Reagents

MERCK

Dyeing Reagents for Thin Layer and Paper Chromatography
Dyeing of thin layer chromatograms*)

Spraying:
Remove the eluants by drying and cool the thin layer chromatograms (glass plates or sheets). Place it nearly vertically into a spraying box or a hood and protect the surroundings by filter paper or the like. Apply the spray solution from a distance of about 30 cm until the layer is uniformly moist but do not rinse the substance. Mostly different kinds of treatment follow which are described in the individual preparation directions. If not otherwise stated, the plates are finally dried at room temperature.

Spray reagents ready for use:
For your convenience, the following ready for use spray reagents are available from Merck:

- **Aniline Phthalate**
  Spray reagent for chromatography
  Art. no. 1266

- **Bromocresol Green**
  Spray reagent 0.05% for chromatography
  Art. no. 1998

- **2',7'-Dichlorofluorescein**
  Spray reagent 0.1% for chromatography
  Art. no. 9677

- **4-Dimethylaminobenzaldehyde**
  Spray reagent 0.5% for chromatography
  Art. no. 3056

- **Molybdatophosphoric Acid**
  Spray reagent 3.5% for chromatography
  Art. no. 531

* For detection of paper chromatograms see:
Ninhydrin
Spray reagent 0.1 % for chromatography
Art. no. 6758

Rhodamine B
Spray reagent 0.1 % for chromatography
Art. no. 7596

Propellent gas sprayers:
Most practical is the three-part applicator, “Spray-Gun”. The propellent gas container and the spraying solution can be changed as desired.
Glass sprayers:
Spraying with a reagent solution with glass sprayers is the most widely used technique. They can be connected with compressed nitrogen in steel cylinders or with a pressure pipe via a reducing valve. By means of a small diaphragm pump oil-free compressed air can be produced.

With throat sprayers customary in paper chromatography, or even with rubber bulb sprayers, a uniform and finely atomised spray is rarely achieved.

Hood:
When spraying with aggressive reagents which are widely used in thin layer chromatography, a well functioning hood is essential. If not available, a small spraying cabinet made of acid resistant material and attached to the tube of a hood might be taken.

Heating:
The optimum colour development (after spraying with the visualisation reagent) is often attained after heating. An adjustable drying oven is usually used for this purpose. For uniform heating the plate is placed vertically. Small drying ovens for plates 20×20 cm are commercially available.

Dipping:
Especially for quantitative evaluation a uniform dyeing of the plates is needed. For optical measurements (transmission or remission) dipping of the chromatograms usually is the best method. For this technique glass plates, aluminium or plastic foils ready for use have proved to be specially satisfactory because of their good layer adhesion.
Instruments for the techniques mentioned are supplied by:
CAMAG Chemie-Erzeugnisse und Adsorptionstechnik AG Muttenz, Hombreger Str. 24, Switzerland; 1000 Berlin 45, Baseler Str. 65, Germany
C. Desaga GmbH, 6900 Heidelberg 1, Postfach 407, Germany
Shandon Scientific Comp. Ltd., 65 Pound Lane, Willesden London NW 10, Great Britain

1. Acetic anhydride — sulfuric acid for Δ3-3-sterols, (cholesterol and esters), steroids and triterpene glycosides (Liebermann-Burchard reagent).

Spray solution: Mix carefully and with cooling freshly before use 5 ml acetic anhydride with 5 ml 97% sulfuric acid and add the mixture with cooling to 50 ml ethanol.

Treatment: Heat 10 min at 110° C.

Characteristic fluorescence in long-wave UV light.


Chemicals:
- Acetic anhydride GR, art. no. 42
- Sulfuric acid 95—97% (1.84) GR, art. no. 731
- Ethanol abs. GR, art. no. 972

2. Alizarin for cations.

Spray solution: Saturated ethanolic alizarin solution.

Treatment: Place the moist chromatogram into a chamber with ammonia vapour.


Chemicals:
- Alizarin GR and indicator, art. no. 1016
- Ethanol abs. GR, art. no. 972
- Ammonia solution min. 25% (0.91) GR, art. no. 5432

3. Aluminium chloride for flavonoids.

Spray solution: 1% ethanol solution of aluminium chloride. Yellow fluorescence in long-wave UV light.


Chemicals:
- Aluminium chloride extra pure cryst., art. no. 1084
- Ethanol abs. GR, art. no. 972

4. 4-Aminoantipyrine — potassium hexacyanoferrate(III) for phenols (Emerson reaction).

Spray solution I: 2% ethanol solution of 4-aminoantipyrine.

Spray solution II: 8% aqueous potassium hexacyanoferrate (III) solution.

Procedure: Spray with I, then with II, and subsequently place the chromatogram into a chamber saturated with ammonia vapour.

Red-orange to salmon pink spots.
5. o-Aminodiphenyl — phosphoric acid for sugars (modif. reagent acc. to Lewis-Smith).

**Spray solution:** Dissolve 0.3 g o-aminodiphenyl and 5 ml 85% phosphoric acid in 95 ml ethanol.

**Treatment:** Heat 15—20 min at 110° C.

Sugars show brown spots.


**Chemicals:**
- o-Aminodiphenyl
- Ortho-phosphoric acid min. 85%/o (1.71) G R, art. no. 573
- Ethanol abs. G R, art. no. 972

6. 4-Aminohippuric acid for reducing sugars.

**Spray solution:** 0.3%/o ethanolic 4-aminohippuric acid solution.

**Treatment:** Heat 8 min at 140° C.

Characteristic spots in long-wave UV light.

**Literature:** L. Sattler, F. W. Zerban, Anal. Chem. 24, 1862 (1952).

**Chemicals:**
- 4-Aminohippuric acid, art. no. 84
- Ethanol abs. G R, art. no. 972

7. o-Aminophenol — phosphoric acid for sugars.

**Spray solution:** Dissolve 0.15 g o-aminophenol in 20 ml ethanol shortly prior to use. Add 10 ml 50%/o phosphoric acid to the solution.

**Literature:** L. Víguyáød—Vámös, Magyar Kém. Folyóirat 50, 253 (1953).

**Chemicals:**
- 2-Aminophenol, art. no. 419
- Ethanol abs. G R, art. no. 972
- Ortho-phosphoric acid min. 85%/o (1.71) G R, art. no. 573

8. Ammonia for tetracyclines.

**Procedure:** Place the chromatogram into a chamber saturated with ammonia vapour. Tetracyclines show yellow spots in long-wave UV light.


**Solution a:** 1% solution of ammonium cerium(IV) nitrate in 0.2 N nitric acid.

**Solution b:** Dissolve 1.5 g N,N-dimethyl-p-phenylenediamine dihydrochloride in 128 ml methanol, 25 ml water and 1.5 ml glacial acetic acid.

**Spray solution:** Mix 1 part a with 10 parts b freshly before use.

**Treatment:** Heat 10 min at 105° C.

Yellowish green spots on red background.


**Chemicals:**
- Ammonium cerium(IV) nitrate G R, art. no. 2276
- N,N-Dimethyl-p-phenylenediamine dihydrochloride G R, art. no. 3067
- Methanol G R, art. no. 6009
- Acetic acid glacial min. 96%/o G R, art. no. 90062
- Nitric acid min. 65%/o G R, art. no. 454

10. Ammonium cerium(IV) nitrate — nitric acid for α-hydroxy acids, α-keto acids and mercaptans, PC.

**Dip solution:** Dissolve 20 g ammonium cerium(IV) nitrate in 50 ml 0.5 N nitric acid. Dilute freshly before use 1 part of this solution with 3 parts water.

**Procedure:** After drying dip the chromatogram into the dip solution and place it on a clean filter paper.

White spots on yellow background.


**Chemicals:**
- Ammonium cerium(IV) nitrate G R, art. no. 2276
- Nitric acid min. 65%/o (1.4) G R, art. no. 454

11. Ammonium cerium(IV) sulfate for Vinca alkaloids.

**Spray solution:** 1% solution of ammonium cerium(IV) sulfate in 85%/o phosphoric acid.


**Chemicals:**
- Ammonium cerium(IV) sulfate G R, art. no. 2273
- Ortho-phosphoric acid min. 85%/o (1.4) G R, art. no. 573
12. Ammonium iron(III) sulfate for flavonoids.
   
   **Spray solution**: 0.2% aqueous solution of ammonium iron(III) sulfate.
   
   
   **Chemicals**: Ammonium iron(III) sulfate G R, art. no. 3776

13. Ammonium iron(III) sulfate for Vinca alkaloids.
   
   **Spray solution**: Dissolve 1 g ammonium iron(III) sulfate in 100 ml phosphoric acid (75 or 85%).
   
   **Spray the reagent on to the heated chromatogram (100°C C)
   
   
   **Chemicals**: Ammonium iron(III) sulfate G R, art. no. 3776

14. Ammonium molybdate – crystal violet for phosphoric acid. PC.
   
   **Spray solution**: Mixture of 5 ml 1% aqueous ammonium molybdate solution, 5 ml 25% hydrochloric acid and 90 ml acetone.
   
