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REVIEW ARTICLE

Safety and Efficacy: The Role of Chemistry, Manufacturing, and Controls in Pharmaceutical Drug Development

Thomas J. DiFeo, Ph.D.*

ChemPharm CMC Sciences & Dossier Management, Johnson & Johnson Pharmaceutical Research and Development, L.L.C., Spring House, Pennsylvania, USA

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INTRODUCTION

There is an intrinsic link between the chemistry, manufacturing, and controls (CMC) attributes of the pharmaceutical product and the safety and efficacy of the clinical therapy. The quality, identity, purity, and strength of the drug substance and drug product provide a foundation for the demonstrated safety and efficacy of the dosage form. Product safety is studied throughout the development process with a specific focus on safety found in the early phases of drug development. The early exploration of safety allows for appropriate risk assessment as clinical exposure to the drug increases in development. As development progresses, the impact of CMC on efficacy of the dosage form becomes the focal point of the development process. This article reviews the critical quality attributes of drug substance and drug product essential to pharmaceutical drug development and the relationship to the safety and efficacy of the clinical therapy.

DEVELOPMENT PHASES

Preclinical

Prior to the introduction of the drug in humans, nonclinical safety studies are performed on animals. These studies include toxicokinetic and pharmacokinetic studies, single dose toxicity studies, and repeated dose toxicity studies.[1,2] The nonclinical studies provide a means to determine the safe starting dose and any clinical parameters that should be monitored with regard to potential adverse effects.[3] In general, the toxicity studies are used to evaluate the effects of systemic exposure of the drug.[4] The route of administration is selected to provide a realistic presentation of the drug relative to the proposed clinical route of administration. The nonclinical study examines the effect of various dose levels. Low-dose or no-toxic-effect levels are examined initially. Subsequently, intermediate-dose levels, which may represent multiple

*Correspondence: Thomas J. DiFeo, Ph.D., ChemPharm CMC Sciences & Dossier Management, Johnson & Johnson Pharmaceutical Research and Development, L.L.C., Spring House, PA 19002, USA; E-mail: tdifeo@prdus.jnj.com.

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Figure 1. Examples of nonclinical safety studies performed for new products.

or fractions of the low-dose level, are pursued followed by high-dose level studies. The examination of multiple doses is particularly of interest for drugs following nonlinear kinetics. The importance of determining drug exposure/dose relationships is integral in the data interpretation of safety studies. For example, negative results of in vivo genotoxicity studies would typically require evidence of adequate exposure of the drug to the indicator tissue.[4] There are a variety of nonclinical safety studies that are pursued to support the marketing approval of a drug application. An overview is provided in Fig. 1.

Phase 1

Phase 1 studies provide for the first introduction of the investigational drug into humans.[5,6] The studies are normally conducted with healthy volunteers although some investigational drugs may be used to treat patients for ''immediately life-threatening'' diseases.[7] Phase 1 studies typically entail human pharmacology studies to explore the pharmacokinetics and pharmacodynamics of the investigational drug. Pharmacokinetic studies provide information on the quantification of the drug and its metabolites as a function of time. Pharmacodynamic studies relate the action of the drug as a function of the drug or metabolite concentration.[8] The pharmacodynamic study can be confounded by the complexity of mechanisms responsible for the elimination of the active drug/metabolite or competing biological mechanisms via alternate biological pathways as exemplified in the hysteresis seen in the dose/response results of the acid inhibitory effect study of ranitidine.[9] Phase 1 studies establish the dose that is tolerated in humans and consist of single-dose and multiple-dose studies including dose escalation protocols that examine any potential side effects. The starting clinical dose is typically 1% of the no-effect level seen for toxicity in animal studies.[10]

Phase 2

Phase 2 studies are referred to as therapeutic exploratory studies and entail examination of the targeted indication, estimates of dose for future studies, as well as endpoints and methodologies to demonstrate further the safety and efficacy of the drug.[11] Phase 2 may be subdivided into phase 2a and phase 2b in order to clarify the types of clinical studies performed.[12] Phase 2a studies are pilot clinical studies used to evaluate efficacy and safety in patients and are also referred to as proof of concept studies. These studies comprise 100 or fewer patients and are used to determine the mechanism of action. An example of a typical Phase 2a protocol is seen in the study of 73 patients with type 1 diabetes mellitus.[13] This clinical study was able to demonstrate the ability to dose successfully patients with inhaled intrapulmonary delivery of insulin. In the study, levels of glucose and glycosylated hemoglobin were followed over time. Phase 2b studies are well-controlled clinical trials that also evaluate efficacy and safety. These studies may enroll a larger number of patients and are randomized and blinded.[14] Phase 2b determines dose response in order to plan the ultimate dosing regimen for the pivotal phase 3 studies. Phase 2b studies are useful in confirming the mechanism of action.

