Drug Substance/Product Quality Analysis (Quality Assessment)

11

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Abstract

Multiple analytical studies are required on drug substances/products to maintain the quality during their lifecycle. One key regulatory change that has happened over the last few years is a heightened focus on the analysis of micro/trace components, which include not only synthetic impurities and degradation products but also metabolites, drug remnants in environment samples, drugs as adulterants, etc. Strategies have been proposed in the literature for the characterization of each type, and owing to their low concentrations, emphasis is on the use of sophisticated hyphenated instruments. While the pharmaceutical industry is duty bound to carry out the desired analyses, the regulatory directives also offer a good opening for research in academia. The objective of this chapter is to highlight the regulatory requirements for the characterization of the micro/trace components, to discuss the practical steps and protocols involved, and to provide a detailed discussion of the opportunities for academic scientists.

Keywords

Pharmaceuticals · Quality assessment · Micro/trace analysis · Nitrosamine impurity · Drug substance · Protocols

11.1 Introduction

The discovery to development to market is a long journey traversed by a new molecule. To make sure that a quality drug product of a new drug reaches the hands of caregivers and patients, there is a requirement for a string of analytical activities (Table 11.1).

Over the years, the demand for drug substance and drug product analysis has grown tremendously, as regulatory expectations have become expanded and stringent. Rather the whole success of regulatory approval, especially in the case of complex generics, is guided by an effective analytical characterization program [1, 2]. The methodology and techniques involved during discovery to market journey depend upon kind of the drug, the step of development, the nature of investigation being undertaken, and the type of product(s) chosen for marketing. Fortunately, there have been advancements in instrumentation, whose range has also expanded to cater to every regulatory directive. Most modern instruments are sophisticated, offering high sensitivity, resolution, and throughput. Irrespective of their cost, all innovative companies rely on the

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Table 11.1 Analytical activities during new drug substance and product development

1. Spectral data acquisition and structural characterization of new chemical entity
2. Semi-preparatory and preparatory purification, method development for purity determination
3. Characterization of impurities and degradation products, their synthesis/isolation and quantitation
4. Solid-state analysis
5. Bioanalysis, pharmacokinetics, metabolic profiling
6. Pre-formulation studies
7. Assay method development for phase-appropriate formulations, the applicability of compendial quality evaluation and functionality tests
8. Validation of developed methods and equipment qualification (DQ, IQ, OQ, CQ, PQ, CSV, etc.)
9. Stability testing, followed by an analysis of the samples
10. Defining key performance indicators (KPIs) or key quality indicators (KQIs) and setting specifications
11. Analytical methodology transfer
12. Commercial batch testing (starting materials, intermediates, APIs, and finished formulations)
13. Regulatory compliant documentation
14. Implementation of innovative analytical platforms and technologies for automated/continuous manufacturing, if applicable

Table 11.2 Micro/trace components of interest during the life cycle of drug candidates and their products

Drug life cycle	Micro/trace component(s)
Drug candidate synthesis and manufacturing in later stages	Impurities (organic, residual solvents, elemental, genotoxic, cohorts of concerns (nitrosamines, nitrosamine drug substance related impurities (NDSRIs), azido impurities), etc.
Pharmacological, toxicology and clinical research	Proteomics, metabolomics, biomarkers
DMPK investigations	Drugs (initial and clearance stages of PK profile) and metabolites
Formulation development and stability testing	Drug degradation products; and drug-drug (in case of FDCs), drug-excipient, drug-packaging interaction products
Environmental pollution profiling	Drug traces in the environment

best analytical tools available at a given time, so that the final product quality exceeds normative expectations.

It may be pertinent to highlight here an emphasis by International regulatory, which has brought a sea change in quality testing operations in the industry. It is the requirement of qualitative and quantitative micro/trace analysis (Table 11.2), applicable today not only to new drug substances and drug products but also to generics. Pharmaceutical manufacturers often face recall orders, if any impurity (IMP) or degradation product (DP) exceeds the defined limits. Table 11.3 provides a list of types of IMPs, their applicability, and regulatory/compendial requirements issued for them. Since mid-2018, a spate of recalls has happened owing to nitrosamine IMPs, and there is the latest focus and recalls happening owing to nitrosamine drug substance-related IMPs (NDSRIs) and azido IMPs in marketed drugs. One case example of NDSRI is depicted in Fig. 11.1. These IMPs are considered cohorts of concern, because of their carcinogenic and toxicogenic features. The current regulatory directives require proper control of their levels in the final products [3–7]. This chapter delves into the regulatory requirements for the characterization of the micro/trace components, provides a brief on the practical steps involved, and describes in detail the prospects for academic researchers.

