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Rubber — Identification of accelerators in cured and uncured compounds

Caoutchouc — Identification des accélérateurs dans les mélanges vulcanisés ou non

Reference number
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Foreword

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Rubber — Identification of accelerators in cured and uncured compounds

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

1.1 This International Standard specifies methods using gas chromatography (GC) and thin layer chromatography (TLC) for the separation and identification of the following classes of accelerators in vulcanized and unvulcanized compounds:

- thiazoles
- sulfenamides
- thiurams and dithiocarbamates
- guanidines
- dithiodimorpholine

1.2 When 2-mercaptobenzothiazole (MBT) is identified and no sulfenamides are present, it is not possible to establish if the original accelerator was MBT and/or its salts or 2,2'-dibenzothiazoledisulfide (MBTS) as each of these accelerators may be produced from the others during the vulcanization process.

1.3 When sulfenamides are identified, it is not possible to establish if MBT and/or its salts and MBTS are present as these accelerators may be produced from 2-mercaptobenzothiazole sulfenamides during the vulcanization process.

1.4 The methods do not distinguish thiurams and dithiocarbamates derived from the same amines.

1.5 From the morpholine identification it is not possible to determine if the initial accelerator is 2-morpholinothiobenzothiazole (MBS) or dithiomorpholine as morpholine may be formed from MBS and dithiodimorpholine during the vulcanization process.

1.6 The separation of accelerator compounds from unvulcanized compounds is relatively straightforward whereas separation from vulcanized compounds is difficult due to the lesser ability of solvents to penetrate the vulcanized matrix.

1.7 Some compounding ingredients may interfere with method B. In such cases methods A or C shall be used.

2 Principle

2.1 Method A — Identification of amines via GC and thiazoles via TLC

2.1.1 A portion of the sample compound is refluxed with hydrochloric acid (HCl) to hydrolyze sulfenamides, thiurams and dithiocarbamates. The resulting amine hydrochlorides are separated and purified. After purification, the amines are separated by GC as their trifluoroacetamide derivatives. Identification may also be effected by comparison of GC retention times of sample and standard trifluoroacetamides, prepared and analyzed under the same analysis conditions.

2.1.2 The HCl reflux solubles and insolubles, remaining after amines are separated by distillation, are combined and refluxed with sodium hydroxide (NaOH). The resultant sodium salts of the thiazoles formed from free thiazoles and from MBT produced during the HCl reflux of sulfenamides and other MBT derivatives are separated by extraction and purified. After purification, the thiazoles are separated by TLC and qualitatively detected by comparison of R_f and colours of the sample TLC spots with R_f and colours of standard thiazole spots prepared and analyzed under the same analysis conditions.

2.2 Method B — Identification of amines, guanidines, thiazoles and dithiocarbamates via TLC

2.2.1 Accelerators are extracted from the sample with suitable solvents.

2.2.2 A portion of the extract is hydrolyzed with HCl and the resultant amines separated and qualitatively detected by TLC through comparison of R_f and colours of sample TLC spots with standard TLC spots prepared and analyzed under the same analysis conditions.

2.2.3 A second portion of the extract is hydrolyzed with ammonium hydroxide (NH_4OH) and the ammonium salts of the thiazoles, dithiocarbamates and sulfenamides are separated and qualitatively detected by TLC through comparison of R_f and colours of sample TLC spots with standard TLC spots prepared and analyzed under the same analysis conditions.

2.3 Method C — Identification of thiurams, thiazoles, sulfenamides and guanidines via TLC

2.3.1 Accelerators are extracted from the sample with suitable solvents.

2.3.2 The extracted accelerators are separated and qualitatively detected by TLC through comparison of R_f and colours of sample TLC spots with standard TLC spots prepared and analyzed under the same analysis conditions.

3 Method A — Identification of amines via GC and thiazoles via TLC

3.1 Apparatus

Ordinary laboratory apparatus and

3.1.1 Several GC column types and **operating conditions** may be used providing column type and operating conditions are chosen which give good separation of the trifluoroacetamides from other eluting components. An example of trifluoroacetamide separation is given in figure 1 together with column type and GC operating conditions.

3.1.2 TLC plates covered with a silica gel layer.

NOTE — TLC plates HPTLC Kieselgel 60, 10 cm x 10 cm, supplied by Merck, have been found to be suitable. Other plates with similar qualities may be used.