Phase 3

Phase 3 studies are therapeutic confirmatory studies[11] consisting of multicenter programs with large numbers of patients (typically 1000–3000). Phase 3 studies establish the safety and efficacy of the drug. The studies are well controlled, randomized, and typically involve comparative assessment of the investigational drug with currently approved treatments. Phase 3 studies provide an overall risk-benefit analysis of the drug and provide data to determine the appropriate labeling for the drug product.[15] The clinical studies section of the label provides a summary of the data supporting the efficacy of the drug for its purported indication. The summary should include design aspects of the clinical study, the populations studied, as well as the endpoints measured during the clinical investigation.[16] Safety data is described in the adverse reactions section of the label.[17] The safety data should include serious adverse reactions, the most commonly occurring adverse reactions, and those adverse reactions that most frequently require clinical

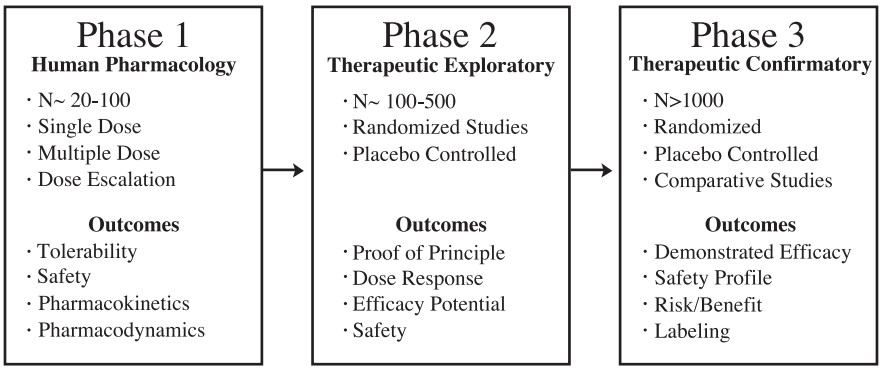

Figure 2. Drug development phases—objectives and study outcomes.

intervention. Fig. 2 depicts a summary flow diagram of the phases of development with study objectives and outcomes.

CHEMISTRY, MANUFACTURING, AND CONTROLS

Drug Substance

The Food and Drug Administration (FDA) highlights some important CMC issues for first-in-human studies in their guidance document on Investigational New Drug Application (IND) meetings.[18] These issues include drug substance and drug product physicochemical characteristics, method of preparation, quality control, formulation aspects, and stability information.

The information provided in this CMC discussion is important in all phases of development with a more detailed understanding of the CMC characteristics of the drug substance and drug product growing over time. The discussion here will focus on the CMC aspects of interest throughout development with emphasis on phase 1/phase 2 studies. The degree of data development for each CMC topic for a particular phase of development will vary depending upon the complexity of the molecule and its intrinsic safety and bioavailability.

One of the key aspects of assurance entails demonstration of proof of structure of the drug entity. The structural formula, including the absolute stereochemistry, the molecular formula, and the molecular mass, should be demonstrated. Structural elucidation studies include elemental analysis, mass spectrometry, liquid chromatography/mass spectrometry (LC/MS),[19] nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy, stereochemical analysis, configurational/conformational analysis and x-ray analysis. Mass spectrometry studies provide structural information based upon the fragmentation patterns of the molecule. Nuclear magnetic resonance studies can be performed on the drug substance in the solid state or in solution. In phase 1 studies, regulatory authorities increasingly are requesting data that demonstrate the structure of the investigational drug. The confirmation of structure is essential in providing evidence that the compound identity is well known and the drug under study in humans is verified to be the same chemical species tested in preclinical animal studies. Regarding the absolute stereochemistry of the molecule, it is known that enantiomers may differ in pharmacodynamic and pharmacokinetic properties and hence can play an important role in the ultimate safety and efficacy of the compound.[20] Enantiomers may be metabolized at different rates or may even compete for the enzyme responsible for metabolism.[21] The use of specific enantiomers in drug therapy provides a means to increase the specificity of the drug treatment and can provide increased therapeutic benefits.[22] In addition, there may be differences in adverse effects between the enantiomers.[23]

