Amalic acid test (test for xanthines)

Method

Add to the sample a few drops of 10 M hydrochloric acid followed by a few crystals of potassium chlorate, and evaporate the mixture to dryness. Observe the colour of the residue then add two or three drops of 2 M ammonium hydroxide and again observe the colour.

Indications

A red, pink, orange or yellow residue, which changes to pink, red or violet after the addition of ammonium hydroxide indicates the presence of a xanthine.

Ammoniacal silver nitrate

Reagent

To 20 mL of 0.1 M silver nitrate add sufficient strong ammonia solution to dissolve the initial precipitate.

Method

Dissolve the sample in a minimum amount of water, with the addition of ethanol if necessary, add an equal volume of the reagent and note any colour that develops. Heat the mixture in a water–bath at 100° for 30 s.

Indications

Red, yellow, brown or black colours (especially at room temperature) indicate potent reducing power, which occurs when adjacent carbon atoms in a ring each bear a hydroxyl group. There is no response when the hydroxyl groups are meta to each other, but there is some restoration of reducing power when they are para to each other. Some colour production is also obtained with ethynyl bonds, but not with ethylenic bonds. Ethchlorvynol and ethinylestradiol both give a white precipitate that turns yellow on heating. Carbidopa
gives a silver mirror on heating.

**Antimony pentachloride**

**Reagent**

Dry some antimony trichloride over phosphorus pentoxide, melt the dried material (m.p. 73°), and pass dry chlorine gas into the melt until a yellow fuming liquid is obtained. Add this liquid to about ten times its volume of chloroform, filter the solution into a dark glass-stoppered bottle and store in a desiccator.

**Method**

Place one drop of an ethanolic solution of the sample on a filter paper, add one drop of the reagent and dry in a current of warm air. Alternatively, the test may be carried out by adding one drop of the reagent to the sample on a white tile.

**Indications**

Various colours are obtained with the cardiac glycosides, their aglycones and certain oestrogens and corticosteroids. No colour is obtained with beclometasone, cortisone, fluocinolone, fludroxycortide, prednisolone, prednisone, progesterone, testosterone or triamcinolone.

**Aromaticity**

**Method 1**

Place a portion of the sample in each of two ignition tubes, and to one tube add some solid sodium hydroxide. Heat both tubes carefully, allow the water vapour to escape, insert into the vapours in each tube an open capillary tube that contains Marquis reagent, and observe the colour of the reagent.
Indications

Red or orange colours indicate that the sample is aromatic in nature. The colours probably result from the liberation of traces of aromatic hydrocarbons, phenols, etc. Colours obtained after heating with sodium hydroxide generally indicate the presence of aromatic acids. Colours obtained after heating without sodium hydroxide generally indicate the presence of phenols, phenolic acids and aldehydes that contain more than one hydroxyl group.

A negative result does not necessarily imply that the substance is non-aromatic.

Method 2

Add two or three drops of nitric acid to the sample, heat in a water-bath at 100° for 1 min, cool the mixture, dilute three to four times with water and make the solution alkaline by the addition of a 40% (w/v) solution of sodium hydroxide.

Indications

A change from colourless or yellow in acid solution to darker colours (e.g. orange or red-orange) after the addition of sodium hydroxide indicates the presence of a benzene ring in the molecule, probably though the production of a nitrophenol or other nitro compound.

Certain compounds (e.g. diazepam, methaqualone) give a negative result. Orange colours are given by certain non-aromatic corticosteroids (e.g. cortisone), by substances that contain sulfur and by compounds that already contain an aromatic nitro group (e.g. nifursol).

Certain substances give distinct colours with cold nitric acid, but the colours fade on heating.

Benedict’s reagent

Reagent

Dissolve 1.73 g of copper sulfate in 10 mL of water. Dissolve 17.3 g of trisodium citrate and 10 g of anhydrous sodium carbonate in 80 mL of water with the aid of heat; pour this solution into the copper sulfate solution and dilute the mixture to 100 mL.
Method

Add 0.5 mL of the reagent to the sample and heat in a water-bath at 100° for 3 min.

Indications

The formation of red cuprous oxide occurs with strong reducing agents, such as ascorbic acid, dithionites, certain phenolic compounds that contain two hydroxyl groups para to one another and compounds that contain at least four hydroxyl groups on a non-aromatic ring (e.g. glucose, tetracyclines).

A weak response (orange-brown or brown colours) is given by streptomycin, hydroxylamine and substituted hydrazines (e.g. phenelzine). No colour is obtained with beclometasone, cardiac glycosides and estriol (two hydroxyl groups) or clindamycin (three hydroxyl groups).

Carbon disulfide

Method

Mix the sample with 1 mL of water and 0.1 mL of a 1% (w/v) solution of sodium tetraborate, add 0.2 mL of a 10% v/v solution of carbon disulfide in ethanol and heat in a water-bath at 100° for 3 min; cool the solution and add three drops of 0.1 M silver nitrate.

Indications

A brown colour indicates the presence of a dithiocarbamate, which suggests that the original substance was an aliphatic or heterocyclic primary or secondary amine. The original sample should be tested to ensure that it does not give a brown colour with silver nitrate alone.

Chromotrope acid
Reagent

Dissolve 20 mg of chromotropic acid in 10 mL of sulfuric acid.

Method

Add a small amount of sample, either solid or in solution, to 1 mL of the reagent. Note any colour that may be produced, and then add the solution dropwise to 0.5 mL of water, with cooling. Substances that give a colour with cold sulfuric acid must be excluded.

