

## 2.3. IDENTIFICATION

01/2008:20301

### 2.3.1. IDENTIFICATION REACTIONS OF IONS AND FUNCTIONAL GROUPS

#### ACETATES

- a) Heat the substance to be examined with an equal quantity of *oxalic acid R*. Acid vapours with the characteristic odour of acetic acid are liberated, showing an acid reaction (2.2.4).
- b) Dissolve about 30 mg of the substance to be examined in 3 ml of *water R* or use 3 ml of the prescribed solution. Add successively 0.25 ml of *lanthanum nitrate solution R*, 0.1 ml of 0.05 M *iodine* and 0.05 ml of *dilute ammonia R2*. Heat carefully to boiling. Within a few minutes a blue precipitate is formed or a dark blue colour develops.

#### ACETYL

In a test-tube about 180 mm long and 18 mm in external diameter, place about 15 mg of the substance to be examined, or the prescribed quantity, and 0.15 ml of *phosphoric acid R*. Close the tube with a stopper through which passes a small test-tube about 100 mm long and 10 mm in external diameter containing *water R* to act as a condenser. On the outside of the smaller tube, hang a drop of *lanthanum nitrate solution R*. Except for substances hydrolysable only with difficulty, place the apparatus in a water-bath for 5 min, then take out the smaller tube. Remove the drop and mix it with 0.05 ml of 0.01 M *iodine* on a tile. Add at the edge 0.05 ml of *dilute ammonia R2*. After 1 min to 2 min, a blue colour develops at the junction of the two drops; the colour intensifies and persists for a short time.

For *substances hydrolysable only with difficulty* heat the mixture slowly to boiling over an open flame and then proceed as prescribed above.

#### ALKALOIDS

Dissolve a few milligrams of the substance to be examined, or the prescribed quantity, in 5 ml of *water R*, add *dilute hydrochloric acid R* until an acid reaction occurs (2.2.4), then 1 ml of *potassium iodobismuthate solution R*. An orange or orange-red precipitate is formed immediately.

#### ALUMINIUM

Dissolve about 15 mg of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add about 0.5 ml of *dilute hydrochloric acid R* and about 0.5 ml of *thioacetamide reagent R*. No precipitate is formed. Add dropwise *dilute sodium hydroxide solution R*. A gelatinous white precipitate is formed which dissolves on further addition of *dilute sodium hydroxide solution R*. Gradually add *ammonium chloride solution R*. The gelatinous white precipitate is re-formed.

#### AMINES, PRIMARY AROMATIC

Acidify the prescribed solution with *dilute hydrochloric acid R* and add 0.2 ml of *sodium nitrite solution R*. After 1 min to 2 min, add 1 ml of  $\beta$ -*naphthol solution R*. An intense orange or red colour and usually a precipitate of the same colour are produced.

#### AMMONIUM SALTS

To the prescribed solution add 0.2 g of *magnesium oxide R*. Pass a current of air through the mixture and direct the gas that escapes just beneath the surface of a mixture of 1 ml of 0.1 M *hydrochloric acid* and 0.05 ml of *methyl red*

*solution R*. The colour of the indicator changes to yellow. On addition of 1 ml of a freshly prepared 100 g/l solution of *sodium cobaltinitrite R* a yellow precipitate is formed.

#### AMMONIUM SALTS AND SALTS OF VOLATILE BASES

Dissolve about 20 mg of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 2 ml of *dilute sodium hydroxide solution R*. On heating, the solution gives off vapour that can be identified by its odour and by its alkaline reaction (2.2.4).

#### ANTIMONY

Dissolve with gentle heating about 10 mg of the substance to be examined in a solution of 0.5 g of *sodium potassium tartrate R* in 10 ml of *water R* and allow to cool: to 2 ml of this solution, or to 2 ml of the prescribed solution, add *sodium sulphide solution R* dropwise; an orange-red precipitate is formed which dissolves on addition of *dilute sodium hydroxide solution R*.

#### ARSENIC

Heat 5 ml of the prescribed solution on a water-bath with an equal volume of *hypophosphorous reagent R*. A brown precipitate is formed.