A rudimentary understanding of the physico–chemical properties of the drug substance is important with regard to understanding the ultimate in vivo behavior of the drug. The drug substance characteristics of interest include the following:

Ionization Constant, Partition Coefficient, Solubility Profile

The solution characteristics of the compound may impact the relative bioavailability of the drug.[24] For example, the in vivo dissolution and permeability of the drug may be effected by the solubility characteristics of the drug substance and/or the dosage form

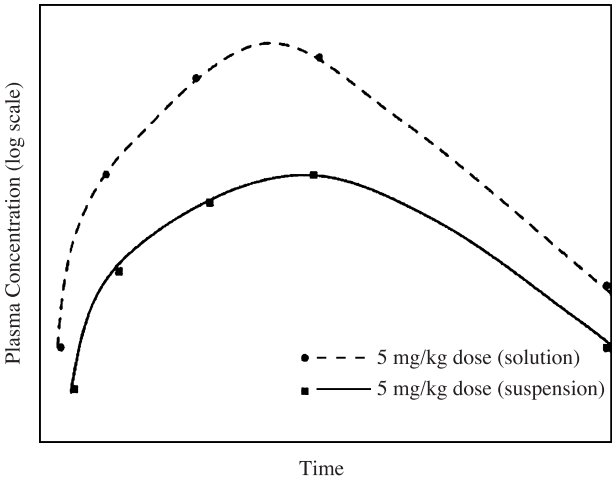

Figure 3. Mean plasma profiles of a poorly soluble drug delivered as a solution and as a suspension.

characteristics. An understanding of the formulation characteristics of the drug are important to the toxicologist reviewing the preclinical safety data used to support the first-in-human studies. Fig. 3 displays a plasma profile curve demonstrating the effect of the dosage form (suspension vs. solution) on plasma levels of the drug substance for a poorly soluble drug. Predicting systemic exposure in humans requires not only a knowledge of the differences in metabolism between humans and animal models but an understanding of the drug characteristics in order to adequately model human exposure.[25]

Solution Stability

Solution stability of the drug substance can play a role in the systemic exposure of the drug substance. For example, the degree of oral absorption of a drug may be influenced by hydrolytic degradation in the stomach.[26] Studies of the pH stability of the drug substance can provide information on the potential for decreased systemic exposure of the drug due to loss of the drug through degradation and/or the production of toxic degradation products.

Hygroscopicity and Hydrate Formation

The ability of a drug substance to form hydrates can play a role in the ultimate disposition of the drug in vivo. Studies on the bioavailability of ampicillin anhydrate and trihydrate in both animal and human studies suggest significant differences in the rate and extent of bioavailability of the two hydrates.[27,28] The drug substance behavior should be studied over a range of humidities to explore the potential conversion of anhydrates to hydrates and the conversion of lower-order hydrates to higher-order hydrates. The moisture content of successive lots of drug substances should be analyzed as well as the differential scanning and thermogravimetric analysis behavior of the drug.[29]

Polymorphism

Crystal polymorphism is an essential characteristic needed to be fully understood in the drug development process. Polymorphism entails different arrangements of the molecule in the solid state. Crystalline polymorphs differ in crystal structure but are chemically identical having the same liquid and vapor states. Polymorphs of the same chemical substance may display differences in bioavailability.[30] The propensity for the drug substance to form polymorphs should be studied in a variety of crystallization solvents as the development proceeds. In early development, the importance centers on characterization of the solid state so that it is well documented which crystal form was administered in the initial safety and clinical trials. In addition, the intrinsic stability of the polymorph (i.e., its propensity to convert to other crystalline arrangements) should be studied in both the solid state and in solution. Where polymorphs exist, the relative difference in energies (and hence the propensity for conversion) may be studied via solution solubility studies. An examination of the solution solubility data is made to assure that no solvent-mediated transformations occurred during the solubility study.[31,32] Fig. 4 shows a typical van't Hoff plot for an enantiotropic system. These plots can be employed to

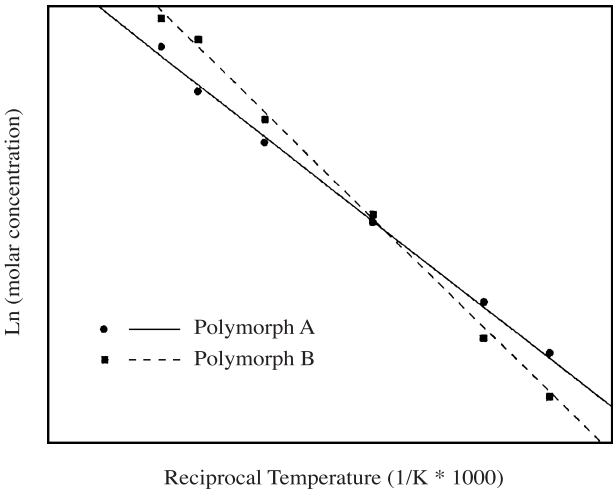

Figure 4. Classical van't Hoff plot of an enantiotropic polymorph system.