   **Solution a**: Dissolve 2 g crystal violet (or brilliant green or iodine green) in 350 ml water.
   
   **Solution b**: Dissolve with heating 4 g ammonium molybdate in water, add 50 ml 10 N hydrochloric acid and fill up to 100 ml with water.
   
   **Dip solution**: Mix a and b, wait at least 3 hours and filter the solution.
   
   **Procedure**: Spray the chromatogram with the spray solution, heat 3–6 min at 85°C, dip into the dip solution and place immediately on a prepared clean filter paper.
   
   **Note**: 0.02 µg of phosphorus are detectable.
   
   - Crystal violet = blue spots on yellow background
   - Brilliant green = green spots on orange background
   - Iodine green = turquoise spots on colourless background

   **Literature**: F. Jungnickel, J. Chromatog. 31, 617 (1967).
   
   **Chemicals**: Ammonium heptamolybdate G R, art. no. 1182
   
   - Crystal violet indicator, art. no. 1408
   - Brilliant green, art. no. 1310
   - Iodine green
   - Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
   - Acetone G R, art. no. 14

15. Ammonium molybdate – perchloric acid (Hanes reagent) for phosphate esters (sugar phosphates).
   
   **Spray solution**: Dissolve 0.5 g ammonium molybdate in 5 ml water, add 1.5 ml 25% hydrochloric acid and 2.5 ml 70% perchloric acid. After cooling to room temperature fill up to 50 ml with acetone. Allow the solution to stand for at least one day prior to use. The solution is stable for about three weeks.
   
   **Treatment**: Irradiate the chromatogram for 2 min with an IR lamp from a distance of 30 cm and subsequently with long-wave UV light for 7 min or heat 5–10 min at 110°C C.
   
   
   
   
   
   **Chemicals**: Ammonium heptamolybdate G R, art. no. 1182
   
   - Hydrochloric acid min. 25% (1.125) G R, art. no. 316
   - Perchloric acid about 70% (1.67) G R, art. no. 519
   - Acetone G R, art. no. 14

   
   **Spray solution I**: 1% aqueous ammonium molybdate solution.
   
   **Spray solution II**: 1% solution of tin(II) chloride in 10% hydrochloric acid.
   
   **Procedure**: Spray with I, dry the chromatogram and spray with II.
   
   
   **Chemicals**: Ammonium heptamolybdate G R, art. no. 1182
   
   - Tin(II) chloride G R, art. no. 7815
   - Hydrochloric acid min. 25% (1.125) G R, art. no. 316

17. Ammonium thiocyanate – iron(II) sulfate for peroxides.
   
   **Spray solution I**: Dissolve 0.4 g ammonium thiocyanate in 30 ml acetone.
   
   **Spray solution II**: Dissolve 1.2 g iron(II) sulfate in 30 ml water.
   
   **Procedure**: Spray with I, dry the chromatogram and spray with II.
   
   
   **Chemicals**: Ammonium thiocyanate G R, art no. 1213
   
   - Iron(II) sulfate G R, art. no. 3965
   - Acetone G R, art. no. 14
18. Aniline — diphenylamine — phosphoric acid for reducing sugars.
   **Spray solution:** Dissolve 4 g diphenylamine, 4 ml aniline and 20 ml 85\% phosphoric acid in 200 ml acetone.
   **Treatment:** Heat 10 min at 85 °C.
   **Characteristics:** o-aldohexose oligosaccharides turn blue.
   S. Schwimmer, A. Bevenne, Science 123, 543 (1956).
   **Chemicals:**
   - Aniline G R, art. no. 1261
   - Diphenylamine G R, art. no. 3086
   - Acetone G R, art. no. 14
   - Ortho-phosphoric acid min. 85\% (1.71) G R, art. no. 573

19. Aniline — phosphoric acid for sugars.
   **Spray solution:** Mix 1 part 2 N aniline solution in 1-butanol saturated with water with 2 parts 2 N phosphoric acid in 1-butanol.
   **Treatment:** Heat the chromatogram 10 min at 105 °C.
   **Chemicals:**
   - Aniline G R, art. no. 1261
   - Ortho-phosphoric acid min. 85\% (1.71) G R, art. no. 573
   - 1-Butanol G R, art. no. 1990

20. Aniline phthalate.
    **Spray reagent for chromatography**
   Visualisation reagent ready for use in aerosol cans.
   **Treatment:** Heat the chromatogram 10 min at 105 °C.
   **Art. no. 1266**

   **Spray solution:** Dissolve 0.93 g aniline and 1.66 g phthalic acid in 100 ml 1-butanol saturated with water.
   **Treatment:** Heat 10 min at 105 °C.
   **Literature:** S. M. Partridge, Nature 164, 443 (1965).
   **Chemicals:**
   - Aniline G R, art. no. 1261
   - Phthalic acid G R, art. no. 9611
   - 1-Butanol G R, art. no. 1990

22. Anisaldehyde — sulfuric acid for sugars, steroids, terpenes.
    **Spray solution:** Prepare freshly before use a solution of 0.5 ml anisaldehyde in 50 ml glacial acetic acid and 1 ml 97\% sulfuric acid.
    **Treatment:** Heat to 100—105 °C until maximal visualisation of the spots.
   The background may be brightened by water vapour. Lichen constituents, phenols, terpenes, sugars and steroids turn violet, blue, red, grey or green.
   **Modified spray solution:** For visualisation of sugars mix freshly before use 0.5 ml anisaldehyde, 9 ml ethanol, 0.5 ml 97\% sulfuric acid and 0.1 ml acetic acid.
   **Treatment:** Heat the sprayed chromatogram 5—10 min at 90—100 °C.
   **Chemicals:**
   - Anisaldehyde, art. no. 1450
   - Acetic acid glacial min. 96\% (1.06) G R, art. no. 90062
   - Sulfuric acid 95—97\% (1.84) G R, art. no. 731
   - Ethanol abs. G R, art. no. 972

23. p-Anisidine for reducing sugars.
    **Spray solution:** Dissolve 1 g p-anisidine hydrochloride in 10 ml methanol, fill up the solution to 100 ml with 1-butanol and shake well after addition of 1 g sodium dichromate.
    **Treatment:** Heat 10 min at 130 °C.
   **Chemicals:**
   - p-Anisidine hydrochloride
   - Sodium dichromate G R, art. no. 6507
   - Methanol G R, art. no. 6009
   - 1-Butanol G R, art. no. 1990

24. p-Anisidine phthalate for reducing sugars.
    **Spray solution:** 0.1 M solution of p-anisidine and phthalic acid in 96\% ethanol.
    **Treatment:** Heat 10 min at 100 °C.
   **Chemicals:**
   - p-Anisidine, art. no. 458
   - Phthalic acid G R, art. no. 9611
   - Ethanol abs. G R, art. no. 972

25. Anthrone for ketoses.
    **Spray solution:** Dissolve 0.3 g anthrone in 10 ml acetic acid and add to the solution 20 ml 96\% ethanol, 3 ml 85\% phosphoric acid and 1 ml water. The solution is stable for several weeks in the refrigerator.
Ketones and oligosaccharides containing ketones show yellow spots.

**Literature:** R. Johnson, Nature 172, 956 (1953).

**Chemicals:**
Antimony, art. no. 1468
Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
Ethanol abs. G R, art. no. 972
Ortho-Phosphoric acid min. 85% (1.71) G R, art. no. 573

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**26. Antimony(III)-chloride for flavonoids.**

**Spray solution:** 10% solution of antimony (III) chloride in chloroform.
Fluorescing spots in long-wave UV light.

**Literature:** L. Höchhammer, H. Wagner, K. Hein, J. Chromatog. 12, 235 (1964).
R. Neu, P. Hagedorn, Naturwissenschaften 40, 411 (1953).

**Chemicals:**
Antimony (III) chloride G R, art. no. 7838
Chloroform G R, art. no. 2445

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**27. Antimony(III)-chloride for vitamin A and D, carotenoids, steroids, sapogenins, steroid glycosides, terpenes (Carr-Price reagent).**

**Spray solution:** Dissolve 25 g antimony (III) chloride in 75 ml chloroform; generally a saturated solution of antimony (III) chloride in chloroform or carbon tetrachloride is used.

