Active Pharmaceutical Ingredient Analysis

Active pharmaceutical ingredients (APIs) are the core of modern pharmaceutical products, and are also the basis of many innovative and effective therapeutic programs that have been applied and are being developed. To comply with these requirements and industry, clinical, and production best practices is absolutely necessary for any participant in the pharmaceutical product supply chain. Quality assurance, clinical trials and user assessment are key parameters for various stakeholders to review.

The latest USP/ USP-NF API analysis contains the identification of the chemical structure and the examination of validity, impurity and safety. Although USP now has removed and reduced properties test, but it can be used as a supplement to identification.

Identification

In APIs analysis, the intension of identification is not to test the exact structure or composition as the medicine requires. Identification is to test the authenticity of the certain batch which mostly requires specificity of the method. As the matter of this, USP uses only 2-4 methods for API identification. Chromatography is now in common use due to the pleasant properties. Chemical and spectrum are also used and perform eligible results as the USP standard.

In general, chemical drugs can be used as a conformation using HPLC (high performance liquid chromatography), GC (gas chromatography) and TLC (thin layer chromatography) according to

the required procedure. In any case, some chemical structures have significant vibration characteristics and can be tested by infrared spectroscopy as demonstrated by USP and ICHQ2.

Impurity

The quality of APIs is the key and source of drug quality control. The research and control of impurities is related to the clinical safety of drugs, so it becomes one of the key links of APIs quality control. Adverse drug reactions in clinical use depend not only on the pharmacological activities of the drug itself, but also on the impurities in the drug, which should be strictly controlled.

Impurities refer to the substances produced or introduced in the process of drug production, transportation and storage, which affect the purity of drugs. In ICH Q3A(R2) Drug impurities are classified into three categories, inorganic impurities, organic impurities and residual solvents. Among them, inorganic impurities mainly refer to the catalysts, inorganic salts, ligands, and reagents added in the preparation process of APIs; organic impurities mainly refer to the process impurities such as intermediates, by-products, and organic substances produced by the degradation, association or reaction between drugs themselves; residual solvents mainly refer to organic solvents in production.

According to the current state of the pharmaceutical industry, DNA reactive(mutagenic) impurities, elementary impurities and antibiotic impurities are the most concerned.

DNA Reactive (Mutagenic) Impurities

In recent years, the research and control of genotoxic impurities have attracted much attention. EMA, FDA and ICH have formulated corresponding guidelines for the identification, research and control of genotoxic impurities.

According to ICH M7(RI), the guidance for DNA Reactive (Mutagenic) Impurities, DNA reactive (mutagenic) Impurities can be classified into 5 groups.

Table 1 Impurities Classification With Respect to Mutagenic and Carcinogenic Potential and Resulting Control Actions

Class	Definition	Proposed action for control (details in Section VII (7) and VIII (8))
1	Known mutagenic carcinogens	Control at or below compound

Class	Definition	Proposed action for control (details in Section VII (7) and VIII (8))
		specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive,* no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

This kind of impurity can induce gene mutation and cause chromosome breakage and rearrangement at very low concentration, so researchers focus on the detection of these impurities in trace analysis means, as well as on the selection of highly specific, sensitive, reproducible and accurate analysis methods.

At present, many methods have been proved affective, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis-mass spectrometry (CE-MS), and liquid chromatography-nuclear magnetic resonance-mass spectrometry (LC-NMR/MS), and LC-MS is the most widely used.

Metal Impurities (Elemental Impurities)

In recent years, the concept of "metallic impurities" has gradually replaced the previously defined concept of "heavy metals"(USP231 ‘heavy metal’ to USP 232 ‘elementary impurities’), including some transition metals and quasi-metals, mainly targeting the residues of reagents, catalysts, ligands in the synthesis and production of drugs and the metal impurities introduced

into drug contact containers. EMA, FDA and ICH have promulgated the relevant guiding principles of metal impurities control, clearly put forward the classification and limits of metal impurities. ICH also sets up a special group of experts on metal impurities control, indicating that the control of such impurities has entered a new height.

USP classifies metal impurities into two categories; one is the metal that can or is likely to cause toxicity to the human body and cause serious environmental pollution, and the other is the metal impurities with lower toxicity but should be controlled.

Classification and limit control of metal impurities have put forward strict requirements for their analysis methods. Traditional heavy metal inspection methods can not meet the requirements of such impurities control. ICP-AES (inductively coupled plasma emission spectrometry), ICP-MS (inductively coupled plasma mass spectrometry) and GFAAS (graphite furnace atomic absorption spectrometry) are becoming more and more popular. USP has specified guidance to technique above.

Antibiotic impurity

Antibiotics are usually obtained through biosynthesis (microbial fermentation) and semisynthesis. Microbial fermentation process is distinctly different from the total chemical synthesis process. The synthesis process is not realized by the breakage and formation of chemical bonds in the reaction, but through the primary or secondary metabolism of microorganisms. This life process is where biological cells are based on their inherent genetic information.

Under the condition of culture, complex and subtle dynamic biochemical reactions are carried out. It is difficult for these metabolic processes to be regulated precisely and precisely as chemical reactions. The quality of products can only be controlled by controlling bacterial species, fermentation conditions and technological processes on the basis of studying the effects of various biological, physical, chemical and engineering environmental factors on the development, growth and metabolism of bacteria. Compared with the direct regulation of chemical reactions, this "indirect" process control method has relatively greater risks and difficulties in grasping the quality of drugs, resulting in more complex antibiotic impurity mass spectrometry than general chemical synthetic drugs . Even semi-synthetic antibiotics, starting from microbial fermentation products, have the characteristics of low purity, complex components, more active groups in the molecular structure (such as hydroxyl, amino, aldehyde, etc.), and most of them have configuration problems. As a result, a variety of by-products are produced. Compared with

purified chemically synthesized products, the analysis of hetero-mass spectra of antibiotic products is more complex, more difficult to predict and control.

Till now, FDA does not come up with a standard operational guidance for antibiotic impurity. But yet there is more detailed quality control method in antibiotic drug analysis. As EMA standard, all related compounds are required to use HPLC/MS and HPLC/DAD to give authentic results.

Validity

The validity test of APIs does not require the high quality as medicine as USP demonstrated. But still, the quality of APIs affects the drug quality in a most certain way. Inspection items include specific curl, absorbance, coefficient, ultraviolet wavelength, infrared spectra, X-ray examination etc. Here, we put a exceptional concern to Crystallographic Form.

The polymorphism of drugs is quite common. Different crystalline forms of the same polymorphic drugs may affect their bioavailability due to their different apparent solubility and dissolution rate, which may lead to different clinical effects. The state of a crystalline substance ensures consistency in the quality and clinical role of crystalline drug products.

Single crystal X-ray diffraction method (SXRD) is used only for APIs Crystal type identification in either Absolute method and comparative method. The state identification of solid crystalline substances can be achieved by changing the composition (compound, crystal water or solvent), cell parameters (a, b, c, a, a, beta, v), molecular symmetry (crystal system, space group), molecular bond and mode (hydrogen bond, salt bond, coordination bond), molecular conformation and so on. Powder X ray diffraction (PXRD) is suitable for identification of crystalline and crystalline, crystalline and amorphous, amorphous and amorphous crystals and other crystalline materials. Differential scanning calorimetry (DSC) and IR is suitable for the identification of crystalline substances with different crystalline solvents (or water) or with different amounts and types of crystalline solvents (or water) in the test products.

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