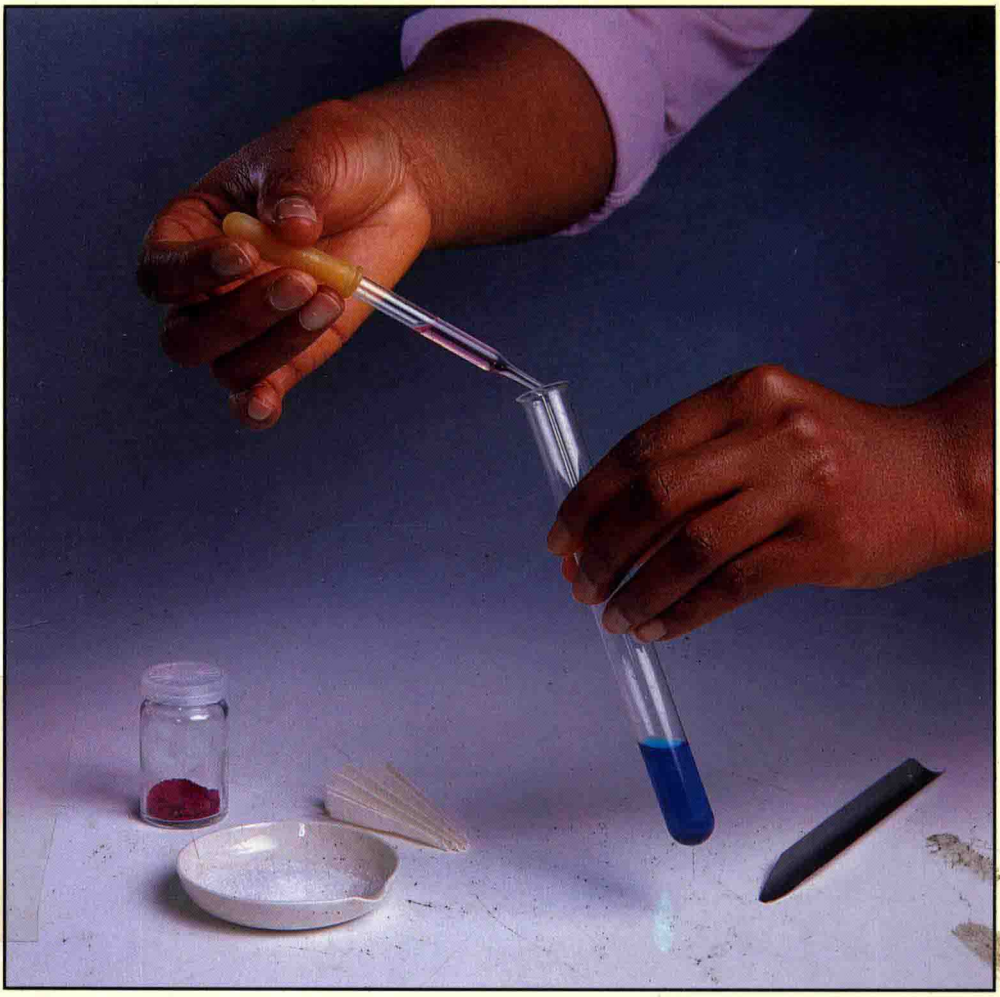
BASIC TESTS FOR PHARMACEUTICAL SUBSTANCES



World Health Organization, Geneva

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The tests described in this manual are intended only to verify the identity of pharmaceutical substances and to detect or exclude gross degradation. They should not be used to replace the monographs contained in the *International Pharmacopoeia*. As a first step, tests have been devised for the majority of drug substances contained in the WHO Model List of Essential Drugs as well as for a number of other widely used drug substances. Analogous tests for substances in dosage forms are in preparation.

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1. INTRODUCTION

The simplified tests (basic tests) for drug substances described in this manual represent one element of quality assurance in the pharmaceutical supply system.