#### BARBITURATES, NON-NITROGEN SUBSTITUTED

Dissolve about 5 mg of the substance to be examined in 3 ml of *methanol R*, add 0.1 ml of a solution containing 100 g/l of *cobalt nitrate R* and 100 g/l of *calcium chloride R*. Mix and add, with shaking, 0.1 ml of *dilute sodium hydroxide solution R*. A violet-blue colour and precipitate are formed.

#### BENZOATES

- a) To 1 ml of the prescribed solution add 0.5 ml of *ferric chloride solution R1*. A dull-yellow precipitate, soluble in *ether R*, is formed.
- b) Place 0.2 g of the substance to be examined, treated if necessary as prescribed, in a test-tube. Moisten with 0.2 ml to 0.3 ml of *sulphuric acid R*. Gently warm the bottom of the tube. A white sublimate is deposited on the inner wall of the tube.
- c) Dissolve 0.5 g of the substance to be examined in 10 ml of *water R* or use 10 ml of the prescribed solution. Add 0.5 ml of *hydrochloric acid R*. The precipitate obtained, after crystallisation from warm *water R* and drying *in vacuo*, has a melting point (2.2.14) of 120 °C to 124 °C.

#### BISMUTH

- a) To 0.5 g of the substance to be examined add 10 ml of *dilute hydrochloric acid R* or use 10 ml of the prescribed solution. Heat to boiling for 1 min. Cool and filter if necessary. To 1 ml of the solution obtained add 20 ml of *water R*. A white or slightly yellow precipitate is formed which on addition of 0.05 ml to 0.1 ml of *sodium sulphide solution R* turns brown.
- b) To about 45 mg of the substance to be examined add 10 ml of *dilute nitric acid R* or use 10 ml of the prescribed solution. Boil for 1 min. Allow to cool and filter if necessary. To 5 ml of the solution obtained add 2 ml of a 100 g/l solution of *thiourea R*. A yellowish-orange colour or an orange precipitate is formed. Add 4 ml of a 25 g/l solution of *sodium fluoride R*. The solution is not decolorised within 30 min.

#### BROMIDES

- a) Dissolve in 2 ml of *water R* a quantity of the substance to be examined equivalent to about 3 mg of bromide (Br<sup>-</sup>) or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid R* and add 0.4 ml of *silver nitrate solution R1*.

Shake and allow to stand. A curdled, pale yellow precipitate is formed. Centrifuge and wash the precipitate with three quantities, each of 1 ml, of *water R*. Carry out this operation rapidly in subdued light disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate obtained in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate dissolves with difficulty.

b) Introduce into a small test-tube a quantity of the substance to be examined equivalent to about 5 mg of bromide ( $\text{Br}^-$ ) or the prescribed quantity. Add 0.25 ml of *water R*, about 75 mg of *lead dioxide R*, 0.25 ml of *acetic acid R* and shake gently. Dry the inside of the upper part of the test-tube with a piece of filter paper and allow to stand for 5 min. Prepare a strip of suitable filter paper of appropriate size. Impregnate it by capillarity, by dipping the tip in a drop of *decolorised fuchsin solution R* and introduce the impregnated part immediately into the tube. Starting from the tip, a violet colour appears within 10 s that is clearly distinguishable from the red colour of fuchsin, which may be visible on a small area at the top of the impregnated part of the paper strip.

#### CALCIUM

a) To 0.2 ml of a neutral solution containing a quantity of the substance to be examined equivalent to about 0.2 mg of calcium ( $\text{Ca}^{2+}$ ) per millilitre or to 0.2 ml of the prescribed solution add 0.5 ml of a 2 g/l solution of *glyoxal-hydroxyanil R* in *ethanol (96 per cent) R*, 0.2 ml of *dilute sodium hydroxide solution R* and 0.2 ml of *sodium carbonate solution R*. Shake with 1 ml to 2 ml of *chloroform R* and add 1 ml to 2 ml of *water R*. The chloroform layer is coloured red.

b) Dissolve about 20 mg of the substance to be examined or the prescribed quantity in 5 ml of *acetic acid R*. Add 0.5 ml of *potassium ferrocyanide solution R*. The solution remains clear. Add about 50 mg of *ammonium chloride R*. A white, crystalline precipitate is formed.