3.1.3 Desiccator for storing activated TLC plates.

3.1.4 Developing tanks for TLC.

3.1.5 Sprayers for spraying the spray reagents.

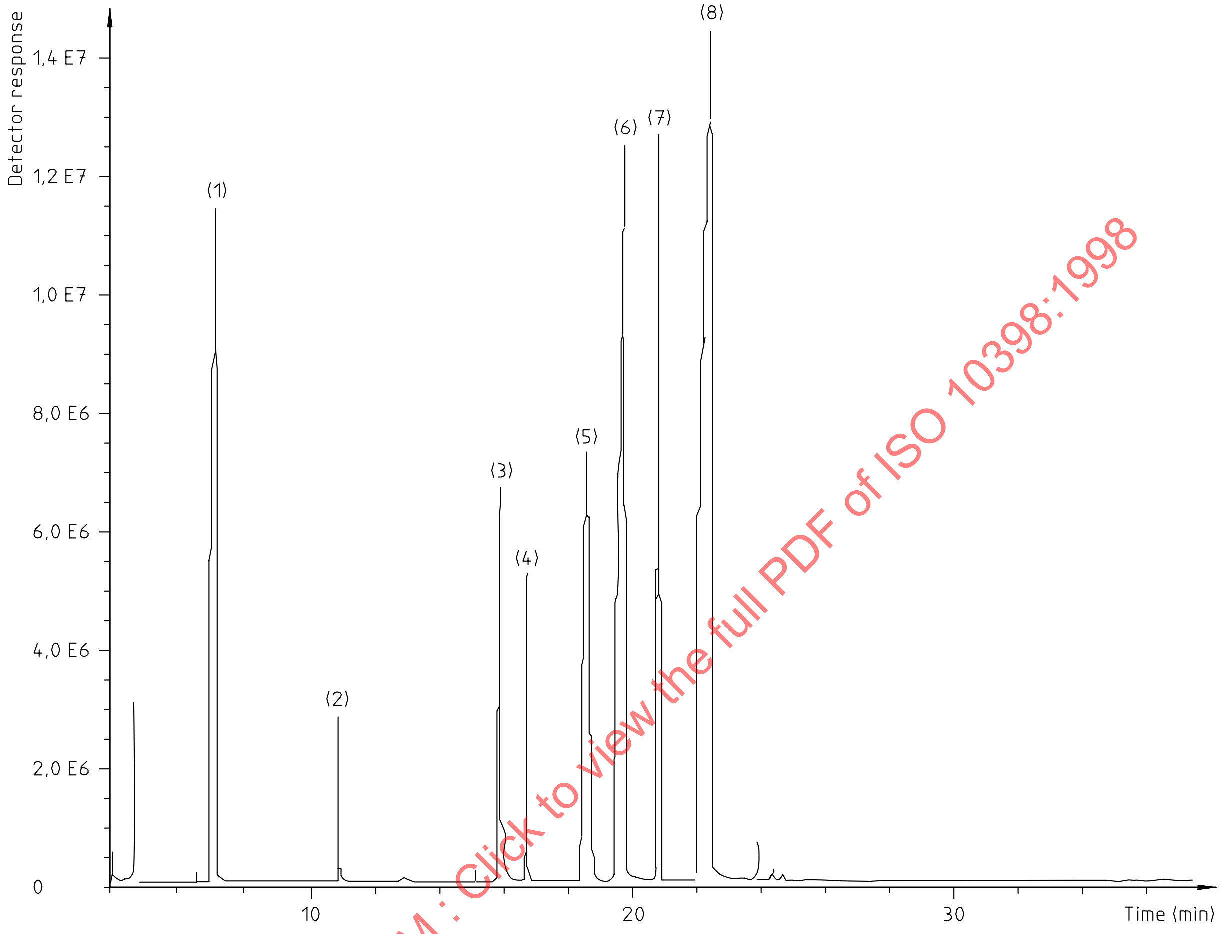
3.1.6 Microsyringe for GC, capacity 10 mm^3 (µl).