They have been devised with the following objectives:

(*a*) to provide a simple and readily applicable method for verifying the identity of a drug substance, using a limited range of widely available reagents, when the labelling and physical attributes of the substance do not provide adequate confirmation;

(*b*) to provide a practicable means for confirming the identity of a drug, when a fully equipped laboratory is not available;

(*c*) to indicate if gross degradation has occurred in certain substances that are known to decompose readily under adverse conditions.

Basic tests are not, in any circumstances, intended to replace the requirements of pharmacopoeial monographs. The latter give an assurance of quality whereas basic tests merely confirm identity.

In several European countries, simple tests have already been endorsed by the national pharmaceutical associations for use at peripheral levels of distribution (wholesale premises, pharmacies) to verify the identity of pharmaceutical substances whenever the possibility of confusion arises and sometimes to exclude gross degradation or adulteration.

Degradation during storage and transportation is of particular importance in tropical countries. Indeed, an expiry date determined for a temperate climate may be inappropriate in a tropical region even when adequate containers are used. For this reason, the stability characteristics of most of the substances referred to in this manual have been determined and tests to indicate gross degradation are presented for the least stable substances.

Basic tests need not be carried out by fully qualified pharmacists or chemists, but they should be performed by persons who have some understanding of analytical chemistry such as that acquired in courses for pharmaceutical assistants.

Several tests are described for most substances. Not all of these need to be applied to any one sample. One test concerned with melting

behaviour, together with two test-tube reactions, will suffice in most circumstances. If, however, there is any reason to suspect that the product is mislabelled or substandard, all tests described should be performed. By their nature, simplified tests cannot be completely reliable. An adverse result, even in one test, should be taken as a warning of potential unsuitability of a drug. In these circumstances, a final conclusion should not be drawn until a full analytical examination has been carried out in a properly equipped analytical laboratory.

The reagents and equipment required for these tests have been kept to a minimum. Reagents that are unstable, corrosive, expensive or difficult to obtain have been excluded.

The use of visual or similar characteristics for initial assessment is self-evident. Attributes such as colour and characteristic odour of the sample should always be noted.

Some of the individual tests, such as those referring to a change in the physical aspect of the test substance, call for comparison against a standard. Each laboratory where basic tests are routinely performed should therefore retain a collection of authentic samples of frequently analysed substances. Such a collection may be established from small amounts of substances previously tested and found to be satisfactory. These provide a standard of comparison both for visual characteristics and test-tube reactions.

In general, the following types of test have been preferred:

—classical chemical techniques such as colour reactions, the formation of precipitates with specific reagents, the evolution of gas and its identification, and the behaviour of substances on heating. In some instances these reactions lack specificity since drugs with similar functional groups may not be distinguishable by simple chemical reactions;

—the appearance of a concentrated solution of a substance in selected solvents. This can be useful for detecting degradation products and the presence of some other impurities.

The techniques used to determine melting characteristics are described in particular detail. They provide a basis not only for confirming the identity of a substance but also for detecting possible contamination, whether arising from poor manufacture, adulteration, cross-contamination during storage, or degradation.

The capillary method has been selected for use in basic tests. Other techniques, such as the microscopic hot bench or melting block, are applicable, but the results of different methods are not directly comparable.

It should be noted that the terms used to describe the melting characteristics are different from those adopted in the *International Pharmacopoeia*, since pharmacopoeial specifications are based on more rigorous methods.

Basic tests for finished pharmaceutical forms are planned to follow this publication.

Comments on the tests described are invited and should be addressed to:

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1211 Geneva 27,
Switzerland.

2. DETERMINATION OF MELTING CHARACTERISTICS

2.1 Determination of melting point

2.1.1 *Definition*

The melting point is determined in a capillary tube. The expression "melts about . . . " means that the temperature at which the substance is completely melted, as indicated by the disappearance of the solid, will be in the range ±4 °C from the stated value, unless otherwise indicated.