**Treatment:** Heat 10 min at 100°C.
Inspect the chromatogram in long-wave UV light.

**Literature:** E. Stahl, Chemiker-Ztg. 82, 323 (1958).

**Chemicals:**
Antimony (III) chloride G R, art. no. 7838
Chloroform G R, art. no. 2445
Carbon tetrachloride G R, art. no. 2222

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**28. Antimony(III)-chloride — acetic acid for steroids and diterpenes.**

**Spray solution:** Dissolve 20 g antimony trichloride in a mixture of 20 ml glacial acetic acid and 60 ml chloroform.

**Treatment:** Heat 5 min at 100°C.
Diterpenes show red-yellow to blue-violet spots.
Inspect in long-wave UV light.


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**29. Antimony(III)-chloride — sulfuric acid for bile acids.**

**Spray solution:** Dissolve 20 g antimony (III) chloride in 50 ml anhydrous 1-butanol, and mix this solution with 10 ml 97% sulfuric acid and 20 ml glacial acetic acid. The solution should be prepared freshly before use.

**Treatment:** After drying for 15 min in the air, heat the chromatogram: conjugated bile acids for 25–30 min, free bile acids for 45–50 min at 110°C. Colour from yellow to green.


**Chemicals:**
Antimony (III) chloride G R, art. no. 7838
1-Butanol G R, art. no. 1990
Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
Sulfuric acid 95–97% (1.84) G R, art. no. 731

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**30. Antimony(V)-chloride for vitamin A, D and E, terpenes, oils, resins, steroid sapogenins.**

**Spray solution:** Mix freshly before use 1 part antimony (V) chloride with 4 parts carbon tetrachloride or chloroform.

**Treatment:** Heat the chromatogram until the spots appear.
Inspect in long-wave UV light.

E. Stahl, Chemiker-Ztg. 82, 323 (1958)

**Chemicals:**
Antimony (V) chloride G R, art. no. 7837
Carbon tetrachloride G R, art. no. 2222
Chloroform G R, art. no. 2445

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**31. Aurin tricarboxylic acid (Aluminon) for aluminium, chromium, and lithium ions.**

**Spray solution:** 0.1% solution of aurin tricarboxylic acid ammonium salt in 1% aqueous ammonium acetate solution.

**Treatment:** Place the chromatogram into a chamber saturated with ammonia vapour.

32. Benzidine for persulfates.

*Spray solution:* Dissolve 0.05 g benzidine in 100 ml 1 N acetic acid.

Persulfates show blue spots immediately after spraying.

**Literature:** Y. Servigne, C. Duval, Compt. Rend. 245, 1803 (1957).

**Chemicals:**
- Benzidine G R, art. no. 1762
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

33. Benzidine for terpene aldehydes, flavonoids, carbohydrates.

*Spray solution:* Dissolve 0.5 g benzidine in 20 ml glacial acetic acid and 80 ml ethanol.

**Treatment:** Heat 15 min at 100°C.

Spraying with dilute hydrochloric acid after heating intensifies the colour of the spots of some substances.


**Chemicals:**
- Benzidine G R, art. no. 1762
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Ethanol abs. G R, art. no. 972
- Hydrocholoric acid min. 25% (1.125) G R, art. no. 316

34. Benzidine diazotised for phenols.

**Benzidine solution:** Dissolve 5 g benzidine in 14 ml 37% hydrochloric acid and fill up to 100 ml with water.

**Nitrite solution:** Freshly prepared 10% aqueous sodium nitrite solution.

*Spray solution:* Mix 20 ml of the benzidine solution with 20 ml of the nitrite solution at 0°C with permanent stirring.

**Note:** The reagent is stable for 2–3 hours. The colours appear very rapidly or after some hours depending on the phenol present.


**Chemicals:**
- Benzidine G R, art. no. 1762
- Sodium nitrite cryst. G R, art. no. 6549
- Hydrocholoric acid min. 37% (1.19) G R, art. no. 317

35. Benzidine — peroxide for chromium and manganese ions.

*Spray solution I:* 5% aqueous sodium peroxide solution.

*Spray solution II:* 1% benzidine solution in glacial acetic acid.

**Procedure:** Spray consecutively with I and II.

**Literature:** I. M. Ladenbauer, L. K. Bradacs, F. Hecht, Mikrochim. Acta 1954, 388

**Chemicals:**
- Sodium peroxide G R, art. no. 6551
- Benzidine G R, art. no. 1762
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

36. Benzidine — trichloroacetic acid for sugars.

*Spray solution:* Dissolve 0.5 g benzidine in 10 ml glacial acetic acid, add 10 ml 40% trichloroacetic acid solution and fill up to 100 ml with ethanol.

**Treatment:** Irradiate the chromatogram 15 min with UV light.

Sugars show greyish-brown to deep red-brown spots.

**Literature:** J. S. D. Bacon, J. Edelmann, Biochem. J. 48, 114 (1951).


**Chemicals:**
- Benzidine G R, art. no. 1762
- Trichloroacetic acid G R, art. no. 807
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Ethanol abs. G R, art. no. 972

37. 2,2'-Bipyridine — iron(III) chloride for phenols, vitamin E and other reducing compounds.

*Solution a:* 0.5% ethanolic iron (III) chloride solution. Keep in the dark.

*Solution b:* 0.5% ethanolic solution of 2,2'-bipyridine.

**Spray solution:** Mix equal parts of a and b before use.

**Literature:** G. M. Barton, J. Chromatog. 20, 189 (1965).


**Chemicals:**
- 2,2'-Bipyridine G R, art. no. 3098
- Iron (III) chloride G R, art. no. 3943
- Ethanol abs. G R, art. no. 972

38. Bismuth chloride for sterols.

**Spray solution:** 33% ethanolic bismuth(III) chloride solution.

**Treatment:** Heat at 110°C until maximal fluorescence of the spots in long-wave UV light.
Spray solution: Dissolve 0.5 g boric acid and 0.5 g citric acid in 20 ml methanol.
Treatment: Heat 10 min at 100°C.
Inspect in UV light.

40. Bromine — fluorescein — silver nitrate for insecticides.
Spray solution: Fill up 1 ml of a 0.25% solution of fluorescein in N,N-dimethylformamide to 50 ml with ethanol.
Spray solution II: Dissolve 1.7 g silver nitrate in 5 ml water, add 10 ml ethylene glycol monophenyl ether and fill up the solution to 200 ml with acetone.
Procedure: Place the chromatogram 30 sec into a chamber with a 5% solution of bromine in carbon tetrachloride. Spray the chromatogram with I, then with II and irradiate 7 min with long-wave UV light.

41. Bromocresol green.
Spray reagent 0.05% for chromatography
Visualisation reagent ready for use in aerosol cans. Art. no. 1998

42. Bromocresol green — indicator reagent.
Spray solution: Dissolve 0.04 g bromocresol green in 100 ml ethanol. Add 0.1 N sodium hydroxide solution until blue colour appears.

Solution a: Dissolve 0.075 g bromocresol green and 0.025 g bromophenol blue in 100 ml ethanol.
Solution b: Dissolve 0.25 g potassium permanganate and 0.5 g sodium carbonate in 100 ml water.
Spray solution: Mix 9 parts a and 1 part b prior to use and spray immediately. The mixture is stable for 5—10 minutes only.

44. Bromocresol purple for dicarboxylic acids on polyethyleneglycol impregnated layers.
Spray solution: Dissolve 0.04 g bromocresol purple in 100 ml 50% ethanol and adjust the solution to pH 10.0 with 0.1 N sodium hydroxide solution (glass electrode).
Procedure: Develop the chromatogram with the eluent di-iso-propylether — formic acid — water (90+7+3) and heat subsequently 10 min at 100°C. Spray after cooling on room temperature. Yellow spots on blue background.

45. Bromocresol purple for halogen ions.
Indicator reagent for use of acetone—1-butanol—ammonia (25%) — water (65 + 20 + 10 + 5) as eluent.
Spray solution: 0.1% ethanolic bromocresol purple solution. Adjust the solution with some drops of 10% ammonia solution until the colour change just appears.
47. Bromophenol blue — methyl red — Pauly reagent for phenols.

Spray solution I: Mix 100 ml 0.12% aqueous bromophenol blue solution, 100 ml 0.06% ethanolic methyl red solution and 100 ml phosphate buffer acc. to Sorensen (pH 7.2).

Spray solution II: See sulfanilic acid diazotised (p. 94).

Procedure: Spray the chromatogram consecutively with I and II.


Chemicals:
- Bromophenol blue indicator, art. no. 8122
- Methyl red indicator, art. no. 6076
- 1/15 M Potassium dihydrogen phosphate solution, art. no. 4875
- 1/15 M di-Sodium hydrogen phosphate solution, art. no. 6587
- Ethanol abs. G R, art. no. 972


Spray solution I: Dissolve 0.01 g N-bromosuccinimide in 100 ml glacial acetic acid.

Spray solution II: Dissolve 0.01 g fluorescein in 100 ml ethanol.

Procedure: Spray consecutively with I and II.

Inspect in day light and in long-wave UV light.