Table 11.3 Types of impurities, their applicability, and regulatory guidelines/compendial general chapters for each (restricted to ICH/USFDA/EMA/USP/EP/JP)

Impurity type	Applicability	Major guidelines/compendial chapters
Organic impurities – Starting materials – Byproducts – Intermediates – Degradation products – Reagents, ligands, and catalysts – Geometric and stereoisomers	Drug substances	– ICH, Q3A – USFDA, ANDAs: impurities in drug substances – USP, <1086> impurities in drug substances and drug products – EMA, control of impurities of pharmacopoeial substances – EP, 5.10 control of impurities in substances for pharmaceutical use – JP, <G0-3-172> concept on impurities in chemically synthesized drug substances and drug products
Degradation products Components arising from – Drug degradation – Drug-impurity interaction – Drug-excipient interaction – Drug-excipient impurity interaction – Drug-residual solvent interaction – Degradation product-residual solvent interaction – Drug-microbe interaction – Drug/degradation product-packaging component interaction – Drug-drug and all other possible interactions in fixed-dose combinations	Drug products	– ICH, Q3B – USFDA, ANDAs: impurities in drug products – USP, <1086> impurities in drug substances and drug products – JP, <G0-3-172> concept on impurities in chemically synthesized drug substances and drug products
Residual solvents	Mainly drug substances but also drug products	– ICH, Q3C – USP, <467> residual solvents – EP, 2.4.24 identification and control of residual solvents – EP, 5.4 residual solvents – JP, 2.46 residual solvents
Elemental impurities – Reagents, ligands, and catalysts – Heavy metals or other residual metals – Inorganic salts – Other materials (e.g., filter aids, charcoal)	Drug substances and products	– ICH, Q3D – USP, <232> elemental impurities-limits – USP, <233> elemental impurities-procedures – EP, 2.4.20 determination of elemental impurities – EP, 5.20 elemental impurities – JP, 2.66 elemental impurities
Mutagenic/genotoxic impurities	Drug substances and products	– ICH, M7
Nitrosamines and other cohorts of concern (nitrosamine drug substance related impurities (NDSRIs) or active substance-derived nitrosamines, azido impurities)	Mainly drug substances but also drug products when the said impurities are the result of degradation	– ICH, M7 – USFDA, control of nitrosamine impurities in human drugs – USP, <1469> nitrosamine impurities – EMA, nitrosamine impurities – EMA, questions and answers for marketing authorisation holders/applicants on the CHMP opinion for the article 5(3) of regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal

(continued)

Table 11.3 (continued)

Impurity type	Applicability	Major guidelines/compendial chapters
		products – EP, 2.5.42 N-nitrosamines in active substances

ICH International Council for Harmonisation, *USFDA* United States Food and Drug Administration, *EMA* European Medicines Agency, *USP* United States Pharmacopeia, *EP* European Pharmacopoeia, *JP* Japanese Pharmacopoeia, *ANDA* Abbreviated New Drug Application

11.2 The Requirement and Steps Involved in the Characterization of Micro/Trace Components

When an unidentified IMP/DP peak is encountered in a chromatogram, the first requirement is its characterization. Then only comes organizing availability of its standard (usually prepared through synthesis once the structure is known) and eventually its quantitation. The conventional approach to the characterization of any micro/trace component involves isolation/enrichment to enough quantity, which is suitable for mass, nuclear magnetic resonance (NMR) and infrared (IR) spectral analysis. The modern approach focuses on the use of a variety of sophisticated hyphenated techniques (Table 11.4). The benefit is acquisition of spectral data by direct transfer of the peak of interest (with on-line enrichment, as required) to interfaced mass, NMR and IR spectroscopic systems. The hyphenated mass and NMR tools are the mainstay instruments that have been employed for the characterization of even other minor components, like metabolites, biomarkers, etc. Mass spectrometers, which allow tandem mass analysis, are more popular for the quantitation of micro/trace components. Therefore, one finds their mention in regulatory recommendations for the determination of nitrosamines and NDSRIs [8–10]. Table 11.5 lists various kinds of mass tools and their applicability.