#### CARBONATES AND BICARBONATES

Introduce into a test-tube 0.1 g of the substance to be examined and suspend in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 3 ml of *dilute acetic acid R*. Close the tube immediately using a stopper fitted with a glass tube bent twice at right angles. The solution or the suspension becomes effervescent and gives off a colourless and odourless gas. Heat gently and collect the gas in 5 ml of *barium hydroxide solution R*. A white precipitate is formed that dissolves on addition of an excess of *hydrochloric acid R1*.

#### CHLORIDES

a) Dissolve in 2 ml of *water R* a quantity of the substance to be examined equivalent to about 2 mg of chloride ( $\text{Cl}^-$ ) or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid R* and add 0.4 ml of *silver nitrate solution R1*. Shake and allow to stand. A curdled, white precipitate is formed. Centrifuge and wash the precipitate with three quantities, each of 1 ml, of *water R*. Carry out this operation rapidly in subdued light, disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate dissolves easily with the possible exception of a few large particles which dissolve slowly.

b) Introduce into a test-tube a quantity of the substance to be examined equivalent to about 15 mg of chloride ( $\text{Cl}^-$ ) or the prescribed quantity. Add 0.2 g of *potassium dichromate R* and 1 ml of *sulphuric acid R*. Place a filter-paper strip impregnated with 0.1 ml of *diphenylcarbazide solution R*

over the opening of the test-tube. The paper turns violet-red. The impregnated paper must not come into contact with the potassium dichromate.

#### CITRATES

Dissolve in 5 ml of *water R* a quantity of the substance to be examined equivalent to about 50 mg of citric acid or use 5 ml of the prescribed solution. Add 0.5 ml of *sulphuric acid R* and 1 ml of *potassium permanganate solution R*. Warm until the colour of the permanganate is discharged. Add 0.5 ml of a 100 g/l solution of *sodium nitroprusside R* in *dilute sulphuric acid R* and 4 g of *sulphamic acid R*. Make alkaline with *concentrated ammonia R*, added dropwise until all the sulphamic acid has dissolved. Addition of an excess of *concentrated ammonia R* produces a violet colour, turning to violet-blue.

#### ESTERS

To about 30 mg of the substance to be examined or the prescribed quantity add 0.5 ml of a 70 g/l solution of *hydroxylamine hydrochloride R* in *methanol R* and 0.5 ml of a 100 g/l solution of *potassium hydroxide R* in *ethanol (96 per cent) R*. Heat to boiling, cool, acidify with *dilute hydrochloric acid R* and add 0.2 ml of *ferric chloride solution R1* diluted ten times. A bluish-red or red colour is produced.

#### IODIDES

a) Dissolve a quantity of the substance to be examined equivalent to about 4 mg of iodide ( $\text{I}^-$ ) in 2 ml of *water R* or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid R* and add 0.4 ml of *silver nitrate solution R1*. Shake and allow to stand. A curdled, pale-yellow precipitate is formed. Centrifuge and wash with three quantities, each of 1 ml, of *water R*. Carry out this operation rapidly in subdued light disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate does not dissolve.

b) To 0.2 ml of a solution of the substance to be examined containing about 5 mg of iodide ( $\text{I}^-$ ) per millilitre, or to 0.2 ml of the prescribed solution, add 0.5 ml of *dilute sulphuric acid R*, 0.1 ml of *potassium dichromate solution R*, 2 ml of *water R* and 2 ml of *chloroform R*. Shake for a few seconds and allow to stand. The chloroform layer is coloured violet or violet-red.

#### IRON

a) Dissolve a quantity of the substance to be examined equivalent to about 10 mg of iron ( $\text{Fe}^{2+}$ ) in 1 ml of *water R* or use 1 ml of the prescribed solution. Add 1 ml of *potassium ferricyanide solution R*. A blue precipitate is formed that does not dissolve on addition of 5 ml of *dilute hydrochloric acid R*.

b) Dissolve a quantity of the substance to be examined equivalent to about 1 mg of iron ( $\text{Fe}^{3+}$ ) in 30 ml of *water R*. To 3 ml of this solution or to 3 ml of the prescribed solution, add 1 ml of *dilute hydrochloric acid R* and 1 ml of *potassium thiocyanate solution R*. The solution is coloured red. Take two portions, each of 1 ml, of the mixture. To one portion add 5 ml of *isoamyl alcohol R* or 5 ml of *ether R*. Shake and allow to stand. The organic layer is coloured pink. To the other portion add 2 ml of *mercuric chloride solution R*. The red colour disappears.