Chemicals:
- N-Bromosuccinimide, art. no. 1949
- Fluorescein, art. no. 3990
- Acetic acid glacial min. 96%/0 (1.06) G R, art. no. 90062
- Ethanol abs. G R, art. no. 972

49. Bromosuccinimide — fluorescein for sulfur containing compounds.

Spray solution I: 0.035% solution of N-bromosuccinimide in 1.1.1-trichloroethane.

Spray solution II: Fill up 3 ml 0.33%/0 solution of fluorescein in 0.1 N sodium hydroxide solution to 100 ml with ethanol.

Procedure: Spray with I, dry at room temperature and spray with II.


Chemicals:
- N-Bromosuccinimide, art. no. 1949
- Fluorescein, art. no. 3990
- 0.1 N Sodium hydroxide solution Titrisol®, art. no. 9959
- Ethanol abs. G R, art. no. 972
- 1.1.1-Trichloroethane

50. Bromothymol blue for lipoids.

Spray solution: Dissolve 0.04 g bromothymol blue in 100 ml 0.01 N sodium hydroxide solution.

51. Cacotheine for vitamin C.

Spray solution: 2% aqueous cacotheine solution.

Treatment: Heat at 110°C.

Violet spots.


Chemicals:

Cacotheine G R, art. no. 4795

52. Carbazole — sulfuric acid for sugars.

Spray solution: Dissolve 0.5 g carbazole in 95 ml ethanol and add 3 ml 97%/ sulfuric acid. Prepare freshly before use.

Treatment: Heat 10 min at 120°C.

Violet spots on blue background.


Chemicals:

Carbazole

Ethanol abs. G R, art. no. 972

Sulfuric acid 95–97%/ (1.84) G R, art. no. 731

53. Carmine for polysaccharides. PC.

Stock solution: Heat 1 g carmine, 0.5 g anhydrous aluminium chloride and 2 ml water 2–3 min, add the solution to 100 ml 50%/ ethanol and filter after 24 hours. The filtrate must be stored at 5°C.

Spray solution: Dilute 5 ml of stock solution with 17 ml ethanol and 3 ml water.

Procedure: Before drying it is advantageous to fix the polysaccharides. Dip the chromatogram 15 min into a mixture of 20 ml formaldehyde and 80 ml ethanol and dry at room temperature.


Chemicals:

Carmine, art. no. 2233

Aluminium chloride anhydrous, art. no. 1082

Ethanol abs. G R, art. no. 972

Formaldehyde solution 35%/ G R, art. no. 4003

54. Cerium(IV) sulfate — arsenite for organic and inorganic iodine containing compounds. PC.

Solution a: Add 10 g cerium(IV) sulfate to 100 ml 1 N sulfuric acid, which has been cooled to 0–5°C. The mixture is cooled for another hour and then filtered or centrifuged. Store the clear solution until use in the refrigerator.

Solution b: Dissolve 5 g sodium arsenite in 30 ml 1 N sodium hydroxide solution. Add the solution dropwise with stirring to 65 ml 2 N sulfuric acid cooled to 0–5°C and fill up to 100 ml with water.

Spray solution: Mix equal parts of a and b prior to use.

Procedure: Spray the chromatogram with the spray solution by placing it on a glass plate. This permits uniform spraying. Place a second glass plate with equal size over the moistened chromatogram and press. Within 30 minutes white spots on yellow background will appear at the sites of iodine compounds. Potassium iodide turns chocolate-brown.

Treatment: For greater contrast the chromatogram may be sprayed before drying with 1%/ solution of o-phenylenediamine in acetone. Thus the entire chromatogram turns brown and the white spots are more pronounced. Dry the chromatogram in iodine-free air.


Chemicals:

Cerium (IV) sulfate G R, art. no. 2274

Sodium metaarsenite G R, art. no. 6287

o-Phenylenediamine, art. no. 9721

1 N Sulfuric acid Titrisol®, art. no. 9984

Sulfuric acid 95–97%/ (1.84) G R, art. no. 731

1 N Sodium hydroxide solution Titrisol®, art. no. 9956

Acetone G R, art. no. 14

55. Cerium(IV) sulfate — nitric acid for polyphenyles.

Spray solution: Dissolve 0.3 g cerium(IV) sulfate in 100 ml 65%/ nitric acid.

Treatment: Heat 15–20 min at 120°C.

Inspect in long-wave UV light.


Chemicals:

Cerium (IV) sulfate G R, art. no. 2274

Nitric acid min. 65%/ (1.4) G R, art. no. 454

56. Cerium(IV) sulfate — sulfuric acid for alkaloids and iodo-organic compounds (modified reagent acc. to Sonnenschein).

Spray solution: Shurry 0.1 g cerium(IV) sulfate in 4 ml water. After addition of 1 g trichloroacetic acid boil and add dropwise 97%/ sulfuric acid until the solution becomes clear.
57. Cerium(IV) sulfate — sulfuric acid for solanum steroid alkaloids and steroid sapogenins.

**Spray solution**: Saturated solution of cerium(IV) sulfate in 65% sulfuric acid.

**Treatment**: Heat 15 min at 120°C.

**Note**: Not applicable with aluminium oxide layers.


**Chemicals**:
- Cerium(IV) sulfate G R, art. no. 2274
- Sulfuric acid 95–97% (1.84) G R, art. no. 731

58. Chloramine T for caffeine.

**Spray solution I**: 10% aqueous chloramine T solution.

**Spray solution II**: 1 N hydrochloric acid.

**Procedure**: Spray with I and after short drying with II. Heat at 96–98°C until the smell of chlorine has disappeared. Place the chromatogram into a chamber saturated with ammonia vapour and heat subsequently for a short time until the maximal visualisation of the spots.

**Literature**: H. Gänshirt, A. Malzacher, Arch. Pharm. 293, 925 (1960).

**Chemicals**:
- 1 N Hydrochloric acid Titrisol®
- Chloramine T GR, art. no. 2426
- Ammonia solution min. 23% (0.91) G R, art. no. 5432

59. Chloramine T — trichloroacetic acid for digitalis glycosides.

**Spray solution**: Mix 10 ml of a freshly prepared 3% aqueous chloramine solution with 40 ml 25% solution of trichloroacetic acid in ethanol. Trichloroacetic acid solution is stable for several days.

**Procedure**: Heat 7 min at 110°C. Bluish and yellow fluorescence in long-wave UV light.

**Literature**: D. Waldi, Arch. Pharm. 292, 206 (1959).

**Chemicals**:
- Chloramine T G R, art. no. 2426
- Trichloroacetic acid G R, art. no. 807
- Ethanol abs. G R, art. no. 972

60. Chlorine — pyrazolone — cyanide for indoles, amides, sulfonamides.

**Chlorination**: Place the chromatogram for about 2–3 min into a chlorine atmosphere (prepared from potassium permanganate and 25% hydrochloric acid).

To remove excess chlorine heat the plate at 100°C.

**Spray solution**: Equal volumes of 0.2 M solution of 1-phenyl-3-methyl-5-pyrazolone in pyridine and 1 N solution of potassium cyanide.

**Procedure**: After removal of the excess chlorine spray the chromatogram until beginning transparency. Caution, poisonous!

The respective compounds show bright red spots which turn blue after 2 min.


**Chemicals**:
- Potassium permanganate G R, art. no. 5082
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316
- 1-Phenyl-3-methyl-5-pyrazolone
- Pyridine G R, art. no. 9728
- Potassium cyanide GR, art. no. 4967

61. Chlorine — toidine for compounds convertible into chloramines.

**Chlorination**: Place the chromatogram into chlorine atmosphere; 5–10 min with chlorine from a bomb, 15–20 min with chlorine prepared from a 1.5% solution of potassium permanganate and 10% hydrochloric acid (1+1).

For removing excess chlorine allow the plate to stand for 5 min in the air.

**Spray solution**: Dissolve 0.16 g o-tolidine in 30 ml glacial acetic acid, fill up the solution to 500 ml with water and add 1 g potassium iodide.

**Note**: Spray a corner of the chromatogram to establish that chlorine has been removed completely. If no blue colour appears spray the whole plate.


**Chemicals**:
- Potassium permanganate G R, art. no. 5082
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- o-Tolidine G R, art. no. 8311
- Potassium iodide G R, art. no. 5043

*Spray solution I*: 2% aqueous solution of potassium hypochlorite.

*Spray solution II*: Mix before use equal volumes of saturated solution of o-tolidine in 2% acetic acid and 0.83% aqueous potassium iodide solution.

**Procedure**: Spray lightly with I, dry at room temperature for 1–2 hours, and spray with II.


**Chemicals**:
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Potassium iodide G R, art. no. 5043
- Potassium hypochlorite
- o-Tolidine G R, art. no. 8311

63. Chlorocyan – 4-aminobenzoic acid for pyridine compounds with at least one free o-position.

*Spray solution*: 5% methanolic solution of 4-aminobenzoic acid.

