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CHAPTER 1

GENERAL METHODS AND TECHNIQUES OF PHARMACEUTICAL DRUGS QUALITY CONTROL

CHEMICAL TESTS FOR DRUG IDENTIFICATION

Identification means confirming with an acceptable degree of certainty that a drug, excipient, or material corresponds to the information on the label. Chemical and physicochemical methods of analysis are used for this purpose.

Chemical methods (qualitative reactions) have a long history of use in pharmaceutical analysis. However, due to the rapid development of instrumental methods, chemical tests were largely displaced from practice. Nowadays, infrared spectroscopy is the priority method for confirming the identity of drugs. UV spectroscopy and chromatographic techniques are also widely used.

Chemical identification tests included up to date in Pharmacopoeias are relatively simple, they do not require expensive equipment, the conditions of their implementation are well studied and described. Therefore, qualitative chemical reactions are often more simple and reliable compare to instrumental methods of analysis.

Identification requires a chemical reaction to be specific, i.e., able to detect a substance or ion in the presence of other substances unambiguously. However, highly specific reactions are relatively rare, so group-selective tests are used more often. In this case, the test provides for additional steps to identify the substance unambiguously. For example, we can determine solubility or insolubility of the precipitate, color change or decolorization after a certain reagent is added. The test sensitivity is the minimum amount of a substance or an ion in a sample that can be detected under the specified test conditions. In the pharmaceutical analysis, when performing an identification test, sensi-

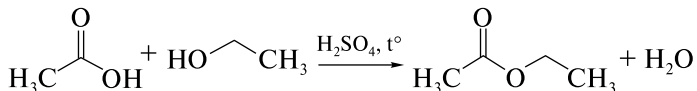
tivity is less important than specificity and selectivity because the substance or the ion is usually present in high concentration in the sample.

Minor fluctuations and changes in any reaction conditions inevitably arise during laboratory practice. Reproducibility and ruggedness of the method are highly required. Therefore, the Pharmacopoeia always clearly and unambiguously regulates the order of reagents addition, concentrations of solutions and other reaction conditions. Moreover, the Pharmacopoeia provides short, but clear and comprehensive expected results descriptions.

Many drug substances contain the same ions or functional groups. There are unified methods of identification for these ions and groups. The general monograph "General identification tests" has been introduced to avoid repeating the same methodology in each monograph. This general monograph exists in all Pharmacopoeias. For example, if a drug contains an ion or a functional group with a unified method of identification, a monograph will refer to the methodology in the general monograph. In some cases, there is also a brief explanation for the test solution preparation.

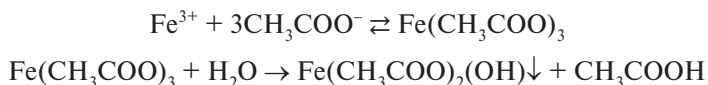
Further, we will discuss the tests included in the general monograph "General identification tests" in SPhRF XIV, Ph. Eur. 10, and Ph. EAEU. Additionally, there will be given some remarks about similar tests particularities according to USP 43 and Ph. Int 10.

Acetates. Acetates are identified after esterification with ethanol. Ethyl acetate has the specific apple odor.



The test is described only in the SPhRF. This reaction identifies both acetate ion and organic acetyl group.

Another test for acetate ion is included in the SPhRF, the Ph. EAEU and the USP. Ferric (III) chloride solution is added to the neutral acetate solution that yields red solution of ferric acetate or hydroxyacetate, which is destroyed by the addition of mineral acids.



There is one more test for acetate ion included in the USP, the Ph. Eur. and the EAEU. If lanthanum nitrate solution, diluted ammonia solution and iodine are added to acetate test solution, then heated until boiling, the formation of a blue precipitate or a blue coloration of the solution indicates a positive

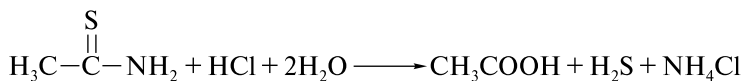
reaction. The mechanism of the reaction is not quite clear, but since iodine forms blue complexes with many colloidal solutions of substances, including starch, one theory is that the physical colloidal structure of the lanthanum basic acetate precipitate is responsible for the ability to form the complex.

The Ph. Eur. and the Ph. EAEU also suggest the reaction with oxalic acid. The test solution heated with the oxalic acid solution liberates acetic acid vapors indicated by the specific odor and acidic reaction.