2.1.2 *Details of the procedure*

The following technique is adequate for the determination of melting point:

Grind about 50 mg of the substance to be tested in a small mortar. Place the ground substance in a vacuum desiccator over silica gel or phosphorus pentoxide at room temperature and dry for about 24 hours (unless another drying procedure is given in the test sheet). Place the substance in a dry capillary tube of 1-mm internal diameter forming a column about 3 mm high. Heat the melting-point apparatus to a temperature 5–10 °C below the expected temperature of melting and adjust the heating so that the temperature in the chamber rises about 1 °C per minute. Introduce the capillary with the substance into the heated chamber, and note the temperature when the sintered substance becomes completely transparent; this is considered to be the melting point, as defined in section 2.1.1.

2.1.3 *Discussion*

The difference between the purely theoretical definition of the melting temperature and the results obtained in practice is now widely recognized. A precise physical definition exists only for the so-called triple point, i.e., the temperature at which all three phases (solid, liquid and gaseous) are in equilibrium. The measurement of the triple point is done in a highly complicated experiment. Many compendia do not use this temperature, but describe melting intervals as observed in practice,

when the formation of droplets, the softening of the substance or its sintering are considered to be the beginning of the melting process, while the formation of a clear and transparent drop of liquid is taken to be the end of the melting process.

In the case of pure substances that melt without decomposition, the beginning of melting can be observed with some certainty. For impure substances, the beginning of the melting process will vary, depending on the nature of the impurities. Therefore it has been proposed that in the basic tests the following definition of melting point be used. This definition is similar to that used in the *International pharmacopoeia*,[a] to describe melting temperature:

> The melting point denotes the temperature at which the substance has just completely melted; this is indicated by the disappearance of the solid phase and complete transparency of the melt.

This approach has the disadvantage that, if impurities are present, their presence can only be deduced from the lowering of the melting-point value, as no observation is made of the melting interval. An increase in the latter usually indicates low purity of a substance. These considerations, however, have less importance for basic test identification, where this disadvantage is fully offset by increased reproducibility of the values of melting point determined according to the above procedure.

2.2 Melting behaviour

2.2.1 *Definition*

The expression "melting behaviour" used in the basic tests denotes the melting point of substances that melt with decomposition. It is also used for melting points above 250 °C to indicate that the reproducibility of the value may be low.

2.2.2 *Discussion*

It is necessary to bear in mind that a difference exists between true melting points (or melting ranges) and the temperature of decomposition. Ideally, in the case of a true melting point, no chemical change occurs in the substance. However, when some substances are heated, decomposition takes place either before or during the process of melting, being indicated by a change in the colour of the substance or by the evolution of a gas. In such situations, the observed temperature of

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[a] *The international pharmacopoeia*. Third edition. Volume 1: *General methods of analysis*. Geneva, World Health Organization, 1979; Volume 2: *Quality specifications*. Geneva, World Health Organization, 1981.

melting is not a true melting point of the substance but the melting point of a mixture with decomposition products. It is obvious that the temperature of decomposition cannot be considered as a physical property of a substance as the amount of decomposition products, and consequently the temperature of decomposition, depend on the length of the period of heating and therefore have low reproducibility, even if a standardized procedure is used.

2.3 Determination of eutectic temperature

2.3.1 *Definition*

Eutectic temperature is given as a single value only and designates the beginning of melting, i.e., the temperature at which the solid collapses or forms drops on the wall of the capillary tube. The mixture to be used in the test is usually prepared by thorough mixing of approximately equal parts of the test substance and the accessory substance, unless the use of strictly equal amounts of both substances is specially indicated in the test procedure.

2.3.2 *Details of the procedure*

The following technique is adequate for the determination of eutectic temperature:

Grind equal parts (by weight) of the substance to be tested and the accessory substance, both of them previously dried for about 24 hours at room temperature in a vacuum desiccator over silica gel or phosphorus pentoxide. Fill a dry capillary tube, of 1-mm internal diameter, with the mixture, forming a column about 3 mm high. Heat the melting-point apparatus to a temperature 5–10 °C below the expected temperature of melting and adjust the heating so that the temperature in the chamber rises about 1 °C per minute. Introduce the capillary with the mixture into the heated chamber, and note the temperature at which the solid collapses or forms droplets on the wall of the capillary tube.