**Procedure**: Place the sprayed chromatogram into a chamber with a freshly prepared mixture of 20 ml 28% aqueous shurry of chloramine, 20 ml 1 N hydrochloric acid and 10 ml 10% aqueous potassium cyanide solution.

Caution, poisonous!

The spots will appear after a short time.


**Chemicals**:
- 4-Aminobenzoic acid G R, art. no. 102
- Chloramine T GR, art. no. 2426
- Potassium cyanide G R, art. no. 4967
- 1 N Hydrochloric acid Titrisol®, art. no. 9970
- Methanol G R, art. no. 6009

64. 1-Chloro-2,4-dinitrobenzene – indicator reagent.

*Spray solution*: 0.5% ethanolic solution of 1-chloro-2,4-dinitrobenzene.

**Chemicals**:
- 1-Chloro-2,4-dinitrobenzene G R, art. no. 2427
- Ethanol abs. G R, art. no. 972

65. 1-Chloro-2,4-dinitrobenzene for nicotinic acid, nicotinamide, pyridoxol.

*Spray solution I*: 1% methanolic solution of 1-chloro-2,4-dinitrobenzene.

*Spray solution II*: 3 N sodium hydroxide solution.

**Procedure**: Spray subsequently with I and II.


*Spray solution*: 0.04% ethanolic solution of chlorophenol red. Adjust the solution with 0.1 N sodium hydroxide solution to pH 7.0.


**Chemicals**:
- Chlorophenol red indicator, art. no. 3024
- 0.1 N Sodium hydroxide solution Titrisol®, art. no. 9959

67. Chlorosulfonic acid – glacial acetic acid for triterpenes, sterols, steroids.

*Spray solution*: Dissolve 5 ml chlorosulfonic acid in 10 ml glacial acetic acid with cooling.

**Treatment**: Heat 5–10 min at 130°C C.

Inspect in long-wave UV light.


**Chemicals**:
- Chlorosulfonic acid, art. no. 220
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

68. Chromosulfuric acid as universal reagent for organic compounds.

*Spray solution*: Dissolve 5 g potassium dichromate in 100 ml 40% sulfuric acid.

**Note**: The reagent is suitable for charring organic compounds, in particular, lipids, by heating the chromatogram at 150°C C.


**Chemicals**:
- Potassium dichromate G R, art. no. 4864
- Sulfuric acid 95–97% (1.84) G R, art. no. 731

69. Chromotropic acid for methylenedioxyphenyl-type compounds.

(e.g. narcotine, hydrastine, sesamine and other compounds splitting off formaldehyde).

*Solution a*: 10% aqueous solution of chromotropic acid sodium salt.
70. Cinnamaldehyde – acetic anhydride – sulfuric acid for steroid sapogenins.

**Spray solution I**: 5% ethanolic cinnamaldehyde solution.

**Spray solution II**: Prepare freshly before use a mixture of 12 parts acetic anhydride and 1 part 97% sulfuric acid.

**Procedure**: Spray with I, dry 5 min at 90°C and spray with II. After 1–2 min at room temperature, the chromatogram is heated at 90°C until the spots appear.

**Chemicals**:
- Cinnamaldehyde, art. no. 2505
- Sulfuric acid 95–97%/ (1.84) G R, art. no. 731
- Acetic anhydride G R, art. no. 42
- Ethanol abs. G R, art. no. 972

71. Cinnamaldehyde – hydrochloric acid for indole derivatives.

**Spray solution**: Dissolve 5 ml cinnamaldehyde in 100 ml ethanol and add 5 ml 37% hydrochloric acid freshly before use.

**Treatment**: Place the plate into hydrogen chloride atmosphere. Red spots.

**Literature**: D. Jerschel, R. Müller, Naturwissenschaften 38, 561 (1951).

**Chemicals**:
- Cinnamaldehyde, art. no. 2505
- Ethanol abs. G R, art. no. 972
- Hydrochloric acid fuming min. 37%/ (1.19) G R, art. no. 317

72. Cobalt(II) chloride for organic phosphate esters.

**Spray solution**: 1% anhydrous cobalt(II) chloride solution in acetone.

**Treatment**: Heat at 40–50°C.

Blue spots. The reaction is not sensitive.


**Chemicals**:
- Cobalt(II) chloride G R, art. no. 2539
- Acetone G R, art. no. 14

73. Cobalt(II) – lead nitrite for ammonium and potassium ions. PC.

**Spray solution I**: Dissolve 5 g cobalt(II) nitrate and 5 g lead nitrate in 100 ml water and add 1–2 drops nitric acid.

**Spray solution II**: Saturated sodium nitrite solution in 2 N acetic acid.

**Procedure**: Spray with I, and after drying with II. Then rinse with water and dry again.


**Chemicals**:
- Lead(II) nitrate G R, art. no. 7398
- Cobalt(II) nitrate G R, art. no. 2536
- Sodium nitrite G R, art. no. 6549
- Acetic acid glacial min. 96%/ (1.06) G R, art. no. 90062
- Nitric acid min. 65%/ (1.4) G R, art. no. 454

74. Cobalt(II) nitrate – ammonia for barbiturates (Zwikker reagent).

**Spray solution**: 1% ethanolic cobalt(II) nitrate solution.

**Treatment**: Dry and place into a chamber saturated with ammonia.


**Chemicals**:
- Cobalt(II) nitrate G R, art. no. 2536
- Ethanol abs. G R, art. no. 972
- Ammonia solution 25%/ (0.91) G R, art. no. 5432

75. Cobalt(II) nitrate – lithium hydroxide for barbiturates.

**Spray solution I**: 2% cobalt(II) nitrate solution in absolute methanol.

**Spray solution II**: 0.5% methanolic lithium hydroxide solution.

**Procedure**: Spray with I and after drying at room temperature with II.

**Literature**: H. Weidmann, Dissertation, Berlin 1961

**Chemicals**:
- Cobalt(II) nitrate G R, art. no. 2536
- Lithium hydroxide, art. no. 5691
- Methanol G R, art. no. 6009
- Methanol dried G R, art. no. 6012

76. Cobalt(II) thiocyanate for alkaloids and amines.

**Spray solution**: Dissolve 3 g ammonium thiocyanate and 1 g cobalt(II) chloride in 20 ml water.

**Note**: Alkaloids and amines show blue spots on white to pink background.

The colours grow pale after 2 hours and can be restored by spraying with water or placing the chromatogram into water vapour.
77. Copper acetate — potassium hexacyanoferrate(II) for the identification of higher fatty acids acc. to Kaufmann, PC.

**Procedure:**
- **Dip solution I:** Mix 10 ml saturated aqueous copper acetate solution with 240 ml water.
- **Dip solution II:** Freshly prepared 1.5% aqueous potassium hexacyanoferrate(II) solution.

**Note:** After separation of the fatty acids on petroleum — or undecane — impregnated paper heat the chromatogram 2 hours at 120°C to remove the impregnation. Then place the chromatogram 45 min into dip solution I. Subsequently remove the excess copper acetate with running water by rinsing for 15 min. Then place the chromatogram into dip solution II where the acids show red brown spots.


**Chemicals:**
- Copper(II) acetate G R, art. no. 2711
- Potassium hexacyanoferrate(II) G R, art. no. 4984

78. Copper acetate — rubeanic acid for the identification of higher fatty acids acc. to Kaufmann, PC.

**Procedure:**
- **Dip solution I:** Dilute 10 ml saturated copper(II) acetate solution to 1 l with water.
- **Dip solution II:** 0.1% ethanolic rubeanic acid solution with 0.5% ammonia.

**Note:** After separation of the fatty acids on petroleum — or undecane — impregnated paper heat the chromatogram 2 hours at 120°C to remove the impregnation. Then place the chromatogram 45 min into dip solution I and remove excess copper salt by rinsing with water for 1.5 hours. Dip the moist chromatogram 30 min into II, then rinse again 30 min with running water and dry.


**Chemicals:**
- Copper(II) acetate G R, art. no. 2711
- Rubeanic acid (dithiooxamide) G R, art. no. 629
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25%/e (0.91) G R, art. no. 5432

79. Copper chloride for oximes.

**Spray solution:** 0.5% aqueous copper(II) chloride solution.

**Note:** β-Oxime complex compounds show green spots immediately after spraying, α-oxime complex compounds show weak green spots after heating 10 min at 110°C.

**Literature:** M. Hranisavljević-Jacovljević, I. Pexjkić-Tadić, A. Stojilković, J. Chromatog. 12, 70 (1963).

**Chemicals:**
- Copper(II) chloride G R, art. no. 2733

80. Copper sulfate — benzidine for pyrindine monocarboxylic acids.

**Spray solution I:** Dissolve 0.3 g copper(II) sulfate in 100 ml 45% ethanol.

**Spray solution II:** 0.1% solution of benzidine in 50% ethanol.

**Procedure:** Spray with I, dry the chromatogram at 60°C and spray with II. Blue spots.

**Chemicals:**
- Copper(II) sulfate G R, art. no. 2790
- Benzidine G R, art. no. 1762
- Ethanol abs. G R, art. no. 972

81. Copper sulfate — quinine — pyridine for barbiturates and thiobarbiturates.

**Spray solution I:** Dissolve 0.2 g copper(II) sulfate and 0.02 g quinine hydrochloride in 50 ml water, add 2 ml pyridine and fill up to 100 ml with water.

