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Identification in drug quality control and drug research

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ABSTRACT

Identification is an important step in the quality control of drugs and the research for new drugs. First, this review discusses the identification of bulk drugs and the active ingredients in formulations, based mainly on pharmacopoeial tests. The most important methods for this purpose are infrared (IR) and, to a lesser extent, ultraviolet (UV) spectroscopy, as well as retention matching with standards using high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). The identification of impurities and degradants is based mainly on HPLC-UV, HPLC-mass spectrometry (HPLC-MS) and nuclear magnetic resonance (NMR) spectroscopies. The above methods are also used for identification purposes in drug research. The use of MS and NMR in the research for large-molecule drugs of biotechnological origin and natural products, mainly of plant origin, with special respect to traditional Chinese (and Indian) medicines is also discussed. The review concludes with the identification aspects of the fight against counterfeit drugs.

Contents

1. Introduction

Identification is the first step in the complex procedure of drug quality control and in the quality aspects of the search for new synthetic drugs and natural products. It is also an important tool in the fight against counterfeiting of drugs. It is sometimes difficult to find the borderline between identification and structure elucidation. The European Pharmacopoeia presents a good answer to this question: "*The tests given in the Identification section are not designed to give a full confirmation of the chemical structure or composition of the product; they are intended to give confirmation, with an acceptable degree of assurance that the article conforms to the description on the label*" [1].

Although, especially in the case of new drugs, drug research is the first step followed by production and quality control of the drug, in this review, the role of identification in drug quality control is dealt with first, because this issue is more important when drugs already exist, and, in drug research, structure elucidation is more important than identification.

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2. Identification in drug quality control

2.1. Identification of bulk drugs

This section is mainly based on the latest editions of the European Pharmacopoeia (Ph. Eur.) [1], which is the basis of the pharmacopoeias of the European Union (EU) countries, the US Pharmacopeia (USP) [2] and the Japanese Pharmacopoeia [3].

The monographs of bulk drugs in pharmacopoeias and in other main documents (e.g., Drug Master Files) usually begin with the identification test(s) because simple but reliable identification is of great importance in avoiding the danger of drugs from the manufacturing pharmaceutical company being confusing to the pharmacy where they are sold.

2.1.1. Classical color or precipitation reactions

A few decades ago, when spectroscopic and chromatographic methods were not yet developed enough for drug-identification purposes, usually test-tube methods based on color and precipitation reactions were used for this purpose. Some of these are still used in USP, as a possibility to be used in less developed places as test *B*, *C* or *D*, where *A* is a more up-to-date method. This is characteristic of the treatment of classical drug materials. For example, in the case of morphine sulfate, the following methods are still official in the latest edition of USP [2]: *"**B:** To 1 mg in a porcelain crucible or a small dish add 0.5 mL of sulfuric acid containing, in each mL, one drop of formaldehyde TS: an intense purple color is formed at once, and quickly changes to deep blue-violet (distinction from codeine, which gives at once an intense violet-blue color, and from hydromorphone, which gives at first a yellow to brown color, changing to pink and then to purplish red.) **C:** To a solution of 5 mg in 5 mL of sulfuric acid in a test tube add 1 drop of ferric chloride TS, mix, and heat in boiling water for 2 minutes: a blue color is produced, and when one drop of nitric acid is added, it changes to dark red-brown (codeine and ethyl morphine give the same color reactions, but hydromorphone and papaverine do not produce this color change). **D:** A solution (1 in 50) responds to the test of Sulfate <191>."].*

More or less the same applies to the Ph. Eur. In this case sets of First and Second Identification are included in many monographs: *"Certain monographs have subdivisions entitled 'First identification' and 'Second identification'. The test or tests that constitute the 'First identification' may be used in all circumstances. The test or tests that constitute the 'Second identification' may be used in pharmacies provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the monograph. Certain monographs give two or more sets of tests for the purpose of the first identification, which are equivalent and may be used independently".*

In the case of morphine sulfate, the above-mentioned sulfate test is included in the First and the formaldehyde-sulfuric acid test in the Second Identification set.