Acetyl (acetylated substances). The test identifies the substance to be examined as a compound containing an acetyl group that upon acidic hydrolysis yields acetic acid. The substance undergoes hydrolysis when heated with phosphoric acid solution. The acetic acid vapor is condensed followed by the reaction with lanthanum nitrate and iodine (see Acetates). This test is included in the Ph. Eur., the Ph. Int. and the Ph. EAEU.

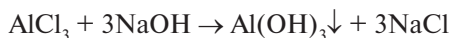
Aluminum. In the first step of analysis, aluminum salt solution is acidified with hydrochloric acid, and thioacetamide solution is added. No precipitate is formed. In the second step, sodium hydroxide solution is added dropwise. A gelatinous white precipitate is formed which dissolves on further addition of sodium hydroxide. Then ammonium chloride solution is added gradually. The gelatinous white precipitate is re-formed.

In the acidic environment, thioacetamide will liberate sulfide ions by hydrolyze, thus it is used as a source of S^{2-} ions.



Aluminum cations do not react with sulfide ions at the low pH, but heavy metal cations would react and corresponding sulfide will be precipitated.

Then, sodium hydroxide solution is added dropwise that leads to the formation of the white gelatinous precipitate of aluminum hydroxide:



Hydroxide precipitation reaction is non-specific because most of metal cations are precipitated with hydroxide ion. However, heavy metal ions would have been precipitated earlier, when the thioacetamide solution is added, so this test is specific for aluminum.

Owing to aluminum's ability to form amphoteric hydroxides, the aluminum hydroxide precipitate dissolves on the further addition of sodium hydroxide as aluminum hydroxo complex is created:



The precipitate of aluminum hydroxide can be reformed by the addition of ammonium chloride solution. This is a result of the downward shift in pH caused by the acid reagent.



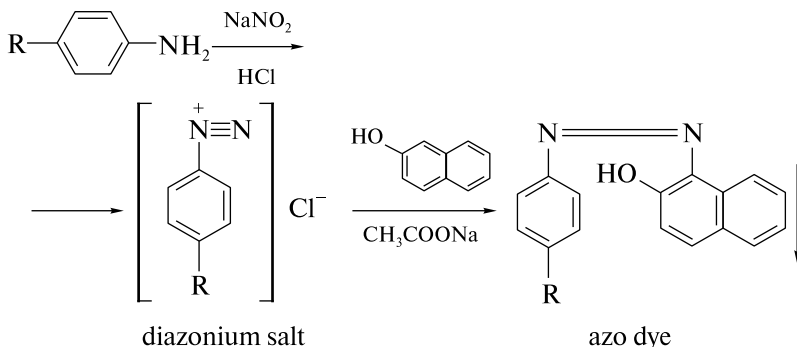
Due to aluminum amphoteric properties, the test is selective. Zinc cations form zinc hydroxide reacting with sodium hydroxide, but would stay in solution when ammonium chloride is added because of formation of soluble complex $[\text{Zn}(\text{NH}_3)_4]^{2-}$.

There is no thioacetamide adding in the USP tests for aluminum. Solutions of aluminum salts yields a gelatinous, white precipitate that is insoluble in an excess of the reagent when ammonium hydroxide solution is added.

The aluminum hydroxide is precipitated by adding sodium hydroxide and dissolves in an excess of the reagent.

Amines, primary aromatic. The reaction with nitrous acid and coupling with β -naphthol yields azo dye and specific for primary aromatic amines.

Acidified with hydrochloric acid test solution with is heated, if necessary, then cooled in ice, and sodium nitrite solution is added. An intense yellow-orange to orange-red precipitate is formed by adding this mixture to the β -naphthol alkaline solutions, containing sodium acetate.



Sodium acetate shifts pH close to neutral leading azo dye precipitation. The Ph. Eur. does not consider adding sodium acetate, thus the colored solution is obtained instead of the precipitate. There are no tests for primary aromatic group in the USP.

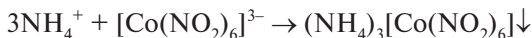
Ammonium salts. According to the Ph. Eur., the USP, the EAEU and Ph. Int., ammonia is liberated from ammonium salts by magnesium oxide:



Then ammonia gas passes through hydrochloric acid solution:

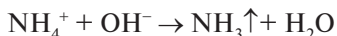


The last step of the ammonium identification is the reaction with sodium cobalt nitrite solution, which yields yellow precipitate of ammonium hexanitrocobaltate (III).



