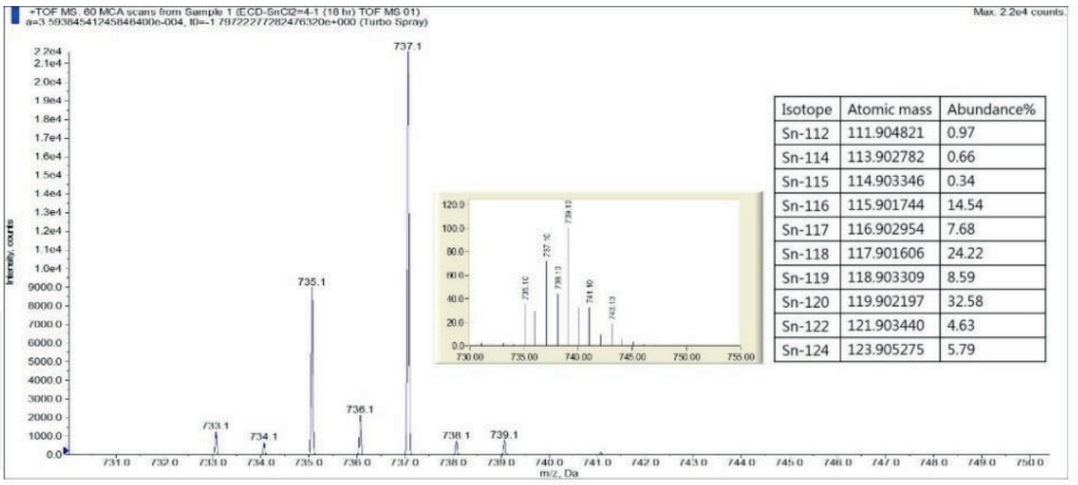
Each element, like a fingerprint, has its own unique stable isotope distribution patterns and natural abundances. Occasionally, stable isotope distribution patterns or natural abundances are available as a unique tool for structure characterization.

Ten, two, and two of uncommon patterns in the MS spectra as shown in **Figure 9(a)–(c)** were clearly indicated in our structure identification of ethyl cysteinate dimer (ECD) cold kit, (R)-N-methyl-3-(2-bromophenoxy)-3-phenylpropanamine (MBPP), and methoxyisobutylisonitrile (sestamibi, or $Cu(MIBI)_4$), respectively. These uncommon patterns were attributed to the contribution of stable isotope distributions of tin (Sn), bromine (Br), and copper (Cu), respectively.

When 7 major (or actually total 10) peaks are shown in the MS spectra, it may strongly mislead the works of structure elucidation as shown in **Figure 9(a)**. However, if it is available to know the presence of some special elements may present in impurity.

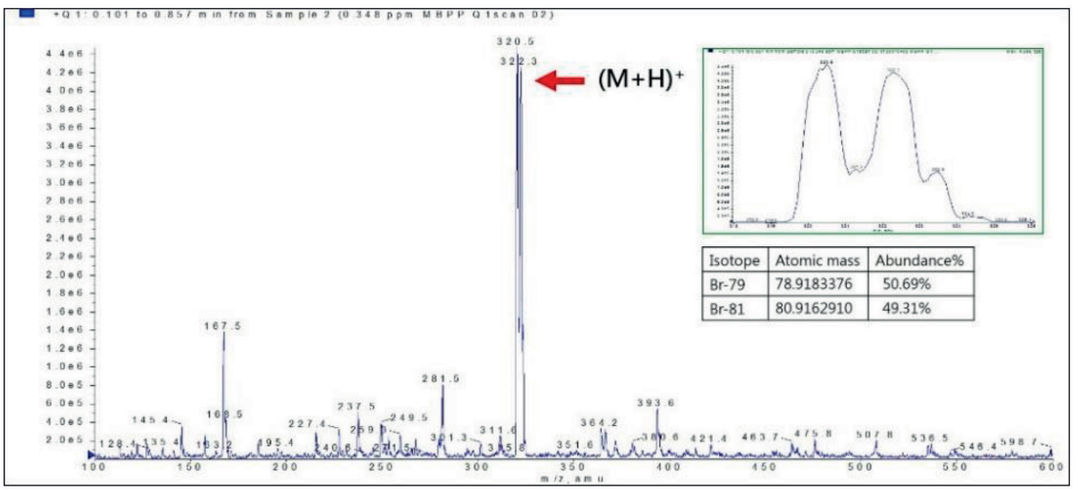
If it is able to presuppose that some special elements may contain in the structure, then it will be easier to elucidate the MS spectra. In other words, when pattern of MS spectra is significantly different from the normal CHO distribution, it may also indicate that a special element exists on the structure.