2.3.3 *Discussion*

The measurement of eutectic temperature has been introduced in the basic tests as an additional criterion of identity. An exact determination of the eutectic melting point requires a set of measurements carried out on mixtures prepared in different ratios. The eutectic melting point thus measured is thermodynamically exactly defined and may be used as a criterion of both identity and purity. Such a procedure is not, however, practical for the basic test project, as it requires a long time and adequate laboratory facilities. For the purpose of basic tests, the determination is carried out at a constant ratio of 1:1. This has the disadvantage that in some cases the melt will not become transparent, so

that the reproducibility of the measurement is low owing to individual errors. Nevertheless, the eutectic temperatures given in the basic tests are usually reproducible to within ±5 °C.

It should be noted that during eutectic temperature determination the beginning of the process of melting is observed, whereas during melting-point determination it is the end of the process that is surveyed.

2.4 Mixed-melting point

2.4.1 *Procedure*

The determination of a mixed-melting point is carried out in a glass capillary as described in section 2.1.2. Equal amounts of the substance to be tested and the authentic substance are mixed and placed in a capillary. A separate capillary is filled with the substance to be tested and a further capillary with the authentic substance. All three capillaries are simultaneously heated in the melting-point apparatus. The melting point of the mixture should not differ by more than ±4 °C from the melting points of the single substances.

2.4.2 *Discussion*

Although mixed-melting-point determinations are not included in the basic tests, this procedure is a highly reliable criterion in deciding whether two substances are identical. The general introduction of mixed-melting-point determination as an identity test would require a wide accessibility of appropriate reference substances, which can sometimes only be arranged on a national basis. Each laboratory can, however, gradually create for itself a collection of authentic substances from incoming consignments of materials of good quality and can then use the mixed-melting point as a strong additional criterion of identity. Such a collection, once established, may further be used in identity tests using the thin-layer chromatography technique.

2.5 Melting-point apparatus

2.5.1 *Type of apparatus*

A number of types of melting-point apparatus are produced. A review of those that are commercially available is given by Büchi & Hasler.[a]

The apparatus employed in the determination should be equipped with a magnifying glass, have a controlled heating arrangement that

[a] BÜCHI, J. & HASLER, C. *Pharmaceutica acta Helvetiae*, **49**: 47 (1974).

permits a heating rate of 1–2 °C/min around the temperature of melting, and be equipped to be used with capillaries of 1-mm inner diameter.

The heating arrangement can take the form of a stirred bath, such as the Thiele apparatus and its modifications,[a] or a heated block, e.g., the Lindström or Culatti modifications.[b]

2.5.2 *Calibration of thermometers*

For the various measurements of melting characteristics to be of any value, it is essential to use accurate thermometers. The thermometer used should preferably be certified by a duly recognized body. Alternatively, it could be calibrated against such a thermometer. Another method of checking the accuracy of the thermometer is by measuring the melting points of a set of WHO melting point reference substances using a 1-mm capillary; if the observed melting points of the reference substances lie within ±2 °C of the melting temperature indicated for that substance, the thermometer may be considered satisfactory. An important requisite, however, is that the geometrical arrangement of the thermometer and capillaries in the apparatus is practically identical in every determination. The length of the column of mercury in the thermometer exposed to room temperature can introduce significant error particularly at high temperature. It is therefore desirable to use thermometers with narrow ranges of temperature such as 0–110 °C, 110–210 °C or 200–300 °C. If this is not possible, a correction factor should be introduced according to the formula given in the *International pharmacopoeia, third edition* (volume 1, p. 22).

2.6 Heating behaviour

The expression "heating behaviour" used in the basic tests denotes the behaviour of the substance (such as colour changes or evolution of gas) when heated in an open test-tube in a flame or in an electrical heater.