Cobalt thiocyanate (see Scott’s test)

Reagent

- A 2% (w/v) solution of cobalt thiocyanate in water
- Phosphoric acid
- 1 g H2PtCl6,6H2O in 20 mL of H3PO4

Mix nine parts of solution 1 and three parts of solution 2, add one part of solution 3 and mix well. Add nine parts distilled water and mix. When the solution turns pink it is ready for use.

Method

Add a few drops of the reagent to the sample to be tested.

Limit of detection (LOD): cocaine HCl 60 μg, methadone HCl 15 μg.

Coniferyl alcohol (primary aromatic amines)

Reagent

Warm 0.1 g of coniferyl alcohol until it melts (m.p. 74°), dissolve in 3 mL of ethanol and dilute to 10 mL with ethanol.
Method

Place one drop of a solution of the sample on a filter paper, add one drop of the reagent and expose the paper to hydrochloric acid fumes.

Indications

An orange colour indicates the presence of an aromatic primary amine in which the amino group is attached directly to a benzene ring.

An anomalous reaction is obtained with diphenylamine (bright orange).

Copper sulfate

Method 1

Dissolve the sample in a minimum volume of 0.1 M sodium hydroxide and add a 1% (w/v) solution of copper sulfate, drop by drop, until the colour change is complete.

Indications

Green, blue or brown colours indicate the presence of a sulfonamide.

Method 2

Add one or two drops of a 1% (w/v) solution of copper sulfate to the sample on a white tile.

Indications

A blue colour indicates the presence of an alkali salt of a fatty acid, such as sodium cromoglicate (1–2 min) or valproate. The colours are not produced by a change of pH (some of the alkali salts will change the pH), as negative results are obtained with sodium bicarbonate.
Cyanogen bromide

Reagent

1. Decolourise bromine solution by the addition of solid potassium cyanide and then add more bromine solution until the solution is pale yellow.
2. Prepare a saturated solution of aniline in water.

Solutions 1 and 2 are stable for 1 week. Mix equal volumes of the two solutions immediately prior to the test.

Method

Add one drop of the mixed reagent to the sample on a white tile.

Indications

Red, orange or yellow colours indicate the presence of a mono-substituted pyridine ring. Increasing chain length of the substituent group weakens the response; a delayed response is obtained when the pyridine ring is substituted by nitrogen adjacent to the ring nitrogen; a weak response is obtained where there is a C=O substituent adjacent to the ring nitrogen. There is no response to the test if the pyridine ring is bound to another ring, if it is substituted in more than one position, or if the nitrogen in the ring is substituted. Anomalous results are obtained with azatadine (pink), bisacodyl (no response) and tropicamide (violet-pink).

Diazotisation

Method

Dissolve the sample in 2 M hydrochloric acid, and to one drop on a white tile add one drop of a 1% solution of sodium nitrite, and one drop of a 4% solution of naphth-2-ol in 2 M sodium hydroxide.
Indications

A bright red or orange-red colour indicates the presence of a primary aromatic amine. Diphenylamine does not give a reaction; aminonitrothiazole (solid) gives a violet colour.

**Dille-Koppanyi reagent modified (a general test for barbiturate-like compounds)**

**Reagent**

1. Dissolve 0.1 g of cobalt(II) acetate dihydrate in 100 mL of methanol. Add 0.2 mL of glacial acetic acid and mix.
2. Add 5 mL of isopropylamine to 95 mL of methanol.

**Method**

Add two drops of solution 1 to the drug, followed by one drop of solution 2.

**Indications**

A light purple (blue-violet) colour indicates the presence of a barbiturate. Other reacting compounds are hydantoins, sulfonamides, pyrimadine, piperidine, methyprylon. The LOD is 25 μg or lower.

**p-Dimethylaminobenzaldehyde (Wasicky reagent or Van Urk reagent; a general test for ergot alkaloids)**

**Reagent**

Dissolve 2.0 g of p-dimethylaminobenzaldehyde (p-DMAB) to 50 mL of 95% ethanol and 50 mL of concentrated hydrochloric acid. The reagent should be freshly prepared.
Method

Add the reagent to the sample in a test tube, warming if necessary. Observe any colour produced, then carefully dilute with water or spray dried spots on filter paper and heat.

Indications

Colours are given by a number of substances, which include ergot alkaloids, dimethyltryptamine, psilocin, psilocybine (gives a violet colour), cannabinoids and certain indoles in which the indole ring is not bonded to another conjugated ring (red changing to violet on dilution), and certain phenols and phenolic amines (red or orange, usually changing to violet on dilution). Some other types of compound also respond.

The LOD for lysergide (LSD) is 6 μg.

Diphenylamine test

Reagent

Mix 0.5 g of diphenylamine in 20 mL of water and dilute to 100 mL with concentrated sulfuric acid.

Method

Apply the reagent to the sample on a white tile or in a test-tube.

Indications

A blue colour indicates the presence of an oxidising agent such as bromate, chlorate, chromate, dichromate, iodate, lead(IV), manganese(III, IV, VII), nitrate, nitrite, permanganate or vanadate.

This test has been modified for use on blood samples to detect ethchlorvynol (Caughlin 1991). Blood (0.5 mL) is mixed with 1.0 mL of acetone and vortex mixed. The sample is centrifuged and 50 μL of the supernatant added to 50 μL of diphenylamine reagent and 25 μL of chloroform. The mixture is vortex mixed and allowed to stand. A pink colour that
develops in the chloroform layer indicates ethchlorvynol.

**Dragendorff reagent (a general reagent for nitrogenous bases)**

**Reagent**

Dissolve 1 g of bismuth subnitrate in 3 mL of 10 M hydrochloric acid with the aid of heat. Dilute to 20 mL with water and dissolve 1 g of potassium iodide in the mixture. If black bismuth triiodide separates, add 2 M hydrochloric acid and more potassium iodide to dissolve it.