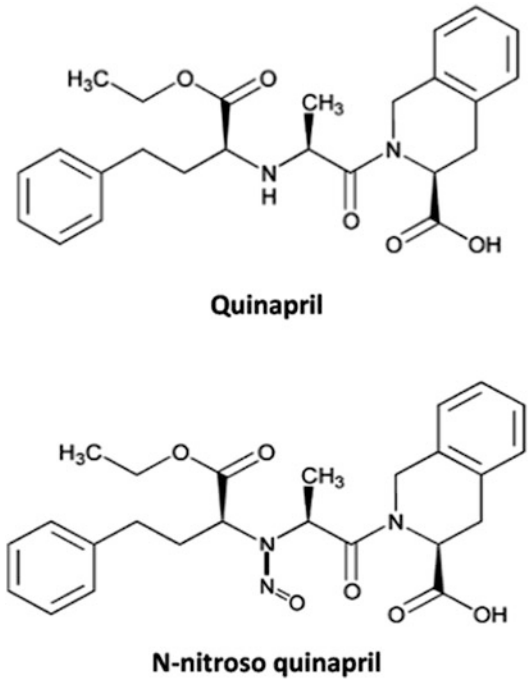

Fig. 11.1 An example of nitrosamine drug substance-related impurity (NDSRI)

While developing a new drug or a generic, the industry ought to perform multiple activities targeted to an analysis of micro/trace components, e.g., (1) their separation from the main constituent(s) on the column or capillary; (2) identification through spiking with pure materials or standards (starting materials, intermediates, reagents, solvents, excipients, etc., as applicable); (3) characterization of unidentified ones through spectral data acquisition; (4) isolation/synthesis of characterized components to best possible purity, or commercial procurement, if the compound of identified structure is pre-known and available; (5) safety evaluation (qualification) through predictive tools, followed by *in vitro* and/or *in vivo* studies; (6) setting of limits and specifications; (7) quantitation and monitoring in laboratory/pilot/ production and routine batch samples, and finally, (8) developing a control strategy. Information

Table 11.4 Variety of sophisticated hyphenated analytical techniques and their role in qualitative and quantitative analysis of the specific category of impurities

Technique	Utility
LC-MS	Separation, mass assessment and quantitation of organic impurities
LC-IR	Separation and IR spectrum recording of organic impurities
LC-NMR	Separation and NMR spectrum recording of organic impurities
CE-MS	Separation, mass assessment and quantitation of organic impurities, including those of chiral nature
CE-NMR	Separation and NMR spectrum recording of organic impurities, including those of chiral nature
HS-GC-MS	Separation, mass assessment and quantitation of residual solvents and volatile impurities
GC-IR	Separation and IR spectrum recording of residual solvents and volatile impurities
ICP-MS	Mass assessment and quantitation of elemental impurities
ICP-OES	Mass assessment and quantitation of elemental impurities

Table 11.5 Various kinds of mass tools and their utility

Variety of LC-MS tools	
LC-MS (single quad)	
LC-MS (triple quad)	
LC-MS-TOF	
LC-MS-Q-TOF	
LC-MS-TOF-TOF	
LC-MS-IT (ion trap)	
LC-MS-Q-IT	
LC-MS-IT-TOF	
LC-MS-Orbitrap	
LC-MS-Q-Orbitrap	
LC-IT-Orbitrap	
LC-MS-FTICR	
LC-MS-Q-FTICR	
LC-IT-FTICR	
Utility	MS type used for the purpose
High resolution mass spectrometry (HR-MS)	TOF, Orbitrap, FTICR
Multiple stage mass spectrometry (MS^n)	Ion trap
Tandem mass spectrometry (MS/MS)	Q-TOF, Q-Orbitrap, Q-IT, IT
Precursor ion, product ion and neutral loss scans	Triple quad
Selected/multiple reaction monitoring (SRM/MRM)	Triple quad
Post-run extracted ion chromatograms	All
Hydrogen/deuterium-exchange mass spectrometry (HDE-MS)	All
Molecular formula generator and RDB calculator	Available with all
Isotopic simulation	Possible with all