3.1.7 Microinjector or **micropipettes** for TLC, capacity 0,01 cm^3.

3.1.8 Filter paper, fast flowing.

3.2 Reagents

3.2.1 Mixture of isopropanol, hydrochloric acid and water, 50:25:25 (*V/V/V*).



Trifluoroacetamides of:

(1) dimethyl-amine
(2) diethyl-amine
(3) morpholine
(4) piperidine
(5) cyclohexyl-amine
(6) aniline
(7) dibutyl-amine
(8) ethyl-phenyl-amine

Column:	Capillary, IIP-5 (cross-linked 5 % phenyl silicon) 25 m x 0,2 mm (0,33 µm film)
Carrier:	Helium, 10 kPa
Injection:	1 mm^3 (µl), on-column
Detector:	Mass selective
Oven programme:	
isothermal 1:	35 °C, 4 min
ramp 1:	6 °C/min
isothermal 2:	200 °C, 0,5 min
ramp 2:	15 °C, 15 min
isothermal 3:	300 °C, 15 min

Figure 1 — Separation of trifluoroacetamides

3.2.2 Isopropanol.

3.2.3 Sodium hydroxide pellets.

3.2.4 Hydrochloric acid solution, prepared by diluting 1 volume of concentrated hydrochloric acid with 1 volume of water.

3.2.5 Trifluoracetic anhydride.

3.2.6 Methylene chloride.

3.2.7 Sodium sulfate, anhydrous.

3.2.8 Sodium hydroxide solution, 40 g/dm^3.

3.2.9 *n*-heptane.

3.2.10 Eluent A: mixture of

n-hexane	55 parts in volume
methylene chloride	35 parts in volume
ethyl ether	35 parts in volume
methanol	15 parts in volume
acetic acid	15 parts in volume

3.2.11 Spray reagent B: 1 % solution of 2,6-dichloroquinone-4-chlorimide in ethanol.

3.2.12 Toluene.

3.2.13 *n*-butanol.

3.2.14 Spray reagent F: 5 % bismuth nitrate solution in 0,5 mol/dm^3 nitric acid.

3.3 Procedure

3.3.1 Identification of the amines

3.3.1.1 Cut 2 g to 10 g of a representative sample into small pieces or thinly sheet about 10 g of a representative sample on a laboratory mill to about 0,25 mm to 0,5 mm thickness. Maintain the sample at the lowest possible temperature during the milling in order to avoid thermal degradation of the accelerators.

3.3.1.2 Reflux the small pieces or thinly sheeted test portion for 2 h with 100 cm^3 of the mixture of isopropanol, hydrochloric acid and water (3.2.1).

3.3.1.3 Filter the boiling solution through fast flowing filter paper (3.1.8) into a conical flask. Wash the reactor vessel and the insoluble portion in the filter with 20 cm^3 of boiling isopropanol (3.2.2) into the same conical flask.

3.3.1.4 Save the washed insoluble portion in the filter.

3.3.1.5 Adjust the solution to a volume of about 10 cm^3 and cool at room temperature.

3.3.1.6 Make the solution strongly alkaline by adding sodium hydroxide pellets (3.2.3).

3.3.1.7 Distil the solution under a slow flow of nitrogen and recover the distillate by bubbling through a few cm^3 of the hydrochloric acid solution (3.2.4) into a conical flask.

3.3.1.8 Stop the distillation when 2 cm^3 to 3 cm^3 of solution remains in the distillation flask.

3.3.1.9 Add to the distillation residue the washed insoluble portion (3.3.1.4) and save the mixture.

3.3.1.10 Concentrate the distilled solution to a volume of 2 cm^3 to 3 cm^3.

3.3.1.11 Transfer the concentrated solution into a 10 cm^3 distillation vessel and heat in a sand bath until only a few drops of solution remain in the vessel, then evaporate the solution to dryness by heating in an oven at 80 °C overnight.

3.3.1.12 Cool the dry residue at room temperature, pour 2 cm^3 of trifluoroacetic anhydride (3.2.5) into the vessel and immediately connect to a reflux condenser.

In order to prevent moisture from entering the vessel, connect the upper end of the reflux condenser with a tube filled with calcium chloride.

3.3.1.13 Reflux for 1 h in an oil bath at 80 °C to 90 °C, disconnect the reflux condenser, concentrate the solution at a volume of about 0,5 cm^3 and cool to room temperature.

3.3.1.14 Add drop by drop 10 cm^3 of water and transfer the solution to a separating funnel.

3.3.1.15 Add 5 cm^3 of methylene chloride (3.2.6), shake, allow the two layers to separate and recover the layer of methylene chloride. Repeat the extraction two times with methylene chloride. Discard the aqueous layer.