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[a] SKAN, E. L. & ARTHUR, J. C. JR. In: Weissberger, A., ed. *Technique of organic chemistry*, New York, Interscience, 1971, vol. I, p. 105.

[b] KIENITZ, H. In: Houben-Weyl, *Methoden der organischen Chemie*, Stuttgart, Georg Thieme Verlag, 1953, Vol. II, p. 788.

3. TESTS

ACETAZOLAMIDE

Identity tests

Description. A white, or almost white, crystalline powder; odourless.
Melting behaviour. About 255 °C with decomposition.
Colour and other reactions
1. Triturate 0.5 g with a mixture of 5 ml of water and 0.5 ml of sodium hydroxide (~ 80 g/l) TS, add 0.2 g of zinc R powder and about 0.5 ml of hydrochloric acid (~ 420 g/l) TS; hydrogen sulfide is evolved and may be detected by its odour (proceed with caution), or by the use of a strip of lead nitrate paper R, which turns black on exposure.
2. Dissolve 25 mg in 5 ml of water and add 0.10 ml of sodium hydroxide (~ 80 g/l) TS and 0.05 ml of copper(II) sulfate (160 g/l) TS; a bluish green colour or precipitate is formed.

ACETYLSALICYLIC ACID

Identity tests

Description. Colourless crystals or a white, crystalline powder; odourless or almost odourless.
Eutectic temperature. With acetanilide R, about 85 °C.
Heating behaviour. Heat a small quantity in a test-tube; it melts quickly. The melt has a strong odour of acetic acid. On further heating, the colour of the melt changes from yellow to brown and finally to black.
Colour and other reactions
Heat 0.05 g in 2.0 ml of water for several minutes, cool and add 1 or 2 drops of ferric chloride (25 g/l) TS; a violet-red colour is produced which does not change on the addition of ethanol (~ 750 g/l) TS.

Degradation test

A strong odour of acetic acid on opening the container of the test substance and non-compliance with the following test usually indicate gross degradation:

Dissolve 0.10 g in sufficient ethanol (~ 750 g/l) TS to produce 50 ml. Transfer 5 ml to a comparison tube. To serve as a reference, dissolve separately 10 mg of salicylic acid R in sufficient ethanol (~ 750 g/l) TS to produce 100 ml. Transfer 1.0 ml of this solution to a second comparison tube and add 4 ml of ethanol (~ 750 g/l) TS. Add 15 ml of water to both tubes. Dilute separately 1.0 ml of ferric chloride (25 g/l) TS to 5 ml and transfer 0.05 ml of this reagent solution to both tubes. Mix and allow to stand for 1 minute; the violet colour produced in the solution of the test substance should not be more intense than that obtained with the reference solution.

ACRIFLAVINIUM CHLORIDE

Identity tests

Description. An orange-red powder; odourless.

Melting behaviour. About 260 °C with decomposition.

Colour and other reactions

1. Dissolve 10 mg in 100 ml of water; an intense yellow colour with a green fluorescence is produced. Add a few drops of hydrochloric acid (~ 70 g/l) TS; the fluorescence disappears. Then add a few drops of sodium nitrite (10 g/l) TS; an intense purple colour is produced.
2. Dissolve 0.04 g in 10 ml of water and use this solution for tests 2, 3 and 4. Dilute 2.0 ml with 6 ml of water and add a few drops of methyl orange/ethanol TS; a red solution is produced.
3. To 2.0 ml of the solution prepared in test 2 add a few drops of sodium salicylate (100 g/l) TS; an orange-yellow precipitate is formed (distinction from fluorescein).
4. To 5 ml of the solution prepared in test 2 add a few drops of formaldehyde TS and 5 ml of sodium nitrite (100 g/l) TS; a brownish precipitate is produced. Allow the mixture to stand for 5 minutes and then filter; the filtrate acquires a cherry red colour (distinction from the methylated diaminoacridine compounds).
5. Dissolve 5 mg in 5 ml of water and filter. To the filtrate add 1.0 ml of nitric acid (~ 130 g/l) TS and a few drops of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~ 100 g/l) TS; the precipitate dissolves.