**Method**

Dissolve the sample in three drops of 2 M hydrochloric acid, add 2 to 3 mL of the reagent and dilute to 10 mL with water.

**Indications**

An orange, red–orange or brown–orange precipitate suggests the presence of an alkaloidal base (precipitated as the alkaloidal bismuth iodide). Primary, secondary, tertiary and quaternary amines give positive results. This reagent is commonly used as a spray or locating agent to detect alkaloids on thin–layer chromatographic plates.

**Duquenois reagent, modified**

**Reagent**

1. Add 2.5 mL of acetaldehyde and 2.0 g of vanillin to 100 mL of 95% ethanol.
2. Concentrated hydrochloric acid.
3. Chloroform.
Method

Place the solid sample, or an evaporated petroleum ether (or other organic solvent) extract of the sample, in a test–tube and add three drops of solution 1. Shake for 1 min and add three drops of solution 2. Agitate gently and observe the colour produced. Add nine drops of solution 3, vortex mix gently and note whether the colour is extracted from the mixture.

Indications

A colour change from grey to green through blue to violet–blue suggests the presence of cannabis, but differentiation from roasted coffee and patchouli oil is required. The colour change is best seen with fresh drug material. The violet colour is extracted into the chloroform layer only when cannabis is present. The LOD is 350 μg of tetrahydrocannabinol (THC). No colour is obtained with other natural products, such as basil, bay leaf, eucalyptus oil, mace, marjoram, rosemary, sage, thyme or tobacco.

Ferric chloride (general reagent for phenols, e.g. salicylates)

Reagent

Dissolve 5 g of anhydrous ferric chloride, or 8.25 g of ferric chloride hexahydrate, in 100 mL of distilled water

Method

Add ferric chloride solution to the sample or an ethanolic solution of the sample.

Indications

Red, orange, green, blue, violet or brown colours indicate the presence of a phenolic compound, fatty acid or a phenylpyrazoline. High quantities of phenothiazines can also cause this test to be positive. Salicylates give a violet colour. Many phenols give no colour with ferric chloride when water is used as a solvent, but give positive tests when anhydrous solvents such as chloroform are used. Aspirin (acetylsalicylic acid) does not give a positive
result unless first hydrolysed with concentrated sodium hydroxide to give salicylate.

**Ferrous sulfate A (test for nitrates and nitrites)**

**Reagent**

To one volume of a 10% (w/v) solution of ferrous sulfate (FeSO₄,7H₂O) add five volumes of sulfuric acid with cooling.

**Method**

Add the sample to 0.5 mL of the reagent.

**Indications**

A red or pink colour is given only by nitrates and nitrites (e.g. glyceryl trinitrate).

**Ferrous sulfate B (test for cyanide)**

**Reagent**

Dissolve 10 g of ferrous sulfate in 100 mL of freshly boiled and cooled water (prepare fresh).

**Method**

Dilute 1 mL of sample with 2 mL of 10% (w/v) sodium hydroxide solution and add 2 mL of ferrous sulfate solution. Add sufficient 10% (v/v) hydrochloric acid to dissolve the ferrous hydroxide precipitate.

**Indications**

A blue colour is given by cyanide. There are no common sources of interference.
**Folin-Ciocalteu reagent (test for phenolic compounds)**

**Reagent**

For the stock solution, dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 800 mL of water in a 1500 mL flask, add 50 mL of phosphoric acid and 100 mL of hydrochloric acid, and reflux for 10 h. Cool, add 150 g of lithium sulfate, 50 mL of water and four to six drops of bromine, and allow to stand for 2 h. Boil for 15 min to remove the excess of bromine, cool, filter and dilute to 1000 mL with water.

This stock solution should be stored at a temperature not exceeding 4° and used within 4 months of its preparation; it has a yellow colour and must not be used if any trace of green colour is present.

For use, dilute one volume of this stock solution with two volumes of water.

**Method**

Add the diluted reagent to the sample and make the mixture alkaline with 2 M sodium hydroxide.

**Indications**

A blue colour indicates the presence of a phenolic compound. The reaction is progressively inhibited with increased halogenation of the phenol nucleus.

**Formaldehyde-sulfuric acid**

**Reagent**

To four volumes of sulfuric acid add six volumes of formaldehyde solution (using a pipette with the tip below the surface of the acid) with stirring and adequate cooling. When the reagent is warm it remains clear for about 1 h. If turbidity develops, this may be dispelled by heating in a water-bath at 100° for about 1 min (note that this reagent is not the same as that used in the Marquis test).
Method
Mix the sample with the reagent and heat at 100° for 1 min.

Indications
Benzodiazepines generally give an orange colour with the exception of bromazepam and clozapine (a benzodiazepine-like compound), which both give yellow, and flurazepam (pink). Other indications include phenothiazines, tetracyclines and thioxanthenes. Tryptamine (brown) and zomepirac (red) also react. No response is obtained with chlordiazepoxide, dimethoxanate or proquamezine. Some of the newer benzodiazepines have not been tested.

Forrest reagent

Reagent
Mix together equal volumes of a 0.2% (w/v) solution of potassium dichromate, a 30% (v/v) solution of sulfuric acid, a 20% (w/w) solution of perchloric acid and a 50% (v/v) solution of nitric acid.

Method
Dissolve the sample in a minimum volume of 2 M hydrochloric acid and add an equal volume of the reagent. To test urine, add 1 mL of reagent to 0.5 mL of urine.

Indications
Red, pink, orange, blue or violet colours are obtained with phenothiazines. A blue colour is obtained with certain dibenzazepines. The blue colour is inhibited by the presence of phenothiazines, so an excess of reagent must be added to overcome this.