determine thermodynamic values for the dissolution of each polymorph. From a physiological standpoint, an important area to be examined is the ability of the drug to convert into a different polymorph in gastrointestinal (GI) fluids. It has been hypothesized for some compounds that differences seen in relative bioavailability compared to in vitro prediction (i.e., dissolution rates) may be as a result of solid-state transformation in vivo.[30]

Particle Size Distribution

The particle size distribution of a drug substance can play a significant role in the bioavailability of the drug,[33] especially for drugs with dissolution rate limited behavior.[34–36] The drug particle size distribution should be determined and compared with the particle size distribution of lots used in preclinical studies to assure similar exposure during the clinical phases of the program. The influence of the formulation on the particle size of the drug substance must also be considered. In the case of metronidazole benzoate suspension, the conversion of the anhydrate to the hydrate form of the drug substance in the formulated product leads to a significant increase in drug particle size with the potential to impact drug bioavailability.[37]

Drug Substance Purity

One of the key areas of focus in early clinical studies concerns the drug substance purity profile. Impurities of drug substances may be classified as organic impurities, inorganic impurities such as heavy metals, and residual solvents.[38] The purity profile of the clinical lots is carefully compared with those lots used in the preclinical safety qualification studies. Reversed-phase high performance liquid chromatography (HPLC) is typically employed for the analysis of organic impurities. Complementary detection techniques should be used to verify the purity of the drug substance. In particular, impurities with weak chromophores may not be detected by conventional UV detection techniques. Alternative detection techniques can be employed including LC-MS, LC-NMR, refractive index, and evaporative light scattering.[39] Alternative separation techniques should also be employed and may include normal-phase HPLC, thin layer chromatography (TLC), and capillary zone electrophoresis (CZE).[40] The importance of adequate detection methods is magnified by the fact that the drug substance process typically undergoes changes during phase 1 and phase 2.

International Conference on Harmonisation (ICH) guidelines delineate reporting thresholds for new drug substances.[38] However, there are examples of toxic effects of compounds at levels below the ICH reporting thresholds.[41,42] These literature examples illustrate important points regarding impurity profiles and safety. Clearly, additional techniques beyond simple HPLC, such as LC-MS should be used to determine if minor impurities are co-eluting with the active component or other impurity peaks.[43] Fig. 5 depicts a hypothetical drug substance analyzed by two different HPLC methods. Method A does not discriminate between lots containing varying levels of impurity X. Method B shows greater specificity and an ability to detect additional impurities including Impurity X. In this case, an analysis of the active peak in Method A by LC-MS would demonstrate the presence of an additional molecular weight component (Impurity X) co-eluting with the active moiety, hence, demonstrating the need for an improved method.

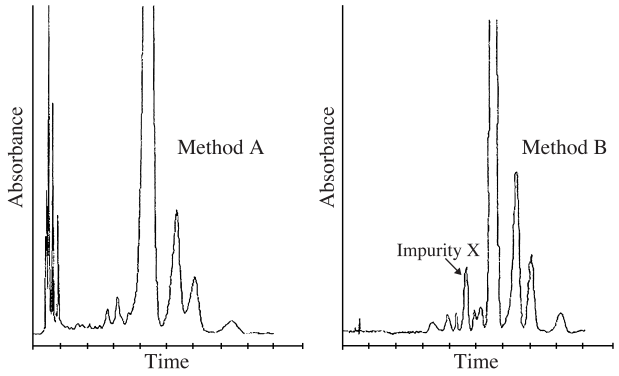

Figure 5. The effect of method specificity on the ability to detect impurities.

Regarding the safety of drug substance batches, it is noteworthy that not only is a comparison of the relative levels of impurities at the reporting threshold in the preclinical and clinical batches important, but impurities at levels below the qualification threshold in the drug substance should be compared. A preclinical study of an antineoplastic drug demonstrated a toxic reaction caused by an impurity at a level of only 0.01%.[42] The above examples demonstrate that method specificity is an important aspect of control in early development where experience with the drug substance manufacturing processes is limited.