c) Dissolve a quantity of the substance to be examined equivalent to not less than 1 mg of iron ( $\text{Fe}^{3+}$ ) in 1 ml of *water R* or use 1 ml of the prescribed solution. Add 1 ml

of *potassium ferrocyanide solution R*. A blue precipitate is formed that does not dissolve on addition of 5 ml of *dilute hydrochloric acid R*.

#### LACTATES

Dissolve a quantity of the substance to be examined equivalent to about 5 mg of lactic acid in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 1 ml of *bromine water R* and 0.5 ml of *dilute sulphuric acid R*. Heat on a water-bath until the colour is discharged, stirring occasionally with a glass rod. Add 4 g of *ammonium sulphate R* and mix. Add dropwise and without mixing 0.2 ml of a 100 g/l solution of *sodium nitroprusside R* in *dilute sulphuric acid R*. Still without mixing add 1 ml of *concentrated ammonia R*. Allow to stand for 30 min. A dark green ring appears at the junction of the two liquids.

#### LEAD

a) Dissolve 0.1 g of the substance to be examined in 1 ml of *acetic acid R* or use 1 ml of the prescribed solution. Add 2 ml of *potassium chromate solution R*. A yellow precipitate is formed that dissolves on addition of 2 ml of *strong sodium hydroxide solution R*.

b) Dissolve 50 mg of the substance to be examined in 1 ml of *acetic acid R* or use 1 ml of the prescribed solution. Add 10 ml of *water R* and 0.2 ml of *potassium iodide solution R*. A yellow precipitate is formed. Heat to boiling for 1 min to 2 min. The precipitate dissolves. Allow to cool. The precipitate is re-formed as glistening, yellow plates.

#### MAGNESIUM

Dissolve about 15 mg of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 1 ml of *dilute ammonia R1*. A white precipitate is formed that dissolves on addition of 1 ml of *ammonium chloride solution R*. Add 1 ml of *disodium hydrogen phosphate solution R*. A white crystalline precipitate is formed.

#### MERCURY

a) Place about 0.1 ml of a solution of the substance to be examined on well-scraped copper foil. A dark-grey stain that becomes shiny on rubbing is formed. Dry the foil and heat in a test-tube. The spot disappears.

b) To the prescribed solution add *dilute sodium hydroxide solution R* until strongly alkaline (2.2.4). A dense yellow precipitate is formed (mercuric salts).

#### NITRATES

To a mixture of 0.1 ml of *nitrobenzene R* and 0.2 ml of  *sulphuric acid R*, add a quantity of the powdered substance equivalent to about 1 mg of nitrate ( $\text{NO}_3^-$ ) or the prescribed quantity. Allow to stand for 5 min. Cool in iced water and add slowly and with mixing 5 ml of *water R*, then 5 ml of *strong sodium hydroxide solution R*. Add 5 ml of *acetone R*. Shake and allow to stand. The upper layer is coloured deep violet.

#### PHOSPHATES (ORTHOPHOSPHATES)

a) To 5 ml of the prescribed solution, neutralised if necessary, add 5 ml of *silver nitrate solution R1*. A yellow precipitate is formed whose colour is not changed by boiling and which dissolves on addition of *ammonia R*.

b) Mix 1 ml of the prescribed solution with 2 ml of *molybdovanadic reagent R*. A yellow colour develops.

#### POTASSIUM

a) Dissolve 0.1 g of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 1 ml of *sodium carbonate solution R* and heat. No precipitate is

formed. Add to the hot solution 0.05 ml of *sodium sulphide solution R*. No precipitate is formed. Cool in iced water and add 2 ml of a 150 g/l solution of *tartaric acid R*. Allow to stand. A white crystalline precipitate is formed.

b) Dissolve about 40 mg of the substance to be examined in 1 ml of *water R* or use 1 ml of the prescribed solution. Add 1 ml of *dilute acetic acid R* and 1 ml of a freshly prepared 100 g/l solution of *sodium cobaltinitrite R*. A yellow or orange-yellow precipitate is formed immediately.