FPN reagent (general reagent for phenothiazines)
**Reagent**

Mix together 5 mL of 5 % (w/v) ferric chloride solution, 45 mL of a 20% (w/w) solution of perchloric acid and 50 mL of a 50% (v/v) solution of nitric acid.

**Method**

Dissolve the sample in a minimum volume of 2 M hydrochloric acid (or use 1 mL of urine) and add an equal volume of the reagent.

**Indications**

A variety of colours, from pink, red, orange, violet to blue, indicate the presence of phenothiazines.

**Froehde reagent**

**Reagent**

Dissolve 1.0 g of molybdic acid or sodium molybdate in 100 mL of hot concentrated sulfuric acid.

**Method**

Add a drop of the reagent to the sample on a white tile.

**Fujiwara test (general reagent for halogenated hydrocarbons)**

**Reagent**

Freshly prepared 20% (w/v) sodium hydroxide solution.
Method
Mix together 2 mL of the reagent and 1 mL of pyridine. Add the sample (1 mL of urine) and heat in a water-bath at 100° for 2 min with shaking.

Indications
A red-pink colour in the pyridine layer indicates the presence of compounds that possess at least two halogen atoms bound to one carbon atom. These include chloramphenicol, chlorbutanol, chloroform, dichloralphenazone, trichloroethane, trichloroethanol, trichloroacetic acid and trichloroethylene. Cloral hydrate and dichlorophenazone do themselves react, but are excreted in urine as trichloroacetic acid. No colour is given by dicophane (DDT) or carbon tetrachloride, although massive exposure to the latter solvent may lead to a positive urine test because of the presence of chloroform as a contaminant. 2,2,2-Trichloroethanol gives a yellow colour. The LOD is 1 mg/L.

Furfuraldehyde (general reagent for carbamates)

Reagent
A 10% (v/v) solution of furfuraldehyde in ethanol.

Method
Dissolve the sample in ethanol, place one drop of the solution on a filter paper, add one drop of the reagent and expose the paper to hydrochloric acid fumes for 2 to 3 min.

Indications
A black spot indicates the presence of non-aromatic carbamates. N-Substituted carbamates do not react. The LOD is 1 μg.

Iodine test
**Method**

Mix the sample with an equal volume of manganese dioxide and heat the mixture carefully to dull redness over a small flame. Repeat the test by heating the sample alone.

**Indications**

The appearance of violet vapour indicates the presence of iodine in the molecule. Better results are sometimes obtained when the manganese dioxide is omitted (e.g. with amiodarone).

**Iodoplatinate test (general test for alkaloids and nitrogenous heterocyclic compounds)**

**Reagent**

Add 2 mL of a 5% (w/v) solution of platinic chloride and 5 g of potassium iodide to 98 mL of water and shake until dissolved. This reagent is often used as a locating agent in TLC.

**Method**

Dissolve the sample in two drops of 2 M hydrochloric acid, add 2 to 3 mL of the reagent and dilute to 10 mL with water.

**Indications**

A violet, blue-violet, brown-violet or grey-violet precipitate suggests the presence of an alkaloidal base (precipitated as the alkaloid–iodoplatinate complex). The clearest colours are obtained with tertiary and quaternary amines; primary amines give indistinct colours and amines of small relative molecular mass generally do not react.

**Koppanyi-Zwikker test**
Reagent

A 1% (w/v) solution of cobalt nitrate in ethanol.

Method

Dissolve the sample in 1 mL of ethanol, add one drop of the reagent followed by 10 μL of pyrrolidine and agitate the mixture.

Indications

A violet colour is given by substances that contain the following structures:

- Imides, in which C=O and NH are adjacent in a ring (e.g. barbiturates, glutethimide, oxyphenisatine and saccharin).
- Sulfonamides and other compounds with free –SO2NH2 on a ring [e.g. clopamide, furosemide, sulfanilamide and thiazides], or with –SO2NH2 in a side-chain (e.g. chlorpropamide), or with –SO2NH2 that links a benzene ring with another ring other than a pyrazine, pyridazine, pyridine or pyrimidine ring (e.g. sulfafurazole and sulfamethoxazole). These latter structures give pink or red-violet colours (e.g. sulfadiazine and sulfadimethoxine).

No response is obtained with compounds with other substituents on the nitrogen atom. Anomalous responses are obtained with paramethadione and theophylline (violet), and with cycloserine, idoxuridine, mephenytoin, niridazole and riboflavin (no response). Note that hydrochlorides give a blue colour before the addition of pyrrolidine.

Liebermann’s reagent

Reagent

Add 1 g of sodium or potassium nitrite to 10 mL of sulfuric acid with cooling and swirling to absorb the brown fumes.
Method

Add two or three drops of the reagent to the sample on a white tile. Occasionally it is necessary to carry out the test in a tube and heat in a water-bath at 100°. Many substances give colours with sulfuric acid alone and the test should be repeated using sulfuric acid instead of the reagent.

Indications

This test was originally developed to give intense colours with phenols:

- Orange colours are given by substances that contain a monosubstituted benzene ring not joined to C=O, N-C(=O)- or to a ring that contains a C=N-O- group.
- Orange or brown colours are given by some substances that contain two monosubstituted benzene rings (or some disubstituted compounds in which fluorine is the second substituent) that are joined either to one carbon atom or to adjacent carbon atoms.
- A wide range of colours is given by compounds that contain -OH, O-alkyl or -O-CH2O-groups attached to a benzene ring or to a ring in a polycyclic structure that contains a benzene ring. The benzene ring must not bear -NO2, nor be halogenated, nor contain an -O- substituent ortho to the oxy groups. Compounds that contain ring sulfur give a similar range of colours.