Drug Substance Manufacturing Process

The drug substance manufacturing process begins at the lab scale and progresses to large-scale factory

production. The early phase 1 studies are often completed using drug produced at lab scale with a process that has yet to be optimized. Throughout the development process, particular attention is given to the final steps of the synthesis as these steps often influence the physico-chemical properties and purity of the drug substance.[44] Adherence to compliance with current Good Manufacturing Practices (cGMPs) in the production of clinical drug substance batches helps to assure that the process is producing batches equivalent to those used in preclinical evaluations. This is particularly important since analysis of the drug substance alone cannot assure quality in the absence of a well-understood and controlled process.

Specifications

Specifications consist of test methods and their associated acceptance criteria. The tests and acceptance criteria discussed below are applicable to all drug substances.[45]

The drug substance appearance test details a qualitative description of the drug substance. The appearance of the drug substance may be an important quality indicator and provide an indication of very low-level impurities.[46] The test method for the drug substance description may include the use of a color chart to compare the color of successive lots. Changes in color between lots may indicate the need to perform additional purity analysis.

Identification testing should distinguish between the drug substance and closely related compounds. Typically, two identification tests are performed with one test being the HPLC retention time match with a reference standard material. The second test is typically a spectroscopic technique such as IR. If the drug substance contains any centers of dissymmetry, an additional stereospecific identification test should be performed.

Assay procedures for drug substances include titration methods and HPLC methods. If a titration method is employed for assay, an additional specific, stability-indicating method should be employed to control impurities in the drug substance. The assay acceptance criteria is typically 98–102%, although in early phases of development this range may be wider. For those drug substances with acceptance criteria that are more broad, careful attention to the mass balance should be given as two lots of drug substance may have similar assay values but demonstrate different mass balance due to unquantitated/undetected impurities[47] with potential safety ramifications.

High-performance liquid chromatography methods are commonly used to control impurities in drug substance. The methods should be specific and stability-indicating. The importance of the level and type of impurities with regard to potential safety risk is highlighted in a recent report detailing a 40% difference in toxicity between two sources of an antituberculosis drug.[48]

Additional specifications that may be applicable depending upon the nature of the drug substance and drug product, include those specifications that may reflect the potential for differences in bioavailability (e.g., particle size, polymorphic form, moisture) and those specifications that may reflect potential safety issues (e.g., melting point and volatile organic impurities). Finally, the importance of analytical variability as applied to the setting of acceptance criteria for impurities has been reviewed.[49] While the uncertainty of the measurement must be taken into account, care should be exercised to establish acceptance criteria that are consistent with historical results from a given

Drug Substance Property	In-Vivo Aspect
· Structure	· Structure-related Metabolism
· Ionization Constant, Partition Coefficient, Solubility	· Systemic Exposure
· Solution Stability	· In-vivo Degradation
· Hygroscopicity	· Hydrate Bioavailability
· Polymorphism	· Polymorph Bioavailability
· Particle Size	· Rate/Extent of Absorption
· Drug Purity	· Toxicity

Figure 6. Drug substance physico-chemical characteristics and their relationship to in vivo parameters.

process. The appearance of unusually large impurities may be a signal that the process is not performing as expected with the risk being the production of other new undetected impurities. Thus the development of acceptance criteria for impurities is a balance of process capability and analytical performance, with the upper limit set by the preclinical safety qualification studies. Fig. 6 provides an overview of drug substance attributes and their potential effect on in vivo attributes.

Drug Product

The formulation chosen for administration of the drug substance may impact the systemic exposure of the drug and therefore can impact the safety profile and ultimately the efficacy of the drug.[25] The role of the formulation in drug disposition should be explored in preclinical studies.

Excipients

The choice of excipients may effect the bioavailability of the drug, an important aspect especially for narrow therapeutic treatments. For example, the change from calcium sulfate to lactose in phenytoin capsules changed the apparent drug exposure in patients by greater than 40%.[50] Other examples in the literature demonstrate that the delivery vehicle may even influence the extent of formation of potential teratogenic metabolites.[51] Even the presence of a lake dye in a solid oral dosage form can impact the in vitro dissolution behavior of a formulation (with the potential to influence in vivo performance).[52]