AJMALINE

Identity tests

Description. A white, or almost white, crystalline powder; odourless.

Colour and other reactions

1. To 10 mg add about 0.5 ml of nitric acid (~ 1000 g/l) TS; a deep red colour is produced, which remains on the addition of 10 ml of water.
2. To 10 mg add 1 ml of sulfuric acid (~ 1760 g/l) TS and 1.0 ml of ammonium molybdate (95 g/l) TS; a red-violet colour is produced, which turns to blue-violet.
3. Dissolve 10 mg in 0.10 ml of hydrochloric acid (~ 70 g/l) TS and add 5 ml of water and 0.1 ml of ferric chloride (25 g/l) TS; a red colour is produced.

ALLOPURINOL

Identity tests

Description. A white, or almost white, crystalline; powder; odourless or almost odourless.

Colour and other reactions

1. Dissolve 0.05 g in 5 ml of sodium hydroxide (~ 80 g/l) TS, and 1.0 ml of alkaline potassio-mercuric iodide TS, heat to boiling and allow to stand; a yellow, flocculent precipitate is produced.
2. Dissolve 0.20 g in a mixture of 2.0 ml of sodium hydroxide (~ 80 g/l) TS and 2.0 ml of water. Add 3.0 ml of citric acid (90 g/l) TS and shake vigorously; a white precipitate is produced.

ALUMINIUM DIACETATE

Identity tests

Description. A white, fine powder; slight odour of acetic acid.

Colour and other reactions

1. Dissolve 0.5 g by heating in 5 ml of sodium hydroxide (~ 80 g/l) TS. To the clear solution add 0.5 g of ammonium chloride R; a white, gelatinous precipitate is produced.
2. Heat 0.10 g with 5 ml of sulfuric acid (~ 100 g/l) TS; an odour of acetic acid is evolved.
3. Shake 1.0 g with 20 ml of freshly boiled and cooled water for

1 minute; the filtrate is slightly acid when tested with pH-indicator paper R.

ALUMINIUM HYDROXIDE

Identity tests

Description. A white, fine, amorphous powder; odourless.

Colour and other reactions

1. Dissolve 0.10 g by heating in 5 ml of sodium hydroxide (~ 80 g/l) TS. To the clear solution add 0.5 g of ammonium chloride R; a white, gelatinous precipitate is produced.
2. Shake 1.0 g with 20 ml of freshly boiled and cooled water for 1 minute and filter; the filtrate is neutral when tested with pH-indicator paper R.

AMILORIDE HYDROCHLORIDE

Identity tests

Description. A pale yellow to greenish yellow powder; odourless or almost odourless.

Melting behaviour. About 292 °C with decomposition (turns brown-black).

Eutectic temperature. With dicyandiamide R, about 178 °C; with phenolphthalein R, about 241 °C.

Colour and other reactions

1. Dissolve 10 mg in 2.0 ml of water, add 1.0 ml of hydrochloric acid (~ 70 g/l) TS and 4–5 drops of sodium nitrite (10 g/l) TS, shake for 2 minutes and add a solution of 10 mg of 2-naphthol R dissolved in 2.0 ml of sodium hydroxide (~ 80 g/l) TS; a reddish brown precipitate is produced. Add a few drops of hydrochloric acid (~ 70 g/l) TS; a greenish yellow precipitate separates.
2. Dissolve 20 mg in 50 ml of freshly boiled and cooled water and add 0.5 ml of nitric acid (~ 130 g/l) TS and a few drops of silver nitrate (40 g/l) TS; a white precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~ 100 g/l) TS; the precipitate dissolves.
3. Dissolve 0.20 g in 25 ml of water and add 4 ml of sodium hydroxide (~ 80 g/l) TS; a light yellow precipitate is produced. Filter, wash the