By comparing the natural abundance of 10 stable isotopes of tin and simulation MS spectra of a promising molecular formula, a series of metal complexes of tin can be verified. In the case for study of impurities in ECD kit, it was an ultimate and effective way to identify all of impurities containing Sn, i.e., DP#4, DP#5, DP#6, DP#6′, DP#6″, DP#7, DP#7′, DP#7″, and DP#8 [25]. Similar case was found in



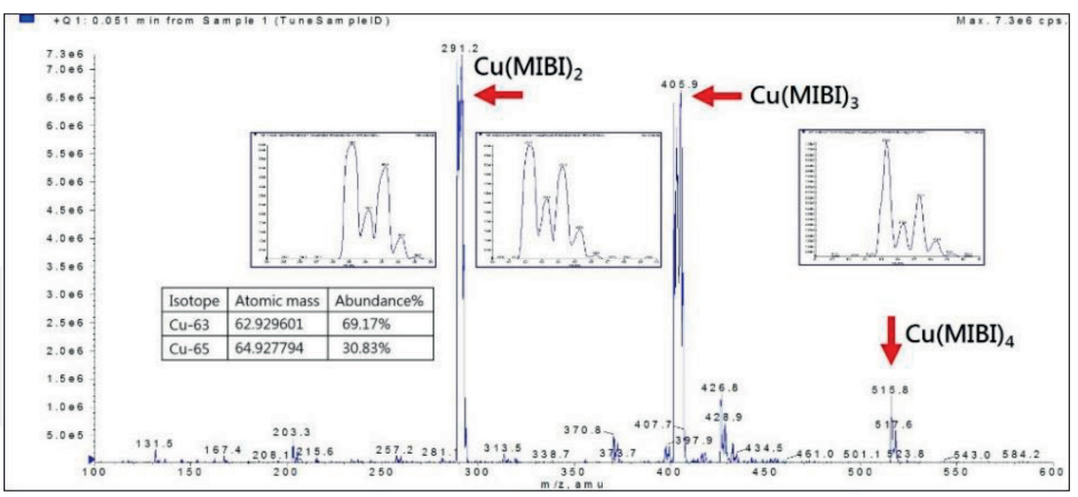
Isotope	Atomic mass	Abundance%
Sn-112	111.904821	0.97
Sn-114	113.902782	0.66
Sn-115	114.903346	0.34
Sn-116	115.901744	14.54
Sn-117	116.902954	7.68
Sn-118	117.901606	24.22
Sn-119	118.903309	8.59
Sn-120	119.902197	32.58
Sn-122	121.903440	4.63
Sn-124	123.905275	5.79

(a)



Isotope	Atomic mass	Abundance%
Br-79	78.9183376	50.69%
Br-81	80.9162910	49.31%

(b)



Isotope	Atomic mass	Abundance%
Cu-63	62.929601	69.17%
Cu-65	64.927794	30.83%

(c)

Figure 9.
Stable isotope distribution patterns and simulation of mass spectra of (a) Sn(ECD)$_2$ (DP#4), (b) (R)-N-methyl-3-(2-bromophenoxy)-3-phenylpropanamine (MBPP), and (c) methoxyisobutylisonitrile (sestamibi).

the structure determination of sestamibi as shown in **Figure 9(c)**. Coordination number (CN = 4) and core metal (Cu) in sestamibi can be clearly verified.

3. Verification by mass balance

When performing a stress study of API, one should determine content of API on each day by using a daily and freshly prepared calibration curve of API reference material, and interpolated within the validated dynamic range. The mass balance is

calculated by summation of the API and total impurity content. It is a tool to justify whether there are impurities unseparated (i.e., same retention time) or undetectable (e.g., without UV-visible chromophores). This topic and several major problems to cause poor mass balance have been detailed by Nussbaum et al. [42]

5. Conclusions

Management of impurities related to APIs in pharmaceutical products must be implemented in strict compliance with the regulatory requirements of pharmaceutical industry due to their quality and safety concerns. An integrated scheme in accordance with the regulatory requirements to establish analytical methods and acceptance criteria of process-related impurities (PRIs) and degradation-related impurities (DRIs) was presented, accordingly. Meanwhile, procedures for the identification and validation/verification of API-related DRIs were proposed. Validation or verification methods to evaluate the reliability of structure identification such as kinetic reactions, stress and stability studies, comparison of retention time(s) and $\Delta m/z$ between experimental and nominal values of targeting peaks, compatibility of MRM pairs with "real samples," stable isotope distribution patterns, and mass balance were demonstrated. Applying of the processes proposed in this article will help to ensure the reliability and quality of the impurity analytical results.

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