#### SALICYLATES

a) To 1 ml of the prescribed solution add 0.5 ml of *ferric chloride solution R1*. A violet colour is produced that persists after the addition of 0.1 ml of *acetic acid R*.

b) Dissolve 0.5 g of the substance to be examined in 10 ml of *water R* or use 10 ml of the prescribed solution. Add 0.5 ml of *hydrochloric acid R*. The precipitate obtained, after recrystallisation from hot *water R* and drying *in vacuo*, has a melting point (2.2.14) of 156 °C to 161 °C.

#### SILICATES

Mix the prescribed quantity of the substance to be examined in a lead or platinum crucible by means of a copper wire with about 10 mg of *sodium fluoride R* and a few drops of  *sulphuric acid R* to give a thin slurry. Cover the crucible with a thin, transparent plate of plastic under which a drop of *water R* is suspended and warm gently. Within a short time a white ring is rapidly formed around the drop of water.

#### SILVER

Dissolve about 10 mg of the substance to be examined in 10 ml of *water R* or use 10 ml of the prescribed solution. Add 0.3 ml of *hydrochloric acid R1*. A curdled, white precipitate is formed that dissolves on addition of 3 ml of *dilute ammonia R1*.

#### SODIUM

a) Dissolve 0.1 g of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 2 ml of a 150 g/l solution of *potassium carbonate R* and heat to boiling. No precipitate is formed. Add 4 ml of *potassium pyroantimonate solution R* and heat to boiling. Allow to cool in iced water and if necessary rub the inside of the test-tube with a glass rod. A dense white precipitate is formed.

b) Dissolve a quantity of the substance to be examined equivalent to about 2 mg of sodium ( $\text{Na}^+$ ) in 0.5 ml of *water R* or use 0.5 ml of the prescribed solution. Add 1.5 ml of *methoxyphenylacetic reagent R* and cool in ice-water for 30 min. A voluminous, white, crystalline precipitate is formed. Place in water at 20 °C and stir for 5 min. The precipitate does not disappear. Add 1 ml of *dilute ammonia R1*. The precipitate dissolves completely. Add 1 ml of *ammonium carbonate solution R*. No precipitate is formed.

#### SULPHATES

a) Dissolve about 45 mg of the substance to be examined in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 1 ml of *dilute hydrochloric acid R* and 1 ml of *barium chloride solution R1*. A white precipitate is formed.

b) To the suspension obtained during reaction (a), add 0.1 ml of 0.05 M iodine. The suspension remains yellow (distinction from sulphites and dithionites), but is decolorised by adding dropwise *stannous chloride solution R* (distinction from iodates). Boil the mixture. No coloured precipitate is formed (distinction from selenates and tungstates).

## TARTRATES

a) Dissolve about 15 mg of the substance to be examined in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 0.05 ml of a 10 g/l solution of *ferrous sulphate R* and 0.05 ml of *dilute hydrogen peroxide solution R*. A transient yellow colour is produced. After the colour has disappeared add *dilute sodium hydroxide solution R* dropwise. A violet or purple colour is produced.

b) To 0.1 ml of a solution of the substance to be examined containing the equivalent of about 15 mg of tartaric acid per millilitre or to 0.1 ml of the prescribed solution add 0.1 ml of a 100 g/l solution of *potassium bromide R*, 0.1 ml of a 20 g/l solution of *resorcinol R* and 3 ml of *sulphuric acid R*. Heat on a water-bath for 5 min to 10 min. A dark-blue colour develops. Allow to cool and pour the solution into *water R*. The colour changes to red.

## XANTHINES

To a few milligrams of the substance to be examined or the prescribed quantity add 0.1 ml of *strong hydrogen peroxide solution R* and 0.3 ml of *dilute hydrochloric acid R*. Heat to dryness on a water-bath until a yellowish-red residue is obtained. Add 0.1 ml of *dilute ammonia R2*. The colour of the residue changes to violet-red.