Mandelin’s test (useful test for amfetamines and antidepressants)

Reagent

Dissolve 1.0 g of ammonium vanadate in 1.5 mL of water and dilute to 100 mL with concentrated sulfuric acid.

Method

Add a drop of the reagent to the sample on a white tile.
Indications

When interpreting the result of this test, account should be taken of the colour given by sulfuric acid and by Liebermann’s test. Hydrochlorides give a red colour with this reagent. When the colours differ from those given with sulfuric acid or Liebermann’s test, this indicates an aromatic ring together with a saturated 5-, 6- or 7-membered ring that contains only one nitrogen atom. The heterocyclic ring must not contain a second nitrogen atom or an oxygen atom. It must not be substituted or bound by -CONH- to the aromatic ring. The aromatic ring must not have -CF3 as a substituent. Colours are also produced if sulfur is in a ring, provided that the ring does not contain more than one nitrogen atom.

LOD values are: codeine sulfate 5.0 μg, amfetamine HCl 10.0 μg, diamorphine HCl 20 μg, metamfetamine 150 μg, morphine 5 μg and strychnine 0.05 μg.

Marquis test

The Marquis test is a useful broad-spectrum test used mostly for opium alkaloids and amfetamines.

Reagent

Carefully mix 100 mL of concentrated sulfuric acid with 1 mL of 40% (v/v) formaldehyde solution (stable for several weeks if protected from light).

Method

Add a drop of the reagent to the sample on a white tile.

Indications

Various colours that represent the whole of the visible spectrum are given by a large number of compounds. Structures that tend to maintain the response to the reagent at the violet end of the spectrum are, in decreasing order of efficacy: ring sulfur (with or without aromatic ring); ring oxygen (with aromatic ring); extra-ring oxygen or sulfur (with aromatic ring); aromatic compounds that consist entirely of C, H, N. Thus, there is a tendency for the
response to the Marquis reagent to move gradually towards longer wavelength (i.e. through green to orange and red) as the ratio of C, H, N to the other groups in the molecule rises.

The LOD values are: 1 μg for codeine sulfate, mescaline sulfate, methadone HCl; 5 μg for lysergide tartrate, metamfetamine HCl and morphine; 10 μg for amfetamine HCl and diamorphine HCl.

**McNally’s test**

**Reagents**

1. A 0.5% solution of copper sulfate in 10% acetic acid.
2. A freshly prepared 2% (w/v) solution of sodium nitrite.

**Method**

Dissolve the sample (1 mg) in a few drops of acetone, and add 1 to 2 mL of water. Add three drops of solution 1 and an equal volume of solution 2. Shake and heat in a water-bath at 100° for 3 min.

**Indications**

A red colour indicates the presence of free salicylic acid. Aminosalicylic acid gives a brown precipitate, and diflunisal gives a violet colour. Certain acids produced during the putrefaction of tissues also give red colours in this test: p-hydroxyphenylacetic acid, p-hydroxyphenylpropionic acid and p-hydroxyphenyl-lactic acid.

**Mecke’s reagent (useful test for opium alkaloids)**

**Reagent**

Dissolve 1.0 g of selenious acid in 100 mL of concentrated sulfuric acid.
Method
Add a drop of the reagent to the sample on a white tile.

Indications
An immediate blue or green colour is indicative of opiates.

Melzer’s reagent (general reagent for hallucinogenic mushrooms)

Reagent
Dissolve 1.5 g of iodine in 100 mL of an aqueous solution that contains 5 g of potassium iodide and 100 g of cloral hydrate.

Method
Place a few drops of the reagent on the mushroom spores or mushroom tissue to be tested.

Indications
A blue, bluish-grey or black-grey colour indicates amyloid mushrooms. A slight yellow or no change indicates the mushrooms are non-amyloid. Psilocybes are always non-amyloid.

Mercurous nitrate (general reagent for barbiturate-like compounds)

Reagent
To a saturated solution of mercurous nitrate, add solid sodium bicarbonate until effervescence ceases and the precipitate formed becomes yellow. The precipitate then changes to a biscuit colour. This reagent should be freshly prepared, shaken immediately
before use and should not be kept for more than 1 h.

**Method**

Dissolve the sample in the minimum amount of ethanol, add one drop of the opaque reagent, shake and examine at intervals during 2 min. A blank solution that contains only ethanol and reagent should be treated similarly at the same time.

**Indications**

A dark grey or black colour indicates a ring imide group or sulfonamides with an additional ring. The speed and intensity of the reaction varies between different compounds. The following ring imides react in decreasing order of intensity: barbiturates, bemebride, phenytoin >benperidol, cycloserine, pimoide >glutethimide, oxyphenisatine >saccharin, sulfinpyrazone. In the case of sulfonamides, succinylsulfathiazole, sulfamoxole, sulfanilamide, sulfasomidine and sulfathiazole react with greater intensity than all others. Chlorpropamide and tolbutamide give a moderate response. If used as a spray, the LOD is 1 to 5 μg for barbiturates.

**Methanolic potassium hydroxide**

**Reagent**

A 20% (w/v) solution of potassium hydroxide in methanol.

**Method**

Add a few drops of the reagent to a solution of the sample in methanol and heat if necessary to boiling point to develop the colour.

**Indications**

A change from colourless or from a pale colour to red, orange, yellow, green or blue is given by quinones, diones that possess an aromatic ring, phenols with adjacent hydroxy groups and by compounds that contain nitro groups on a ring. Many of these compounds are
coloured already and give pale or colourless solutions in methanol.