This step eliminates a false positive reaction for volatile bases making the test specific for ammonia.

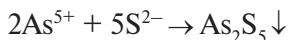
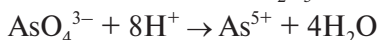
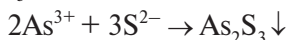
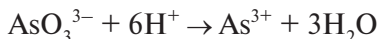
Ammonium salts and salts of volatile bases. Ammonium salts heated with sodium hydroxide solution yield ammonia, which can be identified by its characteristic smell and its alkaline reaction to litmus paper.



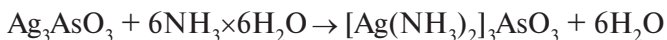
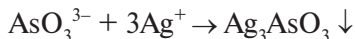
The Ph. Eur., the USP, the Ph. EAEU and the Ph. Int. suggest this reaction for salts of volatile bases.

Arsenic. Arsenic compounds may contain arsenic (III) or arsenic (V), so there are provided identification test for forms in the SPhRF.

Sodium sulfide solution is added to arsenates or arsenite solutions acidified with hydrochloric acid. The yellow precipitate is observed, which is insoluble in concentrated hydrochloride acid, but forms a soluble complex with ammonia solution:



Both ions will give a precipitate with silver nitrate solution, but precipitate color is different. AsO_3^{3-} ions form the yellow precipitate, which dissolves in nitric acid and forms a complex in ammonia solution:

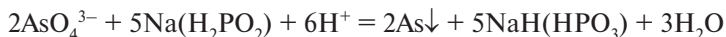
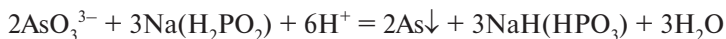


AsO_4^{3-} ions form the brown precipitate, Ag_3AsO_4 , which also dissolves in nitric acid and forms the complex, $[\text{Ag}(\text{NH}_3)_2]\text{AsO}_4$, in ammonia solution.

Arsenates are precipitated with magnesium and ammonium ions. A white crystalline precipitate is formed. It dissolves in diluted hydrochloric acid. This reaction is specific for AsO_4^{3-} ion:



Arsenates and arsenites form the brown precipitate of colloidal metallic arsenic when disodium phosphate is added (Ph. Eur.):



Benzoates. Benzoates in neutral solution yield a dull-yellow precipitate with ferric chloride. The precipitate dissolves in ether:



This test is included in the Ph. Eur., the USP, the SPhRF and the Ph. EAEU.

Benzoic acid releasing from salts takes part in plenty of pharmacopoeial tests.

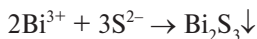
According to the Ph. Eur. and the Ph. EAEU, benzoates are heated with concentrated sulfuric acid and a white sublimate is deposited on the inner well of a tube.

There is one more test: the benzoic acids is precipitated from salts by hydrochloric acid. The melting point of the precipitate is determined after crystallization from water and drying *in vacuo*.

According to the USP, benzoic acid is precipitated by adding sulfuric acid. The precipitate dissolves in ether.

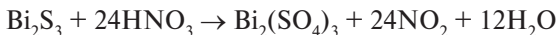
Bismuth. Bismuth (III) salts are hydrolyzed in water, but adding mineral acids inhibit hydrolysis, thus mineral acids are required for all bismuth identification tests.

According to the SPhRF, acidified bismuth (III) ion solution reacts with sulfide ions. The brown precipitate is formed.

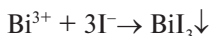


The hydrolysis reaction of bismuth is included in the USP, the Ph. Eur., the Ph. Int. and the Ph. EAEU. Acidified by hydrochloric acid the test solution is heated to boiling, then diluted with water upon a white or slightly yellow precipitate of bismuth oxychloride is formed. Finally, sodium sulfide solution is added to the BiOCl precipitate. The precipitate turns brown, owing to the

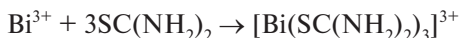
formation of bismuth sulfide. This precipitate is insoluble in the most of mineral acids, except for concentrated nitric acid:



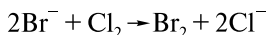
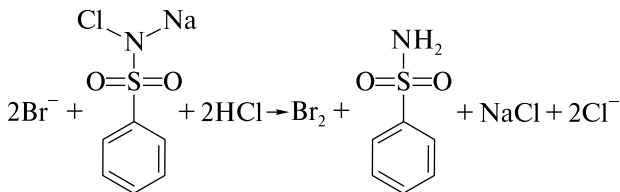
There is a reaction with potassium iodide in the SPhRF and the Ph.Int. soluble bismuth (III) salts are precipitated with potassium iodide solution. The black precipitate dissolves on the further addition of the reagent as an orange-yellow tetraiodobismuthate complex is created.



There is another complexation reaction in the Ph. Eur. and the Ph. EAEU. Bismuth (III) ions form a complex with thiourea. The solution turns yellowish-orange color. The solution should not be decolorized after sodium fluoride is added.



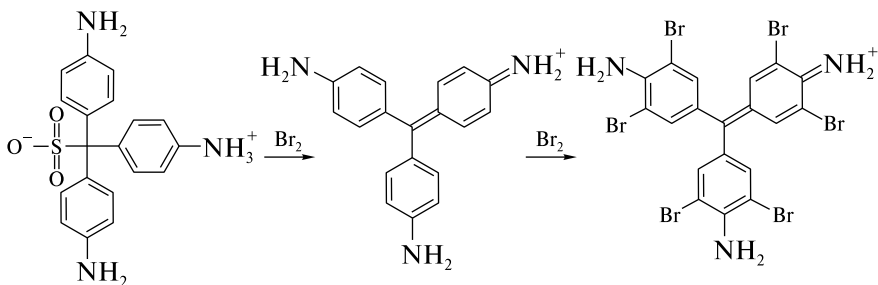
Bromides. Bromides are identified undergoing redox reactions yielding free bromine. Oxidizers can vary depends on Pharmacopoeias. The SPhRF suggests the chloramine B for this purpose, at the same time the USP and the Ph. Int. prefer chlorine solution. The liberated bromine is extracted by chloroform. The chloroform layer turns yellow-brown.



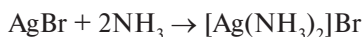
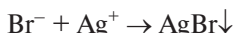
The Ph. Eur. and the Ph. EAEU suggest bromides to oxidize by adding lead dioxide in the acidic environment:



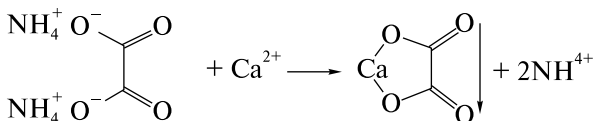
The bromine vapor colors a strip of filter paper impregnated with decolorized fuchsin solution, liberating fuchsine, followed by further reaction with bromine. Violet color appears within 10 s.



Bromides reaction with silver nitrate solution is widely used in Pharmacopoeias (SPhRF, USP, Ph. Int., Ph. Eur. and Ph. EAEU). A curdled, pale yellow precipitate is formed. The precipitate is insoluble in nitric acid, but dissolves in concentrated ammonia solution with difficulty.

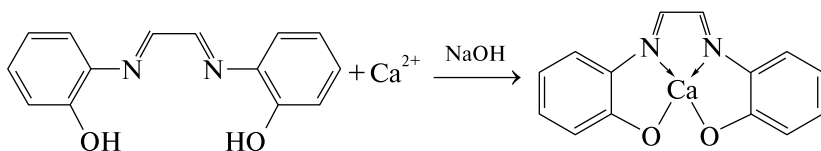


Calcium. Calcium salts are precipitated with oxalate ions. A white precipitate is formed, which is insoluble in acetic acid and ammonia solution, but soluble in diluted mineral acids (SPhRF, Ph. EAEU, USP, Ph. Int.):



Soaked in hydrochloric acid calcium salts produce an intense red color when the solution is introduced into a nonluminous flame (SPhRF).

Calcium ions create a reddish-brown precipitate with glyoxal hydroxyanil solution. The precipitate dissolves in chloroform to give a red solution (Ph. Eur, Ph. Int.).



Calcium salts form a white precipitate with potassium ferrocyanide solution upon the addition of ammonium chloride. The precipitate is not formed in the acetic acid environment (Ph. Eur):