The sulfate test (precipitation with barium chloride) appears in both present official pharmacopoeias. While the importance of the color and precipitation reactions has greatly decreased and is replaced by modern spectroscopic and chromatographic methods to be discussed in the following sections, some of these tests are rather widely used, mainly in the identification of salts based on their counter-ion.

In addition to the above-mentioned sulfate test the identification of chlorides as silver-chloride precipitate is still widely used.

In addition to these, the Ph. Eur. contains identification tests based on color or precipitation reactions to the following anions, cations and some other items: acetate, acetyl group, alkaloids, aluminum, amines (primary aromatic), ammonium, antimony, arsenic, barbiturates, benzoate, bismuth, bromide, calcium, carbonate and bicarbonate, citrate, esters, iodide, iron, lactate, lead, magnesium, mercury, nitrate, phosphate, potassium, salicylate, silicate, silver, sodium, tartrate, xanthines and zinc. The USP contains tests for the same items with the addition of some others, such as barium, borate, chlorate, cobalt, copper, hypophosphite, lithium, manganese, nitrite, oxalate, permanganate, peroxide, sulfite, thiocyanate and thiosulfate.

2.1.2. Spectroscopic methods

Infrared spectroscopy (IR) is undoubtedly the most widely-used identification test for bulk pharmaceuticals in all modern pharmacopoeias and other documents, since this method is rapid and, because IR spectra are very rich in bands of different intensities as a function of the functional groups and their structural environment, it is highly characteristic. In the majority of cases, the potassium-bromide disc method is used: the spectrum of the investigated material should be identical with that of the Reference Standard, obtainable from the related Pharmacopoeia Commissions. It is worth mentioning that, in the case of the Japanese Pharmacopoeia [3], it is not important to scan the spectrum of the Reference Standard, since this pharmacopoeia contains 560 IR spectra for comparison purposes.

Polymorphism, frequently occurring among pharmaceuticals, causes a problem that is not too serious when using IR spectroscopy for identification purposes, since there are minor differences between the IR spectra of the polymorphic modifications taken in the solid state. In the majority of cases, the bioavailability of the pharmaceuticals does not greatly depend on the polymorphic modification. For this reason, in a case of observing minor difference between the spectra of the tested sample and of the Reference Standard, all pharmacopoeias usually prescribe repetition: the two samples should be dissolved in the same volatile solvent and, after evaporation to dryness, the spectra should be scanned again. In the case of equivalence of the spectra, the test is successful.

Although less generally used than IR, ***ultraviolet spectroscopy (UV)*** is also an important method for the identification of drugs in pharmacopoeias. No doubt that the UV spectrum is less characteristic of the investigated compound than the IR spectrum. To increase the reliability of this test, it is sometimes prescribed that the absorbance at the given maximum value should be within ±3% of that of the Reference Standard and, in some cases, the absorbance ratio at two characteristic wavelengths is also limited. An example for the latter is atenolol in the Ph. Eur. [1], where the absorbance ratio at the two characteristic maxima of the phenol-ether moiety (A_{275}/A_{282}) should be within the limits 1.15–1.20.

Nuclear magnetic resonance spectroscopy (NMR) is only used in a few cases in pharmacopoeias for identification purposes, especially for large molecules. Examples are heparin, goserelin and buserelin in the Ph. Eur., and heparin, enoxaparin and oxytocin in the USP.

The determination (with limits) of the ***optical rotation***, mainly at the sodium D-line at 589,3 nm is part of the identification of many optically-active compounds in Ph. Eur., while this is one of the other tests in the USP and the Japanese Pharmacopoeia outside the Identification tests. (The same applies to the non-spectroscopic method, determination of the ***melting point***, also with limits.).

2.1.3. Chromatographic methods

In addition to the progress in spectroscopy, the developments within chromatography [4] have also made great changes in the identification of drugs.

High-performance liquid chromatography (HPLC) is the most generally used method for the assay of bulk drug materials in the USP with lesser but still important contribution in Ph. Eur. and the Japanese Pharmacopoeia. Although the value of the results obtained by HPLC is questionable due to precision problems when these