3.3.1.16 Wash the methylene chloride extract with water to pH 7 to ensure that all the trifluoroacetic acid has been removed.

3.3.1.17 Add about 0,1 g of anhydrous sodium sulfate (3.2.7) to the methylene chloride extract and shake to eliminate any trace of water, filter through filter paper and concentrate the solution to a volume of about 0,5 cm^3.

3.3.1.18 Inject a suitable amount of the solution into the injection port of the gas chromatograph (3.1.1) and record the chromatogram.

3.3.1.19 Compare the retention times of the peaks obtained from the sample solution with the retention times of the peaks obtained from solutions containing known amides under the same gas chromatographic operating conditions.

NOTE — If the trifluoroacetamides of the amines to be identified are not available, it is possible to prepare them by carrying out the procedure described using the pure accelerators to produce the amines to be identified. These standard solutions can be stored at 5 °C for at least 1 year.

3.3.2 Preparation of the TLC plates

3.3.2.1 Activate the TLC plates (3.1.2) by heating in an oven at 105 °C for 2 h or at 80 °C overnight.

3.3.2.2 Cool the activated plates in the desiccator (3.1.3).

NOTE — The activated plates can be stored in the desiccator for 10 days without further activation.

3.3.2.3 Just before use, inscribe a start line on the activated plate 15 mm to 20 mm from the edge of the plate.

3.3.2.4 Different solutions may be applied on the same plate, along the start line. The distance between the two spots shall be at least 25 mm.

3.3.3 Procedure

3.3.3.1 Pour 50 cm^3 of isopropanol (3.2.2) and 50 cm^3 of the sodium hydroxide solution (3.2.8) into the vessel containing the mixture 3.3.1.9 and reflux for 2 h.

3.3.3.2 Filter the boiling solution through filter paper and wash the reaction vessel and the filter with a few cm^3 of boiling isopropanol. Quantitatively transfer the filtered solution and washings into a separating funnel.

3.3.3.3 Extract this solution two times with 2 portions of 25 cm^3 each of methylene chloride (3.2.6), discarding the methylene chloride extracts.

3.3.3.4 Make the solution strongly acidic by adding hydrochloric acid solution (3.2.4).

3.3.3.5 Add 25 cm^3 of methylene chloride, shake, allow the layers to separate and recover the methylene chloride extract. Repeat the extraction with another 25 cm^3 portion of methylene chloride and combine the methylene chloride extracts. Discard the aqueous layer.

3.3.3.6 Concentrate the methylene chloride extract to a volume of 2 cm^3 to 3 cm^3.

3.3.4 Identification of the thiazoles

3.3.4.1 Apply a suitable amount of the methylene chloride extract from the test portion (3.3.3.6) to the start line. Also apply standard solutions of the thiazoles to be identified to the start line.

3.3.4.2 Place the plate in a chromatographic developing tank (3.1.4) containing *n*-heptane (3.2.9) and allow the eluent to run to the opposite edge of the plate.

3.3.4.3 Remove the plate from the developing tank and allow the solvent to evaporate at room temperature.

3.3.4.4 Place the plate in a chromatographic developing tank containing eluent A (3.2.10) and allow the eluent to migrate to 0,5 cm from the opposite edge of the plate.

3.3.4.5 Remove the plate from the developing tank and allow the solvent to evaporate at room temperature.

3.3.4.6 Spray the plate with spray reagent B (3.2.11). Identify the thiazoles by comparing the R_f and colour of the spots obtained from the solution of the sample with the R_f and colour of the spots obtained from the reference solutions.

3.3.4.7 If the identification is not completely satisfactory, the following bi-dimensional technique can be used to confirm the results.

3.3.4.8 Evaporate a suitable amount of the methylene chloride extract on a starting point, near a corner of the TLC plate, and prepare another TLC plate in the usual way by evaporating, on the starting point, a solution containing a mixture of the thiazoles to be identified.

3.3.4.9 Place the two plates in a chromatographic developing tank containing *n*-heptane (3.2.9) and allow the eluent to migrate to the opposite edge of the plates.

3.3.4.10 Remove the plates from the developing tank and allow the solvent to evaporate at room temperature.

3.3.4.11 Place the plates in a chromatographic developing tank containing toluene (3.2.12) and allow the solvent to migrate for 7 cm.