and data generated during all these steps are sought by the United States Food and Drug Administration (USFDA) as part of the Chemistry Manufacturing and Control (CMC) dossier, and as relevant, in annual reports. This applies to both New Drug Applications (NDA) and Abbreviated New Drug Applications (ANDA). Based on its experience of missing data in ANDA applications, USFDA has been forced to issue a Refuse to Receive (RTR) mandate for lack of justification of IMP limits in ANDA submissions [11].

11.3 The Opportunity for Academia

If we look into the activity possible in an academic environment, it is mainly the establishment of degradation chemistry of drugs through stress testing approach; or otherwise characterization of

metabolites, and establishing the fate of a particular drug from the perspective of environmental pollution. Another kind of study that can be pursued in academia is a survey of multi-source drug substances for relative IMPs originated during synthesis, their extents, and the type and extent of DPs present in multi-source drug products. As the drug degradation profile is intrinsic to drug structure and doesn't vary with the manufacturing route, a well-investigated study in literature, which reports drug degradation behaviour under a variety of extrinsic and intrinsic factors, including temperature, humidity, light, oxidation, pH, etc., along with degradation route and mechanisms in each condition, is straight-way useful to all world-wide generic manufacturers of that drug. Certain regulatory agencies mention that there is no need for stress testing by individual generic manufacturers if a good degradation behaviour study has been reported in the literature [12, 13]. The same is the case with metabolite identification studies, and residue analysis of drugs and their remnants in environmental matrices, which are also intrinsic. Practically, the same set of tools finds application in all these mentioned studies. However, it shall be noted that in an academic environment, one can only take projects on generic drugs, as an innovator involved in new drug development will not easily share the newly discovered molecule with academia, provided confidentiality concerns are well settled in advance.

It may be pertinent to add that projects on simple method development and validation for separation of the above enumerated type of components are no more considered challenging unless the investigation involves the characterization of unidentified components by involving relevant tools.

The strategies/protocols for the characterization of IMPs/DPs/metabolites/drug remnants in environmental samples using sophisticated hyphenated tools have been proposed in the literature, including several from our laboratories. There are many firsts to our credit, like we proposed a guideline for stress testing on drugs [14], published a critical review on the establishment of stability-indicating assay methods [15], outlined the process for the characterization of IMPs and DPs using hyphenated tools [16, 17], and offered a comprehensive strategy for metabolite identification during drug discovery and development based on 'high-quality throughput using minimum resources' approach [18]. Also, we laid down a systematic strategy for the identification and determination of pharmaceuticals in the environment at trace levels [19]. An example of our survey investigation has been the screening of herbal healthcare products for adulteration of PDE-5 inhibitors [20], for which we employed the strategy, reported by us in a separate publication [21]. Another survey study encompassed the evaluation of the presence of 25 steroidal and non-steroidal anti-inflammatory drugs in 58 herbal healthcare products collected from various parts of the country [22].

If one critically evaluates the strategy/protocols given in the referenced texts, it will be found that while the analyses part almost remains the same for both characterization and quantitation, the main difference is in the nature of the sample, and hence the sample preparation. It is simple solubilization of a drug substance to optimal concentration in the mobile phase when the target is IMP/DP analysis. This is even the case of forced degradation studies, where the drug is dissolved in the stressor solution, and the prepared samples are subjected to pre-fixed forced degradation conditions, like high temperature and/or humidity, light, oxidative environment, etc., and then diluted/neutralized before analysis. More experimental details can be found in our guidance paper [14]. For drug products, the best way is to follow the sample preparation method and procedure (based on formulation type) suggested in pharmacopeial monographs under the related substance test. The same procedure can even be employed when a particular formulation of a new drug is being developed and the interest is to check for DPs in samples placed on stability. Before and after the analytical run, help can also be taken from Zeneth, which is asserted to be an expert knowledge-based software that quickly yields accurate forced degradation predictions [23]. The software is even claimed to help