**Spray solution II:** 0.5% aqueous potassium permanganate solution.

**Procedure a:** Spray with I and dry at room temperature. White, yellow or violet spots in daylight, dark spots on fluorescent background in long-wave UV light.

**Procedure b:** Spray subsequently with II. Yellow or white spots.


**Chemicals:**
- Copper(II) sulfate G R, art. no. 2790
- Potassium permanganate G R, art. no. 5082
- Quinine hydrochloride G R, art. no. 9728

82. Copper(II) sulfate — sodium citrate for flavonoids and coumarins with o-dihydroxy groups (Benedict's reagent).

**Spray solution:** Dissolve 1.3 g copper(II) sulfate, 17.3 g sodium citrate and 10 g anhydrous sodium carbonate in water and fill up to 100 ml.

**Note:** The fluorescence in long-wave UV light of coumarins with o-dihydroxy groups is quenched by Benedict's reagent. Compounds without o-dihydroxy groups keep or show stronger fluorescence, often connected with change of colour.

83. α-Cyclodextrin for straight-chain lipids.

Spray solution: 30% ethanolic solution of α-cyclodextrin.

Treatment: Dry the chromatogram at room temperature and place it into a chamber containing iodine vapour.


Chemicals:
- Iodine resublimed, G R, art. no. 4761
- Ethanol abs. G R, art. no. 972
- α-Cyclodextrin

84. Cysteine — sulfuric acid for desoxyribonucleosides (modif. reagent acc. to Dicche).

Spray solution: Mix freshly before use 1 part of a 0.5% cysteine hydrochloride solution in 3 N sulfuric acid with 9 parts acetone.

Procedure: Spray the chromatogram with the solution or dip into it, then heat 5–10 min at 85°C.

Desoxyribonucleosides and their phosphates turn green or grey, purines are dyed more rapidly than pyrimidines.


Chemicals:
- L(+)-Cysteimun hydrochloride, art. no. 2839
- Sulfuric acid 95–97% (1.84) G R, art. no. 731
- Acetone G R, art. no. 14

85. 3.5-Diaminobenzoic acid — phosphoric acid for 2-deoxy-sugars.

Spray solution: Dissolve 1 g 3.5-diaminobenzoic acid dihydrochloride in 25 ml 80% phosphoric acid and dilute with 60 ml water.

Treatment: Heat 15 min at 100°C.
The spots fluoresce green-yellow in long-wave UV light. Amounts more than 2 μg are visible as brown spots in daylight.


Chemicals:
- ortho-Phosphoric acid min. 85%/s (1.71) G R, art. no. 573
- 3.5-Diaminobenzoic acid dihydrochloride

86. O-dianisidine for aldehydes and ketones.

Spray solution: Saturated solution of o-dianisidine in glacial acetic acid.

Note: In some cases 2.7-diaminofluorene may be used instead of o-dianisidine. Good differentiation of colours.


Chemicals:
- o-Dianisidine (3,3'-dihaloxybenzidine) G R, art. no. 2953
- Acetic acid glacial min. 96%/s (1.06) G R, art. no. 90062
- 2.7-Diaminofluorene

87. Diazotisation and coupling with 1-naphthol for aromatic primary amines and sulfonamides.

Spray solution I: Freshly prepared 1% sodium nitrite solution in 1 N hydrochloric acid.

Spray solution II: Freshly prepared 0.2% 1-naphthol solution in 1 N potassium hydroxide.

Procedure: Spray with I and after 1 min with II. Dry the chromatogram at 60°C.

Note: Instead of 1-naphthol a 0.4% methanolic solution of N-(1-naphthyl)-ethylenediamine dihydrochloride may be used as coupling agent.


Chemicals:
- 1-Naphthol G R, art. no. 6223
- Sodium nitrite G R, art. no. 6549
- N-(1-Naphthyl)-ethylenediamine dihydrochloride G R, art. no. 6237
- 1 N Hydrochloric acid Titrisol®, art. no. 9970
- 1 N Potassium hydroxide solution Titrisol®, art. no. 9918
- Methanol G R, art. no. 6009

88. 2,6-Dibromoquinone chloride for phenols (Gibbs reagent).

Spray solution: Freshly prepared 0.4% methanolic solution of 2,6-dibromoquine chloride.

Treatment: Spray the chromatogram with 10% aqueous sodium carbonate solution or place it in a chamber saturated with ammonia.

90. 2,7'-Dichlorofluorescein

**Spray reagent 0.1% for chromatography.**

Visualisation reagent ready for use in aerosol cans. Art. no. 9677

91. 2,7'-Dichlorofluorescein - aluminium chloride - iron(III) chloride for free fatty acids (specific detection).

**Spray solution I:** 0.05% ethanolic solution of 2,7'-dichlorofluorescein.

**Spray solution II:** 1% ethanolic solution of aluminium chloride.

**Spray solution III:** 1% aqueous solution of iron(III) chloride.

**Procedure:** Spray with I, dry some minutes at 100°C, spray with II, dry again some minutes at 100°C and spray with III. Pink-violet spots on fallow background.

**Literature:** A. E. Dudzinsky, J. Chromatog. 31, 560 (1967).

**Chemicals:**
- 2,7'-Dichlorofluorescein, art. no. 9676
- Aluminium chloride anhydrous, art. no. 1082
- Iron(III) chloride G R, art. no. 3943
- Ethanol abs. G R, art. no. 972

92. 2,6-Dichlorophenolindophenol - silver nitrate for alkali chlorides.

**Spray solution:** 0.2% ethanolic solution of 2,6-dichlorophenolindophenol sodium salt. Filter after addition of 3 g silver nitrate and shaking. Prepare freshly before use.

**Literature:** T. Barnabas, M. G. Badve, J. Barnabas, Naturwissenschaften 41, 478 (1954).

**Chemicals:**
- 2,6-Dichlorophenolindophenol sodium G R, art. no. 3028
- Silver nitrate G R, art. no. 1512
- Ethanol abs. G R, art. no. 972

93. 2,6-Dichlorophenolindophenol sodium salt for organic acids and keto acids.

**Spray solution:** 0.1% ethanolic solution of 2,6-dichlorophenolindophenol sodium salt.

**Treatment:** After brief warming the acids appear as red spots on light blue background.


**Chemicals:**
- 2,6-Dichlorophenolindophenol sodium G R, art. no. 3028
- Ethanol abs. G R, art. no. 972

94. 2,6-Dichlorophenolindophenol sodium salt for vitamin C (Tillman reagent).

**Spray solution:** 0.05% solution of 2,6-dichlorophenolindophenol sodium salt in 50% ethanol.

**Note:** Colourless spots on blue background.


**Chemicals:**
- 2,6-Dichlorophenolindophenol sodium G R, art. no. 3028
- Ethanol abs. G R, art. no. 972

95. 2,6-Dichloroquinone chlorimide for antioxidants, adrenaline and derivatives, cyanamide and derivatives.

**Spray solution:** Prepare freshly before use a 0.1-1% solution of 2,6-dichloroquinone chlorimide in 100 ml absolute ethanol.

The spots appear after about 15 minutes. Not to be used for urea. Some antioxidants show characteristic change of colours after being sprayed with a 2% solution of sodium tetraborate in 40% ethanol.
96. Dicobalt octacarbonyl for acetylene compounds.

**Spray solution I:** Dissolve 0.5 g dicobalt octacarbonyl in 100 ml petroleum benzine.

**Spray solution II:** 1 N hydrochloric acid.

**Procedure:** Spray with I, wait 10 min, spray with II and remove the layer with Neatan® after drying. Wash out excess reagent with water and place the chromatogram into a bromine atmosphere.

The spots show yellow colours.


**Chemicals:**
- 1 N Hydrochloric acid Titrisol®, art. no. 9970
- Bromine G R, art. no. 1948
- Neatan® for thin layer chromatography, art. no. 6746
- Dicobalt octacarbonyl
- Petroleum benzine boiling range 100–140°C, art. no. 1770


**Spray solution:** Dissolve 0.5 g copper(II) sulfate in 100 ml methanol. Add 3 ml diethylamine to the solution.

**Note:** Shake prior to use; stable for only a few days.

Thiobarbituric acids show green spots.