**Millon’s reagent (general reagent for phenols)**

**Reagent**

Dissolve 3 mL of mercury in 27 mL of fuming nitric acid and add an equal volume of water with stirring.

**Method**

Add 0.5 mL of reagent to the sample and warm the mixture.

**Indications**

A red or orange-red colour indicates the presence of a phenolic substance. Primary aryl amines also react. Some basic compounds that contain a phenolic group do not react to this test; a combination of this test with the Folin-Ciocalteu reagent is therefore advised for phenolic compounds. Phenols that contain more than one hydroxyl group do not give the typical red colour. This reagent does not react with phenols substituted with Cl, Br or I.

**Naphthol-sulfuric acid**

This test should be carried out in conjunction with the sulfuric acid test.

**Reagent**

Mix 1 g of naphth-2-ol with 40 mL of sulfuric acid and heat in a water-bath at 100°, with occasional stirring, until the naphth-2-ol is dissolved.

**Method**

Mix the sample with 1 mL of the reagent, heat in a water-bath at 100° for 2 min and note any colour produced. Cool, add 1 mL of water and note the colour again.
Indications

A range of colours is obtained with steroidal structures. A positive response to this test combined with a positive response to the sulfuric acid test is indicative of the presence of a steroid.

Compounds other than steroids that give colours with this test include cloral hydrate and chloramphenicol (brown–yellow), starch and tartaric acid (green).

Nessler’s reagent

Reagent

1. Dissolve 50 g of mercuric chloride and 35 g of potassium iodide in 200 mL of water and cool.
2. Dissolve 50 g of sodium hydroxide in 250 mL of water and cool.

Add the cold solution 2 to the cold solution 1 and make up to 500 mL. Allow the mixture to stand and decant the clear supernatant (stable for many months) for use. Store in dark brown bottles away from the light.

Method

Add the reagent (three drops) to the sample (three drops), agitate and heat the mixture to 100° in a water-bath, examining it every minute for 10 min. A blank solution should be treated similarly at the same time.

Indications

A brown–orange colour is produced quickly by aliphatic amides and thioamides. The presence of an aromatic ring slows the reaction. The nearer the amide group is to the ring, the more the reaction is inhibited. Substituents in the ring may cause a weak reaction. An immediate black colour is produced by substances that contain ortho or para hydroxy groups and by substances that contain an \(-\text{NH}–\text{NH}\) or \(-\text{NH}–\text{NH}_2\) group in an aliphatic side-chain. Some compounds must be heated to 100° to produce blackening.
**Ninhydrin**

**Reagent**

Dissolve 0.5 g of ninhydrin in 40 mL of acetone.

**Method**

Dissolve the sample in methanol, place one drop of the solution on a filter paper, add one drop of the reagent and dry in a current of hot air.

**Indications**

A violet colour that appears rapidly indicates the presence of an aliphatic primary amine or an amino acid group. The presence of an aromatic ring inhibits the response, and the inhibition increases the nearer the amino group is to the ring, as for amfetamine (pink–orange), procainamide and proxymetacaine (both yellow). If the amino group is associated with a saturated ring, a positive but weak pink–violet colour is obtained (amantadine, rimantadine). Gentamicin gives a violet colour after heating for 4 min.

**Nitric acid, fuming**

**Method**

Mix the sample with three drops of fuming nitric acid, heat at 50° for 30 s and observe any colour produced. Cool the mixture, add two drops of it to 2 mL of sulfuric acid and observe the colour. To the remainder of the cooled mixture, add 2 mL of water followed by 2 M sodium hydroxide, dropwise, until pH 8 is reached (use an indicator paper).

**Indications**

Chlorinated phenols give a series of colours in the three parts of this test.
Nitric-sulfuric acid (Erdmann’s reagent)

Reagent

Mix 1 mL of nitric acid with 30 mL of sulfuric acid.

Method

Dissolve the sample in 1 mL of ethanol, add a pellet of potassium hydroxide and evaporate to dryness (100° in a water-bath). To the residue add 0.5 mL of water and 1 mL of carbon tetrachloride, shake and allow to separate. Decant the lower carbon tetrachloride layer and shake it with 1 mL of the reagent.

Indications

A red colour in the acid layer suggests the presence of clofenotane or its metabolite, dichlorodiphenyldichloroethylene (DDE). The red colour changes to orange and then to green. Weak pink colours are given by aldrin, dieldrin and endrin. A red colour is also given by dichlorodiphenyldichloroethane (DDD, mitotane), but the colour does not change.

Note that the substance should be tested to ensure that it does not give a colour with sulfuric acid alone.

Nitrous acid

Method

Dissolve the sample in a minimum volume of water, and add an amount of solid sodium nitrite equal in volume to the sample followed by a few drops of 2 M hydrochloric acid.

Indications

Orange or yellow colours are given by certain sulfonamides and green, blue or violet colours by certain phenylpyrazolines.
No response is obtained with succinylsulfathiazole, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfadinoxaline, sulthiame or propyphenazone.

**Palladium chloride**

**Reagent**

Dissolve, with the aid of heat, 0.1 g of palladium chloride in 5 mL of 2 M hydrochloric acid and dilute the solution to 100 mL with water. Mix together equal volumes of this solution and 2 M sodium hydroxide. The mixed reagent is stable for several weeks.

**Method**

Mix the sample with 1 mL of the reagent and heat at 100° in a water-bath for 2 min. A blank solution should be treated similarly at the same time.

**Indications**

Red, orange, yellow, brown or black colours are given by aliphatic compounds that have a sulfur atom in the chain, and by aromatic compounds that have a sulfur atom in the side-chain. However, no colour is given when an S-alkyl chain is present, unless the chain is terminated by an halogenated group.