3.3.4.12 Remove the plates from the developing tank and allow to evaporate at room temperature.

3.3.4.13 Place the plates in a chromatographic developing tank containing *n*-butanol (3.2.13) taking care that the elution direction is perpendicular to the direction of the elution with toluene (3.3.4.11). Allow the eluent to migrate for 7 cm.

3.3.4.14 Remove the plates from the developing tank and allow to evaporate at room temperature.

3.3.4.15 Spray the plates with spray reagent F (3.2.14).

Identify the thiazoles by comparing the R_f and colour of the spots developed on the plate corresponding to the methylene chloride extract with the R_f and colour of the spots developed on the plate corresponding to the solution of the known thiazoles.

4 Method B — Identification of amines, guanidines, thiazoles and dithiocarbamates via TLC

4.1 Apparatus

Same as for method A, except 3.1.1 and 3.1.6.

4.2 Reagents

4.2.1 Mixture of methylene chloride and ethanol, 1:2 (*V/V*).

4.2.2 Hydrochloric acid solution, 2 mol/dm^3.

4.2.3 Methylene chloride.

4.2.4 Ammonium hydroxide solution, 4 mol/dm^3.

4.2.5 Eluent 1: mixture of toluene and ethyl acetate, 9:1 (*V/V*).

4.2.6 Eluent 2: mixture of hexane, toluene and ethanol, 30:58:12 (*V/V*).

4.2.7 Eluent 5: mixture of ethanol, acetone and ammonium hydroxide, 15:15:70 (*V/V*).

4.2.8 Eluent 6: mixture of toluene, ethanol and acetic acid, 6:3:1 (*V/V*).

4.2.9 Eluent 7: mixture of acetone and ammonium hydroxide solution, 99,5:0,5 (*V/V*).

4.2.10 Eluent 9: mixture of *n*-butanol, acetic acid and water, 4:1:5 (*V/V*).

4.2.11 Eluent 10: mixture *n*-butanol, formic acid and water, 75:15:10 (*V/V*).

4.2.12 Eluent 11: mixture of hexane and toluene, 1:1 (*V/V*).

4.2.13 Spray reagent A: 5 % solution of cupric sulfate.

4.2.14 Spray reagent B: 1 % solution of 2,6-dichloroquinone-4-chlorimide in ethanol.

4.2.15 Spray reagent E: 2 % solution of ninhydrin in ethanol.

NOTE — The eluents and spray reagents should be freshly prepared.

4.3 Preparation of the TLC plates

Prepare the TLC plates as stated in 3.3.2.

4.4 Procedure

4.4.1 Cut 2 g to 10 g of a representative sample into small pieces or thinly sheet about 10 g of a representative sample on a laboratory mill to about 0,25 mm to 0,5 mm thickness. Maintain the sample at the lowest possible temperature during the milling in order to avoid thermal degradation of the accelerators.

4.4.2 Transfer about 2 g of the sample into a conical flask, add 30 cm^3 of the extraction solvent (4.2.1) and stopper the flask.

4.4.3 Shake the conical flask for about 3 h with a shaking machine or transfer into an ultrasonic bath and extract for about 1 h.

4.4.4 Recover the extract and extract the sample again as described.

4.4.5 Combine the two extracts and evaporate the solvent to a final volume of about 0,1 cm^3 to 0,2 cm^3 on a water bath or in a rotating evaporator at a temperature not greater than 50 °C.

4.4.6 Complete the evaporation to dryness, at room temperature.

4.4.7 Divide the extract into two parts (part A and part B). Part A is used for identification of the amines and guanidines after hydrolysis with hydrochloric acid (4.5, 4.6 and 4.7). Part B is used for identification of thiazoles and dithiocarbamates after hydrolysis with ammonium hydroxide (4.8).

4.5 Hydrolysis with hydrochloric acid

4.5.1 Apply all the procedures described in 4.5.2 to 4.5.6 to part A of the sample extract and to samples of the pure accelerators to be identified in order to prepare the reference solutions.

4.5.2 Add 8 cm^3 to 10 cm^3 of hydrochloric acid (4.2.2) to part A of the extract and reflux for 10 min to 15 min.

4.5.3 Filter the solution through a cotton wool layer and wash the layer with 5 cm^3 to 7 cm^3 of hydrochloric acid (4.2.2).

4.5.4 Combine the filtered solution and the washings and evaporate on a boiling water bath to a volume of 0,1 cm^3 to 0,2 cm^3.