**Literature:** W. Dietz, K. Soehring, Arch. Pharm. 290, 80 (1957).

**Chemicals:**
- Copper(II) sulfate G R, art. no. 2790
- Methanol G R, art. no. 6009
- Diethylamine, art. no. 3010

98. Diethyl malonate for 3,5-dinitrobenzoic acid esters.

**Spray solution I:** 10% ethanolic solution of diethyl malonate.

**Spray solution II:** 10% aqueous sodium hydroxide.

**Procedure:** Spray with I and then with II.

Heat 5 min at 95°C.

Red-violet spots.

**Literature:** J. Cerny, Chem. listy 49, 1899 (1955).

**Chemicals:**
- Diethyl malonate, art. no. 898
- Sodium hydroxide solution 10% (1.109) G R, art. no. 5588
- Ethanol abs. G R, art. no. 972


**Spray solution:** Dissolve 0.3 g 5,5-dimethyl-1,3-cyclohexanedione (dimedone) in 90 ml ethanol and add 10 ml 85% phosphoric acid.

**Treatment:** Heat 15–20 min at 110°C.

In daylight yellow spots on a white background, in long-wave UV light blue fluorescent spots.

**Literature:** S. Adachi, Anal. Biochem. 9, 224 (1964).

**Chemicals:**
- Dimedone G R, art. no. 6013
- Ortho-Phosphoric acid min. 85% (1.71) G R, art. no. 573
- Ethanol abs. G R, art. no. 972

100. 4-Dimethylaminobenzaldehyde.

**Spray reagent 0.5% for chromatography.**

Visualisation reagent ready for use in aerosol cans, Art. no. 3056

101. 4-Dimethylaminobenzaldehyde – acetic acid – phosphoric acid for proazulenes and azulenes (EP-reagent).

**Spray solution:** Dissolve 0.25 g 4-dimethylaminobenzaldehyde in a mixture of 50 g glacial acetic acid and 3 g 85% phosphoric acid. After solution is complete, add 20 ml water. Stable for months in a brown bottle.

**Note:** Azulenes turn deep blue at room temperature. Proazulenes show blue spots only after heating for 10 min at 80°C. The colours grow pale and become green to yellow. By exposure to steam over a water bath the spots show again their intense blue colour.

**Literature:** E. Stahl, Dtsch. Apotheker-Ztg. 93, 197 (1953).


**Chemicals:**
- 4-Dimethylaminobenzaldehyde G R, art. no. 3058
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Ortho-Phosphoric acid min. 85% (1.71) G R, art. no. 573

102. 4-Dimethylaminobenzaldehyde – acetylacetone for amino-sugars (Morgan-Elson reagent).

**Spray solution I:** Add 5 ml of a mixture of 5 ml 50% aqueous potassium hydroxide and 20 ml ethanol immediately prior to use to 10 ml of a solution of 0.5 ml acetylacetone and 50 ml 1-butanol.
**Procedure:** After spraying with I heat 5 min at 105° C, spray with II and dry 5 min at 90° C. Red spots.


**Chemicals:**
- 4-Dimethylaminobenzaldehyde G R, art. no. 3058
- Acetylacetone G R, art. no. 9600
- Potassium hydroxide G R, art. no. 5033
- Ethanol abs. G R, art. no. 972
- 1-Butanol G R, art. no. 1990
- Hydrochloric acid fuming min. 37%/o (1.19) G R, art. no. 317

**103. 4-Dimethylaminobenzaldehyde — hydrochloric acid for amines (Ehrlich’s reagent).**

**Spray solution A:** Dissolve 1 g 4-dimethylaminobenzaldehyde in a mixture of 25 ml 37%/o hydrochloric acid and 75 ml methanol.

**Treatment:** In some cases it is necessary to warm the plate.

**Spray solution B:** 1%/o ethanolic solution of 4-dimethylaminobenzaldehyde.

**Treatment:** Place the sprayed chromatogram 3–5 min in a chamber saturated with hydrochloric acid vapours or spray with 25%/o hydrochloric acid. Sometimes it is necessary to warm the plate.


**Chemicals:**
- 4-Dimethylaminobenzaldehyde G R, art. no. 3058
- Hydrochloric acid min. 37%/o (1.125) G R, art. no. 316
- Hydrochloric acid fuming min. 37%/o (1.19) G R, art. no. 317
- Methanol G R, art. no. 6009
- Ethanol abs. G R, art. no. 972

**104. 4-Dimethylaminobenzaldehyde — hydrochloric acid according to Stahl for indole derivatives (van Urk reagent).**

**Spray solution:** Dissolve 1 g 4-dimethylaminobenzaldehyde in 50 ml 37%/o hydrochloric acid and add 50 ml ethanol.

**Note:** In case of eluents with volatile alkaline reacting components it is necessary to heat the plate to about 50° C, until these compounds have disappeared.

**Procedure:** Spray intensively until transparency. Subsequently blow vapours of aqua regia over the layer.

**Literature:** E. Stahl, H. Kaldewey, Hoppe-Seylers Z. physiol. Chem. 323, 182 (1961)

**Chemicals:**
- 4-Dimethylaminobenzaldehyde G R, art. no. 3058
- Hydrochloric acid fuming min. 37%/o (1.19) G R, art. no. 317
- Ethanol abs. G R, art. no. 972
- Nitric acid min. 65%/o (1.4) G R, art. no. 454

105. 4-Dimethylaminobenzaldehyde — sulfuric acid for ergot alkaloids.

**Spray solution:** Dissolve 0.125 g 4-dimethylaminobenzaldehyde in a cooled mixture of 65 ml 97%/o sulfuric acid and 35 ml water and add 0.05 ml 5%/o aqueous iron(III) chloride solution. Stable for about a week.

**Literature:** M. Zinser, C. Baumgarrel, Arch. Pharm. 297, 158 (1964).

**Chemicals:**
- 4-Dimethylaminobenzaldehyde G R, art. no. 3058
- Sulfuric acid 95–97%/o (1.84) G R, art. no. 731
- Iron(III) chloride G R, art. no. 3943

106. Dimethylaminobenzylidenerhodanine for silver, copper and mercury ions.

**Spray solution:** 1%/o ethanolic solution of 5-(4”-dimethylaminobenzylidene)-rhodanine.

**Treatment:** Spray with 25%/o ammonia solution or place into a chamber saturated with ammonia. Pink to violet spots.

**Literature:** F. W. H. M. Merkus, Pharm. Weekblad 98, 955 (1963).

**Chemicals:**
- 5-(4”-Dimethylaminobenzylidene)-rhodamine G R, art. no. 3059
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25%/o (0.91) G R, art. no. 5432

107. 4-Dimethylaminocinnamaldehyde for indoles.

**Stock solution:** Dissolve 2 g 4-dimethylaminocinnamaldehyde in a mixture of 100 ml 6 N hydrochloric acid and 100 ml ethanol. Store the solution in the refrigerator.

**Spray solution:** 1 part stock solution and 4 parts ethanol.

**Treatment:** Heat 5 min at 105° C. The colours of the spots are intensified by blowing vapours of aqua regia over the layer.

**Note:** Unsuitable with ammonia containing eluents because the background becomes coloured. By brief heating (10 min) at 105° C this can be evaporated before spraying.

108. Dimethyl-p-phenylenediamine dihydrochloride for bromine-containing hypnotics and chlorinated insecticides.

**Spray solution**: Dissolve 0.5 g N,N-dimethyl-p-phenylenediamine dihydrochloride in 100 ml sodium ethoxide (1 g sodium in 100 ml ethanol).

**Procedure**: After spraying moisten the chromatogram with a water spray and irradiate 1 min with unfiltered UV light. This liberates free halogen which oxidises the reagent to Wurster's red.


**Chemicals**:
- N,N-Dimethyl-p-phenylenediamine dihydrochloride G R, art. no. 3067
- Sodium G R, art. no. 6261
- Ethanol abs. G R, art. no. 972


**Spray solution**: Dissolve 1.5 g N,N-dimethyl-p-phenylenediamine dihydrochloride in a mixture of 128 ml methanol, 25 ml water and 1 ml glacial acetic acid.

Peroxides show purple spots.


**Chemicals**:
- N,N-Dimethyl-p-phenylenediamine dihydrochloride G R, art. no. 3067
- Acetic acid glacial min. 96%/ (1.06) G R, art. no. 90062
- Methanol G R, art. no. 6009

110. Dimethyl-p-phenylenediamine dihydrochloride – trichloroacetic acid for methyl-sugars.

**Spray solution**: Dissolve 0.4 g N,N-dimethyl-p-phenylenediamine dihydrochloride in 100 ml 29%/ aqueous trichloroacetic acid solution.

**Treatment**: Heat 1–2 min at 120°C.

**Note**: The colour spots may be eluted for colorimetric determination.


**Chemicals**:
- N,N-Dimethyl-p-phenylenediamine dihydrochloride G R, art. no. 3067
- Trichloroacetic acid G R, art. no. 807

111. 1.3-Dinitrobenzene for 17-ketosteroids.