No response is obtained if the sulfur is in a group that links two rings. Reducing agents such as ascorbic acid, cloral hydrate, chloroform and glucose, and compounds that contain a chain with a hydrazine link (–NH–NH–, –NH–NH2), give a translucent dark grey or black colour, but do not give the gradual yellow to orange to brown colour seen with sulfur-containing compounds. Compounds that contain adjacent hydroxyl groups on an aromatic ring give orange colours that turn brown.

**Phosphorus test**
Method

To the sample add 0.5 mL of nitric acid and 0.2 mL of sulfuric acid and heat at 100° in a water-bath for 30 min. Cool, add 1 mL of a 10% (w/v) solution of ammonium molybdate and replace in the water-bath at 100°C for 5 min. A blank solution should be treated at the same time. For some compounds, the reaction may occur after shorter heating times than those stated above.

Indications

A bright yellow solution or precipitate indicates the presence of phosphorus and suggests an organophosphorus pesticide, especially if the sample is a water-immiscible liquid. Cyclophosphamide and triclofos also react.

Potassium dichromate

Method 1

Dissolve the sample by shaking in 0.5 mL of 2 M hydrochloric acid and add a few crystals of potassium dichromate.

Indications

An immediate brown colour, or a green colour that changes to brown, indicates the presence of an aminophenol or of a phenol that has two or more hydroxy groups in adjacent positions on the ring (see Table 19.29). Monophenols, halogenated phenols and phenols with hydroxyl groups meta to each other react more slowly or not at all.

Method 2

If the sample is a liquid, add one to two drops to 1 mL of water followed by 1 mL of a saturated solution of potassium dichromate in 50% v/v sulfuric acid.
Indications

A green colour is given by acetaldehyde, ethanol, methanol, propan-1-ol and propan-2-ol.

Schiff’s reagent

Reagent

Dissolve 0.2 g of basic magenta (fuchsin, CI 42510) in 120 mL of hot water, cool, add 20 mL of a 10% (w/v) solution of sodium hydrogen sulfite and 2 mL of hydrochloric acid, and dilute to 200 mL. Store at 4° and protect from light.

Method

Add the sample to 1 mL of the reagent.

Indications

A violet colour indicates the presence of an aliphatic aldehyde. The longer the carbon chain, especially if it is branched, the weaker the response to the test.

Scott’s test (see also cobalt thiocyanate test)

Reagent

1. Cobalt thiocyanate dissolved in water (2% w/v) and then diluted 1:1 with glycerine.
2. Concentrated hydrochloric acid.
3. Chloroform.

Method

Add a small amount of the sample to be tested to a test-tube, add five drops of solution 1 and shake. If cocaine is present a blue colour develops at once. Add one drop of solution 2 and shake (the blue colour disappears and a clear pink solution develops). Add several drops
of solution 3.

**Indications**

The chloroform layer develops an intense blue colour if cocaine is present. Methadone also reacts.

The LOD is 60 μg cocaine HCl and 15 μg methadone HCl.

**Simon’s test (modified sodium nitroprusside test):**

**Reagent**

1. Dissolve 1 g of sodium nitroprusside in 100 mL of water and add 2 mL of acetaldehyde to the solution with thorough mixing.
2. Freshly prepared 2% sodium carbonate in distilled water.

**Method**

Add one drop of solution 1 to the sample, followed by two drops of solution 2.

**Indications**

A dark blue colour indicates a secondary amine [e.g. metamfetamine, ephedrine, 3,4-methylenedioxymetamfetamine (MDMA)] or an unsubstituted heterocyclic amine as its free base. A deep blue colour indicates the presence of metamfetamine. Primary amines [e.g. amfetamine, methylenedioxyamfetamine (MDA)] yield a slow pink to cherry-red colour.

**Sodium dithionite**

**Reagent**

A 5% (w/v) solution of sodium dithionite in a 10% (w/v) solution of sodium hydroxide.
Method

Apply the reagent to the sample, either on a white tile or as a solution in a test-tube. A blank solution should be treated similarly at the same time.

Indications

Colours are produced by bis(pyridyl) compounds. Dark colours are likely to be given by certain metallic solutions because of reduction.

Sodium nitroprusside

Reagent

A 1% (w/v) solution of sodium nitroprusside.

Method 1

Add the sample to 2 mL of the reagent followed by one drop of 2 M sodium hydroxide.

Indications

Orange colours are given by ketones and red colours by acetaldehyde.

Method 2

Mix the sample with a minimum volume of 2 M sodium hydroxide, evaporate to dryness, dissolve the residue in two drops of water and add 0.5 mL of the reagent.

Indications

A violet colour is given by substances that contain labile sulfur in the molecule and by unsubstituted dithiocarbamates.
Method 3

Carry out Method 2 above, but after evaporation to dryness heat the residue until it is yellow or orange in colour before proceeding.

Indications

A violet colour is given by certain substances that contain labile sulfur and do not react to Method 2 (e.g. clomethiazole, lincomycin and monosulfiram).

Sodium nitroprusside-acetone

Reagents

1. Dissolve 2 g of sodium nitroprusside in 5 mL of water and add 45 mL of methanol.
2. 2% (w/v) sodium carbonate.
3. Acetone.
4. 10% Acetaldehyde.

Method 1

Add one drop of solution 1 followed by one drop of solution 2 to 3 to 4 mg of sample dissolved in solution 3 on a spot plate. A purple colour is indicative of amfetamine. The LOD is 30 μg.

Method 2

Add one drop of solution 1 followed by one drop of solution 4 to 1 to 2 mg of sample dissolved in solution 3. An immediate blue colour is indicative of metamfetamine. The LOD is 5 μg.