Adequate characterization of the critical quality attributes of selected excipients is crucial to the formulation development process and success of early clinical trials. Trace impurities found in excipients can play a role in the stability of formulations. Impurities in excipients have been shown to be responsible for oxidative degradation of drug products.[53] In this example, oxidative deamination of an aminomethyl phenylalanine group occurred via the presence of oxidizing agent impurities in mannitol. Many compendial monographs do not include impurity profiles and, therefore, conformance to compendial requirements may not be sufficient for adequate characterization of the excipient.[54] In addition, many excipients are not manufactured exclusively for pharmaceutical use and may not have the quality requirements necessary to assure their safe use or consistent physico-chemical characteristics.[55] Noncompendial excipients, such as β-cyclodextrin, may have variable characteristics depending upon supplier, manufacturing process, and process controls.[56]

The identification of critical physico-chemical characteristics via compatibility studies allows for the development of methodologies to control those aspects of the excipient that are critical to product performance and that may ultimately affect the safety and efficacy of the drug.

Manufacturing Process

Adequate control of the manufacturing process in early clinical studies is important in assuring both safety in the clinic and the validity of data concerning the potential efficacy of the drug. Typically in early development, there is little or no known correlation between the physico-chemical characteristics of the drug product and its potential safety or bioavailability. It is important, therefore, that the method used to manufacture clinical supplies is well documented and controlled to assure that materials produced for early studies have reproducible characteristics. For example, many early clinical studies of low-solubility drugs or drugs with saturable absorption mechanisms are delivered in a cyclodextrin complex in order to increase the potential for absorption of the drug. The amount of bound and free drug in the complex can impact the availability of the drug, and therefore the process used to produce the complex must be reproducible in order to assure consistent in vivo performance.[57]

Specifications

Specifications consist of test methods and their associated acceptance criteria. Each specification plays a role in assuring the safety and efficacy of the product. The tests and acceptance criteria detailed below are applicable to all drug products.[45]

A description of the drug product provides a qualitative statement regarding the appearance of the drug product. The appearance specification provides a means to help ensure cGMP compliance with regard to assuring identity of the clinical supplies. In addition, the historical record of the appearance of the product establishes a qualitative means of tracing product performance to qualitative attributes of the product.

Identification testing should distinguish between the drug substance in the drug product and closely related compounds. Typically, two identification tests are performed with one test being the HPLC retention time match with a reference standard material. The second test is typically a spectroscopic technique such

as IR. For products with a stereoisomeric center, the confirmation of the appropriate isomer in the drug substance prior to compounding may be adequate unless the potential exists for the conversion of the drug substance to other stereoisomers within the formulation.

The most common assay procedures for drug products are titration methods and HPLC methods. If a titration method is employed for assay, an additional specific, stability-indicating method should be employed to control impurities in the drug product. The assay of the drug product is closely linked to potential safety concerns. For example, production of a degradation product during preparation of the drug product or during its subsequent storage may affect the safety of the product. Similar to the case with the drug substance, mass balance should be explored to assure that all degradation products have been tested in preclinical safety studies. The assay limits for the drug product may span a large range (e.g., 10–20%), again emphasizing the importance of mass balance in the assay analysis. Establishing mass balance may be difficult for a variety of reasons including lack of detection due to loss of the UV chromophores of the degradation product, loss of degradation product as volatiles, adsorption of the degradation product onto the container, elution or resolution difficulties, or a lack of an appropriate response factor for the degradation product.[58] The above examples demonstrate that the development and validation of stability-indicating methods is an important aspect of quality assurance for the drug product.

High-performance liquid chromatography methods are commonly used to control impurities in drug product. As discussed above, the methods should be specific and stability-indicating to assure appropriate safety margins. It is not sufficient to report ''meets specification'' with regard to impurity or degradation product levels.[59] Specific levels of impurities must be given in order to be able to access potential safety risks.

There are additional specifications that may be applicable depending upon the nature of the drug product. These specifications include:

Disintegration/dissolution/moisture—may impact rate and extent of absorption.
Residual solvents/microbial limits—may impact safety.

For drug product suspensions and solutions, additional physico-chemical characteristics of the drug product may impact the safety and/or the bioavailability of the drug product. These characteristics include pH of solution, particle size of suspended drug, clarity of solution (turbidity), color of solution, viscosity, volume of fill, and preservative testing.

CONCLUSIONS

There is an intrinsic link between the chemistry, manufacturing, and controls (CMC) attributes of the pharmaceutical product and the safety and efficacy of the clinical therapy. The quality, identity, purity, and strength of the drug substance and drug product provide a foundation for the demonstrated safety and efficacy of the dosage form. The degree of development of the various aspects of characterization, manufacture, and control will be dependent, in part, on the complexity of the drug substance molecule and dosage form as well as on the phase of the development program.

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