## ZINC

Dissolve 0.1 g of the substance to be examined in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 0.2 ml of *strong sodium hydroxide solution R*. A white

precipitate is formed. Add a further 2 ml of *strong sodium hydroxide solution R*. The precipitate dissolves. Add 10 ml of *ammonium chloride solution R*. The solution remains clear. Add 0.1 ml of *sodium sulphide solution R*. A flocculent white precipitate is formed.

01/2008:20302

## 2.3.2. IDENTIFICATION OF FATTY OILS BY THIN-LAYER CHROMATOGRAPHY

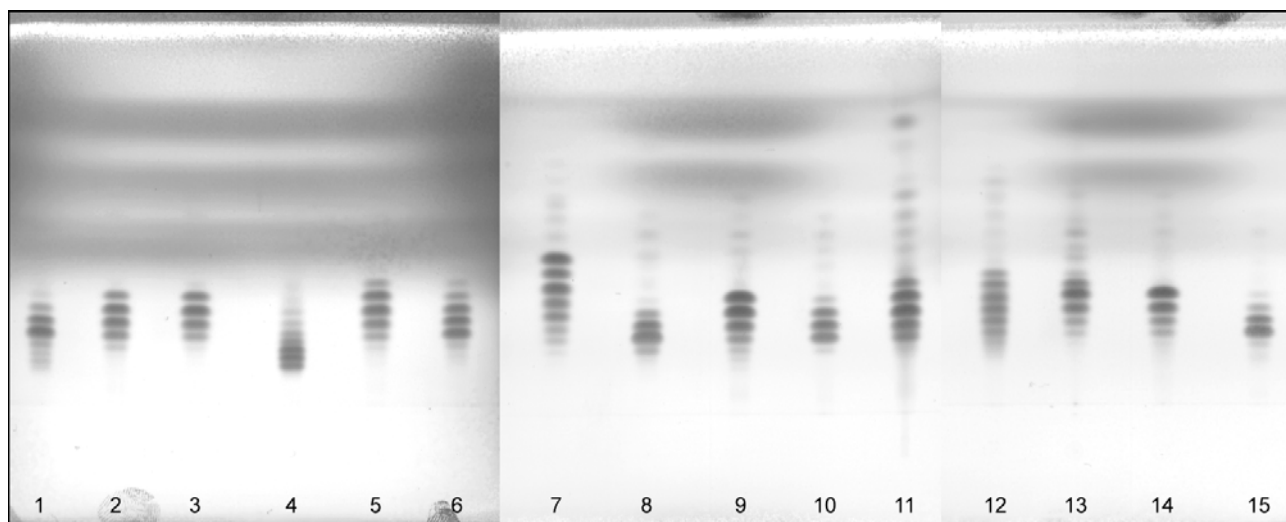
Examine by thin-layer chromatography (2.2.27), using as the coating substance a suitable octadecylsilyl silica gel for high performance thin-layer chromatography.

*Test solution.* Unless otherwise prescribed, dissolve about 20 mg (1 drop) of the fatty oil in 3 ml of *methylene chloride R*.

*Reference solution.* Dissolve about 20 mg (1 drop) of *maize oil R* in 3 ml of *methylene chloride R*.

Apply separately to the plate 1 µl of each solution. Develop twice over a path of 0.5 cm using *ether R*. Develop twice over a path of 8 cm using a mixture of 20 volumes of *methylene chloride R*, 40 volumes of *glacial acetic acid R* and 50 volumes of *acetone R*. Allow the plate to dry in air and spray with a 100 g/l solution of *phosphomolybdic acid R* in *alcohol R*. Heat the plate at 120 °C for about 3 min and examine in daylight.

The chromatogram obtained typically shows spots comparable to those in Figure 2.3.2.-1.



- |                  |                                    |                             |
|------------------|------------------------------------|-----------------------------|
| 1. arachis oil   | 6. rapeseed oil (erucic acid-free) | 11. wheat-germ oil          |
| 2. sesame oil    | 7. linseed oil                     | 12. borage oil              |
| 3. maize oil     | 8. olive oil                       | 13. evening primrose oil    |
| 4. rapeseed oil  | 9. sunflower oil                   | 14. safflower oil (type I)  |
| 5. soya-bean oil | 10. almond oil                     | 15. safflower oil (type II) |

Figure 2.3.2.-1. – Chromatograms for the identification of fatty oils