Sodium picrate (Steyn test)
Reagent

Prepare a solution of 5 g sodium bicarbonate and 0.5 g picric acid in 100 mL of water.

Method

Mix the sample with a few drops of chloroform and sulfuric acid to hasten the reaction while holding a piece of filter paper, impregnated with the reagent, in the vapours that issue from the tube, and heating the contents to 30°.

Indications

The yellow colour of the filter paper changes from orange to brown–orange and then to orange–red or red in the presence of cyanide. Positive results are given by compounds that contain cyanide groups (e.g. cimetidine, diphenoxylate and isoaminile).

Sulfuric acid

Method

Apply sulfuric acid directly to the sample on a white tile or in a test–tube.

Indications

A range of colours is obtained with compounds of various types. Steroids give orange or yellow colours, many of which fluoresce under UV light (λ = 350 nm) either immediately or after dilution. Thioxanthenes give red or orange colours that fluoresce under UV light (λ = 350 nm).

Sulfuric acid–fuming sulfuric acid

Reagent

Mix together 7 mL of sulfuric acid and 3 mL of fuming sulfuric acid.
Method

Dissolve the sample in a minimum volume of toluene and add one or two drops of the reagent.

Indications

A red colour that appears in the lower acid layer indicates the presence of dieldrin (colour develops quickly) or aldrin (colour develops slowly). A pink–orange colour is obtained with endrin.

Tetrabromophenolphthalein ethyl ester

Reagent

Dissolve 50 mg tetrabromophenolphthalein ethyl ester (TBPE) in 100 mL chloroform, shake the solution for 2 min with 1 mL of 10% (v/v) hydrochloric acid and discard the aqueous phase. Dry the organic layer with anhydrous sodium sulfate. Separate the drying agent by filtration. Store the reagent in an amber bottle at 4°.

Method

Place 0.5 mL of sample to be tested in a conical test tube, add 100 μL phosphate buffer (10 mmol/L, pH 8.0) and vortex mix. Add 50 μL of the TBPE reagent and vortex mix. After 2 to 3 min note the colour of the chloroform layer. If the sample to be tested is a solid, dissolve 1 to 2 mg of the material in 0.5 mL of buffer and proceed.

Indications

A deep blue colour indicates quaternary ammonium compounds. An orange, brown, red or purple colour indicates the presence of basic drugs. This test is most sensitive to tertiary amines (e.g. tricyclics, propoxyphene, phenothiazines, diphenhydramine, phencyclidine, methadone, pethidine, etc.). Its LOD is 1 mg/L.
Thalleioquin test

Method

Dissolve the sample in a minimum volume of 2 M hydrochloric acid, add two drops of bromine solution, place one drop of the mixture on a piece of filter paper and expose the paper to ammonia fumes.

Indications

A green colour indicates the presence of a quinine-type structure (e.g. hydroquinidine, hydroquinine, quinidine, quinine). Cinchonidine and cinchonine do not respond.

Trinder’s reagent (see ferric chloride)

Reagent

The solution is prepared as follows: 40 g of mercuric chloride and 40 g of ferric nitrate are dissolved in 850 mL of distilled water. 10 mL of concentrated HCl is added and the solution is diluted to 1 L. This solution is stable for 1 year.

Method

A few drops of the reagent are added to a few drops of urine. A purple colour indicates the presence of a salicylate. This test was devised for the quantitative assay of salicylates in serum, with the mercuric chloride serving as a protein precipitant. The ferric chloride test has been modified for use on blood samples (Asselin and Caughlin 1990). Blood (0.5 mL) is mixed with 1.0 mL of acetone and vortex mixed. The sample is centrifuged, and 50 μL of the supernatant is added to 50 μL of ferric chloride. A purple colour at the interface indicates salicylates.

Vanillin reagent
Reagent

Dissolve 1 g of vanillin in 20 mL of sulfuric acid, warming if necessary.

Method

Add two drops of the reagent to the sample, heat in a water-bath at 100° for 30 s and note any colour that is produced. Dilute the cooled mixture by adding a few drops of water and note any change of colour.

Indications

Many compounds of different chemical structure react with this reagent. However, for barbiturates, the reaction appears to be a steric phenomenon that depends on the structure of the side-chain at the 5-position. Dark colours, which are either dispelled or changed to violet, blue or green by dilution, are produced when either side-chain is greater than two carbon atoms in length or contains a cycloalkene ring. Branching can be proximal to the pyrimidine ring, but not distal. No colour is obtained if both side-chains are less than three carbon atoms in length or if either is branched distally or contains an aryl nucleus. Long, straight, saturated chains also appear to hinder reaction.

Hydroxybarbiturates give positive responses, but bemegride, glutethimide, phenytoin and primidone do not respond. No response is obtained with amobarbital, aprobarbital, barbital, butobarbital, enallylpropymal, hexethal, ibomal, idobutal, metharbital, methylphenobarbital, nealbarbital, phenobarbital or phenylmethylbarbituric acid. With cold reagent, an orange colour is produced by pentobarbital, secobarbital and thiopental, and a brown colour by cyclopentobarbital.

Zwicker reagent (alkaline cobalt test)

This is a general test for barbiturate-like compounds.

Reagent

1. Dissolve 0.5 g of copper(II) sulfate pentahydrate in 100 mL of distilled water.
2. Add 0.5 mL of pyridine to 95 mL of chloroform.

**Method**

Add a few drops of solution 1 to the sample to be tested, followed by a few drops of solution 2 and then heat.

**Indications**

The presence of a violet-blue colour indicates barbiturates. The LOD is 1000 μg for phenobarbital.