4.5.5 Complete the evaporation at room temperature.

4.5.6 Dissolve the dry residue containing the amine chlorides, produced by hydrolysis of the dithiomorpholine, as well as the guanidines with 1 cm^3 to 2 cm^3 of methylene chloride (4.2.3).

4.6 Identification of the guanidines

4.6.1 Place the plate in a chromatographic developing tank containing eluent 5 (4.2.7), eluent 6 (4.2.8) or eluent 7 (4.2.9) and saturate with the eluent vapour.

4.6.2 Allow the eluent to run to the end line, remove the plate from the developing tank and dry at room temperature.

4.6.3 Spray the plate with spray reagent B (4.2.14) and heat the plate in an oven at 200 °C for 5 min to 7 min.

4.6.4 Identify the accelerators by comparing the R_f and colour of the spots obtained from the extract of the sample with the R_f and colour of the spots obtained from the reference solutions.

4.7 Identification of the amine hydrochlorides

4.7.1 Place the plate in a chromatographic developing tank containing eluent 9 (4.2.10), eluent 10 (4.2.11) or eluent 11 (4.2.12) and saturate with the eluent vapour.

4.7.2 Let the eluent run to the end line, remove the plate from the developing tank and dry at room temperature.

4.7.3 Spray the plate with the spray reagent E (4.2.15) and heat the plate in an oven at 120 °C for 5 min to 7 min.

4.7.4 Identify the amines by comparing the R_f and colour of the spots obtained from the extract of the sample with the R_f and colour of the spots obtained from the reference solutions. Solutions of the hydrochlorides of the amines to be identified may be used as reference solutions.

4.8 Identification of the thiazoles and dithiocarbamates after hydrolysis with ammonium hydroxide

NOTE — Solutions of pure thiazoles are used as reference solutions in this procedure.

4.8.1 Add 15 cm^3 to 20 cm^3 of ammonium hydroxide solution (4.2.4) to part B of the extract and reflux for 10 min to 15 min.

4.8.2 Filter the solution through a cotton wool layer.

4.8.3 Evaporate on a boiling water bath to a volume of 0,1 cm^3 to 0,2 cm^3.

4.8.4 Complete the evaporation at room temperature.

4.8.5 Dissolve the dry residue with 1 cm^3 to 2 cm^3 of methylene chloride (4.2.3).

4.8.6 Prepare the TLC plates according to 4.3.

4.8.7 Carry out identification of dithiocarbamates according to 5.6.

4.8.8 Carry out identification of thiazoles according to 5.7.

5 Method C — Identification of thiurams, thiazoles, sulfenamides and guanidines via TLC

5.1 Apparatus

Same as 4.1, plus

5.1.1 **Ultra-violet lamp**, with maximum emission at 253,7 nm.

5.2 Reagents

5.2.1 **Mixture of methylene chloride and ethanol**, 1:2 (*V/V*).

5.2.2 **Petroleum ether**, boiling range 40 °C to 60 °C.

5.2.3 **Eluent 1**: mixture of toluene and ethyl acetate, 9:1 (*V/V*).

5.2.4 **Eluent 2**: mixture of hexane, toluene and ethanol 30:58:12 (*V/V*).

5.2.5 **Eluent 3**: mixture of toluene and dichloromethane, 8:2 (*V/V*).

5.2.6 **Eluent 4**: dichloromethane.

5.2.7 **Eluent 5**: mixture of ethanol, acetone and ammonium hydroxide, 15:15:70 (*V/V*).

5.2.8 **Eluent 6**: mixture of toluene, ethanol and acetic acid, 6:3:1 (*V/V*).

5.2.9 **Eluent 7**: mixture of acetone and ammonium hydroxide solution 99,5:0,5 (*V/V*).

5.2.10 **Eluent 8**: mixture of toluene and diethylether, 7:1 (*V/V*).

5.2.11 **Spray reagent A**: 5 % solution of cupric sulfate.

5.2.12 **Spray reagent B**: 1 % solution of 2,6-dichloroquinone-4-chlorimide in ethanol.

5.2.13 **Spray reagent C**: 10 % solution of potassium iodide.

5.2.14 **Spray reagent D**: add 2 drops of sulfuric acid to 100 cm^3 of 10 % potassium iodide solution.

NOTE — The eluents and spray reagents should be freshly prepared.

5.3 Preparation of the TLC plates

Prepare the TLC plates as stated in 3.3.2.