determine the chemical structures of DPs detected by a mass spectrometer, or other detection methods, and to deduce the likely degradation pathways. A range of filters can be applied to provide a results tree, which is said to be consistent with the experimental findings. Figure 11.2 briefly outlines the activities related to stress testing experiments. The workflow includes *in cerebro* and/or *in silico* prediction of hypothetical DPs, the conduct of experimental stress testing, development of stability-indicating methods, characterization of potential unidentified DPs, establishment of degradation pathway and mechanism of degradation, as well as *in silico* toxicity prediction of each characterized DP. Strategies to control non-mutagenic and mutagenic DPs are also included.

Nowadays, Quality by Design (QbD) is a well-established systematic approach, which is used by pharmaceutical companies to control the DPs. ICH Q11 emphasizes that a control strategy is required for all drug substances and/or existing drug products to limit the levels of DPs within the given acceptance criteria [24]. Also, risk management of DPs and scientific knowledge, such as understanding their formation, fate, and purge (whether the DP is removed *via* stabilization strategy) is considered to be important. Integration of technology, chemistry, risk management, design space, and control tools is required during the manufacturing of the drug substance, whenever a particular DP, characterized during stress studies and formed during processing, is considered a critical quality attribute (CQA) [25]. At the same time, attention is also paid to the stability of the reaction mass to understand the formation of critical DP due to the presence of a residual reagent. The latter is then also considered as a CQA, along with the DP. The IND/NDA stability studies need to be repeated if the manufacturing process with respect to intermediate or reagent is changed markedly.

In the case of drug products also, apart from conventional formulation stabilization approaches, involving the use of stabilizers, excipients and protective packaging, the roles of technology, chemistry, risk management, manufacturing design space, and control tools have assumed importance to keep a check on the DPs [25, 26]. IMPs in the excipients also have attracted significant attention as these may catalyze the degradation of a drug to a pre-characterized DP [27]. Interestingly, a new focus is on the stress testing of excipients per se [28].

Overall, a big problem for the industry is to predict the exact level of DP, whenever it is considered a CQA, so as to avoid recalls from the market in the future. However, the recently launched software, *viz.*, ASAPprime® and Mirabilis allow companies to make better decisions in such situations early in the development process [29, 30]. These also help in quick reformulation and choosing the optimal combination of packaging, formulation, ingredients, and manufacturing process.

For metabolite identification (metID) studies, the metabolites are generated in either *in vitro* systems (microsomes, S9 fractions, hepatocytes, and recombinant enzymes), or *in vivo* where plasma and excreta are the major sample types. The target of sample preparation herein is to obtain concentrated samples free or almost free of biological matrix. This is usually achieved through protein precipitation and solid-phase extraction (SPE). In one of our studies, we employed a novel additional step of freeze-liquid separation to reduce the loss of polar analytes due to the overloading of SPE cartridges [31]. For metabolite characterization, there is an advantage that LC-MS manufacturers provide *in silico* tools, as part of a software bundle, for both prediction and detection of the metabolites. Usually, the prediction software foretells the biotransformation of any molecule by considering all mammalian phase I and phase II enzyme systems. The predicted structures are listed along with the accurate theoretical mass of their protonated and unprotonated species. The metabolite detection software, when supplied with the LC-HRMS system, facilitates the matching of the accurate mass of metabolites eluted, with those predicted. The software-predicted/detected metabolites and those additionally observed in total ion and UV chromatograms are confirmed using the accurate mass values, ring plus double bonds (RDBs)

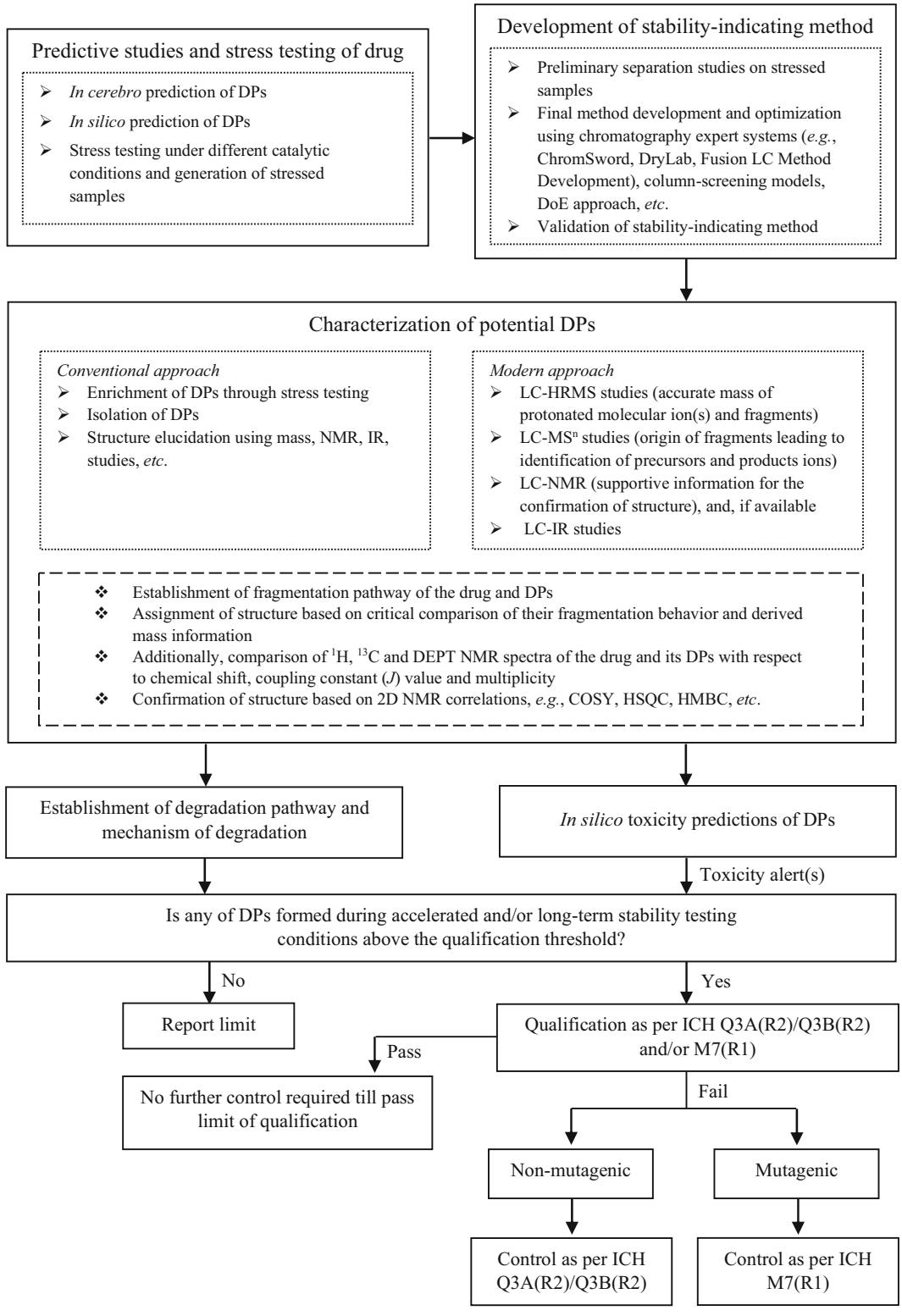

Fig. 11.2 Comprehensive workflow of stress testing and other studies leading to the identification, characterization, toxicity evaluation, and control of degradation products (DPs)

calculations, application of nitrogen rule, and determination of exact mass losses. The site of change in the drug structure because of metabolism is identified through a comparison of MS fragmentation pattern of each metabolite with that of the drug. After structures of metabolites detected in different *in vitro* and *in vivo* samples are elucidated, extracted ion chromatograms (EICs) of the individual metabolites are evaluated to determine their relative amounts.

Pharmaceuticals are produced or consumed in large amounts globally and it is no surprise that environmental pollution with them is rising progressively. Whether consumed or disposed of without use (due to any reason), the potent drug molecules eventually make their way into the environment and may persist as contaminants, either in an intact form, as a DP, or as a metabolite of the parent. Interest in them has got kindled because of tremendous progress in the analytical techniques for trace analysis. Reports exist on the effects of pharmaceutical contaminants on aquatic flora and fauna but long-term eco-toxicological consequences, especially to humans, are still unmapped [32]. There are a few basic questions that one needs to answer before taking up analytical research projects in this area, owing to the vastness of the scope. The first question to answer is - what is the purpose of the study? Is it exploration of major pharmaceutical pollutants (e.g., industrial discharge in lake/water body), or micro/trace level contaminants (e.g., discharge from hospitals/households into sewage). Secondly, whether the interest is in the detection of targeted pollutants only, or to characterize all those present, and so on. The defined purpose hence lays down the extensiveness of subsequent study actions. For example, in our laboratory, we narrowed down the scope to evaluate the presence of residues of forty commonly prescribed drugs in ground drinking water in villages surrounding our institute [19]. The expected concentrations were at micro/trace level, so the first part of sample collection was procuring a minimum required water quantity. The analyte enrichment was the next key step, for which SPE was the chosen method. For identification of the drugs present, the enriched samples were subjected to LC-MS/MS analysis. The identification strategy included matching of retention times against the standards, comparison of MRM transitions, matching of qualifier to quantifier intensity ratio (as suggested by the Environment Protection Agency), comparison of base peak MS/MS profile, and comparison of accurate mass data. Eventually, quantification was done through the use of calibration curves developed using quantifier MRM transitions.

As mentioned earlier, the survey testing done in our laboratory was targeted at observing the adulteration of herbal dietary supplements (HDSs) with synthetic drugs, like phosphodiesterase type-5 (PDE-5)-inhibitors (viz., sildenafil, vardenafil and tadalafil), and multiple steroidal and non-steroidal anti-inflammatory agents. It came to our notice that unapproved analogues of all PDE inhibitor drugs were being found in HDSs, and more seriously, concealed, structurally modified analogues were also being used increasingly. As many of these adulterants are unknown, the likelihood exists of much higher associated risk, because their effects and side effects are not known pre-hand. So, we focused to build a strategy to help identify, not only the approved drugs but also their known and unknown derivatives. This was made possible through a critical study of the reported mass fragmentation behaviour of the drugs and their known derivatives. We could identify one or two common mass fragments, which if observed in the mass spectrum of any peak in the mass chromatogram of the sample, would mean a strong likelihood of an analogue of any one of the PDE inhibitors. In the case of adulteration of AHPs with steroidal and non-steroidal anti-inflammatory agents, the strategy was simpler because of the availability of all involved standard drugs. The study primarily involved comparing ultraviolet and mass spectral data of the standard with similar data for unknown peak(s), comparison of retention time values, and final confirmation through spiking of standard(s) in the sample [22].

We exemplify in Fig. 11.3 a modified strategy/protocol for the characterization of IMPs and DPs over and above the one reported by us earlier [17]. However, it must be understood that, apart

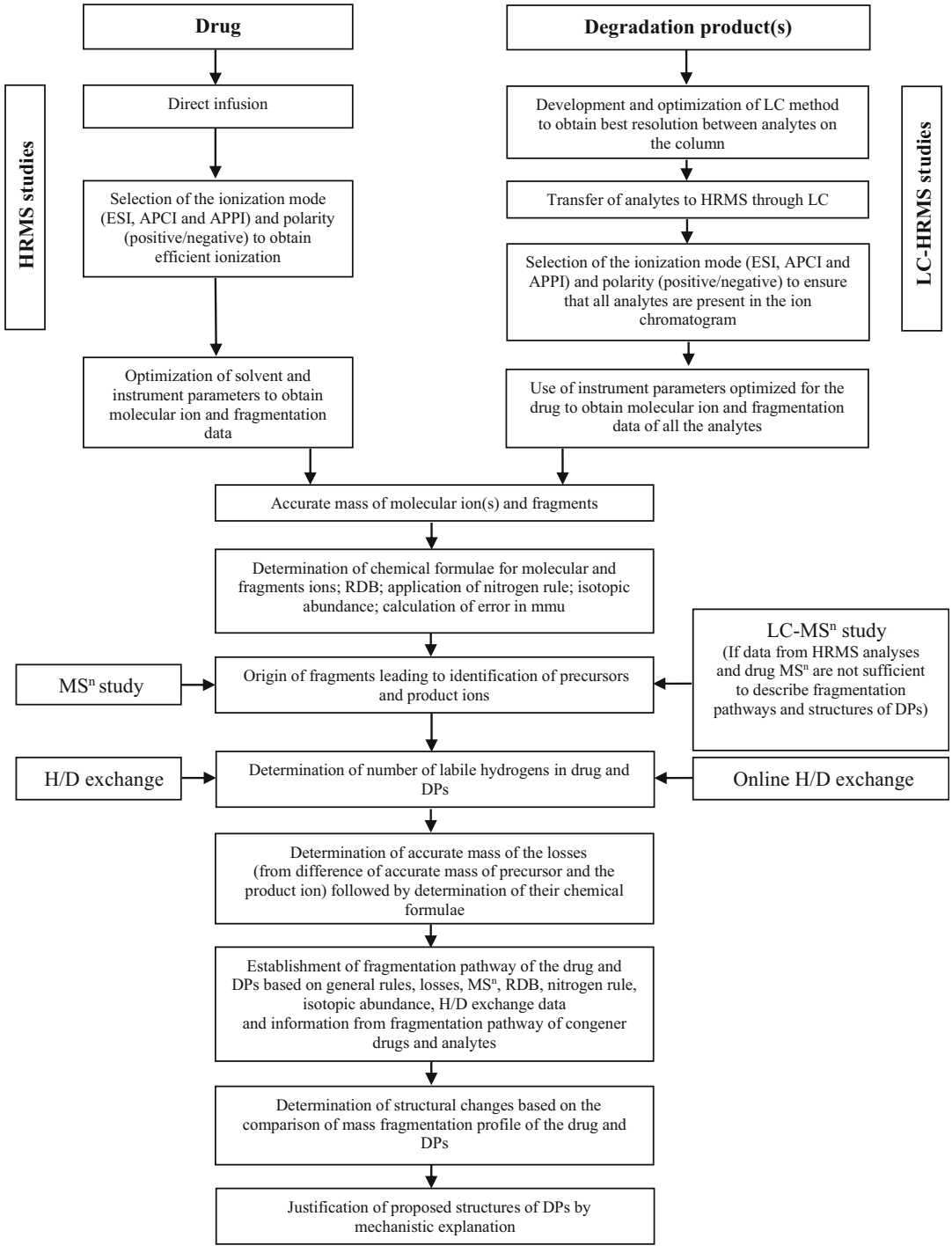

Fig. 11.3 General strategy for the characterization of degradation products (DPs) by LC-MS tools. Duly modified after adaption from [17] with due permission

from a strategy/protocol, there are a lot of practical intricacies involved while carrying out experimental work to generate useful data for the characterization of micro/trace components using sophisticated hyphenated instruments. One can find extensive discussion on these in our published reviews pertaining to data generation using LC-MS [17] and LC-NMR [33]. The nature of sensitivity needed even governs the purchase of instruments for the purpose among various types and models available with the vendors. For example, one can buy simple LC as front-end, or instead UHPLC, or capillary/nano LC systems for much lower analyte concentrations. Similar is the situation with back-end MS and NMR detectors, wherein models are available with ever-improving resolution and sensitivity.

11.4 Concluding Remarks

There is a big advantage of pursuing research in the areas of identification and characterization of micro/trace components, produced either upon transformation (including biotransformation) or when present as contaminants or adulterants. This precursor step is critical to the quantitative assessment of micro/trace components in actual samples, and for exercising controls to comply with stringent regulatory limits. The matter is of deep regulatory interest, as it is the responsibility of regulators worldwide to ensure the availability of high-quality and high-purity products to patients globally.

It is to be acknowledged that the eventual success of the mentioned effort requires a thorough understanding and knowledge of all aspects involved in the steps of planning, execution, and data interpretation. This chapter provides references to resources that can be referred to for the conduct of successful experiments, and to arrive at acceptable inferences. It highlights the nature of investigations possible to be undertaken by scientists in academia.

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Conflict of Interest None.

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