**Solution a**: 2%/ethanolic solution of 1.3-dinitrobenzene.

**Solution b**: 2.5 N methanolic potassium hydroxide solution.

**Spray solution**: Mix equal parts of a and b.

**Treatment**: Heat 1–2 min at 80°C.

**Violet spots**.


**Chemicals**:
- 1.3-Dinitrobenzene, art. no. 3114
- Sodium hydroxide G. R., art. no. 5033
- Ethanol abs. G R, art. no. 972

**Variation for PC**:

**Dip solution I**: Mix 1 part 30%/ aqueous potassium hydroxide with 1 part ethanol.

**Dip solution II**: 2%/ethanolic 1.3-dinitrobenzene solution.

**Procedure**: After dipping into I press off excess between filter paper. Then dip into II, press off and heat slowly at 65°C.

17-Ketosteroids turn violet, 2-ketosteroids blue-violet and 20-ketosteroids brown.


**Chemicals**:
- 1.3-Dinitrobenzene, art. no. 3114
- Potassium hydroxide G R, art. no. 5033
- Ethanol abs. G R, art. no. 972

112. 3.5-Dinitrobenzoic acid for cardiac glycosides.

**A. Spray solution**: Dissolve 1 g 3.5-dinitrobenzoic acid in a mixture of 50 ml methanol and 50 ml 2 N potassium hydroxide solution.

**B. Spray solution I**: 2%/ methanolic solution of 3.5-dinitrobenzoic acid.

**Spray solution II**: 5.7%/ methanolic potassium hydroxide solution.

**Procedure**: Spray lightly with I and then with excess II. The spots show blue violet colours.

113. 3.5-Dinitrobenzoic acid for reducing sugars.

*Spray solution:* 1% solution of 3.5-dinitrobenzoic acid in 2 N sodium carbonate solution.
*Treatment:* Dry 5–10 min at 100° C.
*Chemicals:* 3.5-Dinitrobenzoic acid, art. no. 138
Sodium carbonate G R, art. no. 6392

114. 2,4-Dinitrofluorobenzene for amino acids.

*Spray solution I:* Dissolve 8.4 g sodium hydrogen carbonate in 80 ml water, add 2.5 ml N sodium hydroxide solution and make up to 100 ml with water.
*Spray solution II:* 10% methanolic solution of 2,4-dinitrofluorobenzene.
*Treatment:* Spray with I and subsequently with II.
*Procedure:* Scrape off 5 mm from both sides of the plate. Place two polyethylene strips of same breadth on the margins so that a second glass plate can be laid on the layer. Heat 1 hour at 40° C in the dark, cool the plate and place 10 min in an ether bath. After drying the spots are outlined.
*Chemicals:* 1-Fluoro-2,4-dinitrobenzene G R, art. no. 2966
Sodium hydrogen carbonate G R, art. no. 6329
1 N Sodium hydroxide solution Titrisol®, art. no. 9956
Methanol G R, art. no. 6009
Diethyl ether G R, art. no. 921

115. 2,4-Dinitrophenylhydrazine for free aldehyde and keto groups and ketoses.

A. *Spray solution:* 0.4% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid.
B. *Spray solution:* Add 10 ml 37% hydrochloric acid to 1 g 2,4-dinitrophenylhydrazine in 1000 ml ethanol.
*Treatment:* For distinction of the formed 2,4-dinitrophenylhydrazones (DNPH) spray consecutively with 0.2% solution of potassium hexacyanoferate(III) in 2 N hydrochloric acid. Saturated keto-DNPH show blue colour immediately, saturated aldehyde-DNPH show olive-green colour more slowly. Unsaturated carbonyl derivatives change only slowly or not at all.

*Chemicals:* 2,4-Dinitrophenylhydrazine G R, art. no. 3073
Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
Ethanol abs. G R, art. no. 972
Potassium hexacyanoferate(III) G R, art. no. 4973

116. 3,5-Dinitrosalicylic acid for reducing sugars.

*Spray solution:* 0.5% solution of 3,5-dinitrosalicylic acid in 4% sodium hydroxide solution.
*Treatment:* After brief pre-drying at room temperature heat 4–5 min at 100° C.
*Chemicals:* 3,5-Dinitrosalicylic acid, art. no. 141
Sodium hydroxide G R, art. no. 6498

117. Diphenylamine for glycolipids.

*Spray solution:* Mixture of 20 ml 10% ethanolic diphenylamine solution, 100 ml 37% hydrochloric acid and 80 ml glacial acetic acid.
*Treatment:* Heat 5–10 min at 100° C.
*Blue-grey spots.*
*Chemicals:* Diphenylamine G R, art. no. 3086
Ethanol abs. G R, art. no. 972
Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

118. Diphenylamine – palladium(II) chloride for nitroamines.

*Spray solution:* Mix 5 parts 1.5% ethanolic diphenylamine solution and 1 part 0.2% sodium chloride solution containing 0.1 g palladium(II) chloride.
*Treatment:* After exposure to short-wave UV light the substances show violet spots.
*Chemicals:* Diphenylamine G R, art. no. 3086
Palladium(II) chloride, art. no. 7110
Ethanol abs. G R, art. no. 972
Sodium chloride G R, art. no. 6404
119. Diphenylamine – zinc chloride for chlorinated insecticides.
Spray solution: Dissolve 0.5 g diphenylamine and 0.5 g zinc chloride in 100 ml acetone.
Treatment: Heat 5 min at 200° C.
Colour reaction.
Chemicals:
  Diphenylamine G R, art. no. 3086
  Zinc chloride G R, art. no. 8816
  Acetone G R, art. no. 14

120. Diphenylboric acid-β-aminoethyl ester for α- and γ-pyrones (Neu reagent).
Spray solution: 1% methanolic diphenylboric acid-β-aminoethyl ester solution.
Procedure: Spray about 10 ml of the solution and inspect the fluorescence in long-wave UV light.
Chemicals:
  Diphenylboric acid-β-aminoethyl ester
  Methanol G R, art. no. 6009

121. Diphenylcarbazide for silver, lead, mercury, copper, tin, zinc, and calcium ions.
Spray solution I: 1—2% ethanolic diphenylcarbazide solution.
Spray solution II: 25% ammonia solution or a chamber saturated with ammonia.
Note: For mercury acetate adducts heat some minutes at 80° C, causing the spots to turn blue-violet.
Chemicals:
  Diphenylcarbazide G R, art. no. 3091
  Ethanol abs. G R, art. no. 972
  Ammonia solution min. 25% (0.91) G R, art. no. 5432

122. Diphenylcarbazone for addition compounds of unsaturated fatty acids.
Spray solution: 0.2% ethanolic solution of diphenylcarbazone.
Note: Addition compounds of unsaturated acids (e.g. with Hg) are dyed purple.
Colour intensification may be obtained by respraying with 0.05 N nitric acid in ethanol.

123. Diphenylcarbazone for cations.
Spray solution: Saturated solution of diphenylcarbazone in methanol.
Chemicals:
  Diphenylcarbazone, art. no. 3087
  Methanol G R, art. no. 6009

124. Diphenylpicrylhydrazyl for essential oils.
Spray solution: Dissolve 0.06 g diphenylpicrylhydrazyl in 100 ml chloroform.
Treatment: Heat 5–10 min at 110° C.
Yellow spots on violet background.
Chemicals:
  2,2′-Diphenyl-1-picrylhydrazyl
  Chloroform G R, art. no. 2445

125. 2,5-Diphenyl-3-(4-styrylphenyl)-tetrazolium chloride (TPTZ) for reducing steroids (corticosteroids).
Solution a: Freshly prepared 1% methanolic solution of TPTZ.
Solution b: 3% aqueous sodium hydroxide solution.
Spray solution: Mix equal parts of a and b freshly before use.
Chemicals:
  2,5-Diphenyl-3-(4-styrylphenyl)-tetrazolium chloride
  Methanol G R, art. no. 6009
  Sodium hydroxide solution 10% (1.109) G R, art. no. 5588

126. Dipicrylamine for choline (non-specific).
Spray solution: Dissolve 0.2 g dipicrylamine in a mixture of 50 ml acetone and 50 ml water.
Note: Choline and its derivates appear as red spots on yellow background.

Stock solution: Add 1 g dipicrylamine to 0.12 g magnesium carbonate and 15 ml water, heat the mixture 15 min on a boiling water bath and filter.

Spray solution: Add to 0.2 ml of the dipicrylamine solution 50 ml methanol, 49 ml water and 1 ml 25%/solution ammonia solution.


Chemicals:
- Dipicrylamine G R, art. no. 3089
- Acetone G R, art. no. 14

128. Dithizone for ions of heavy metals.

Spray solution I: 0.05%/solution of dithizone in carbon tetrachloride.

Spray solution II: Spray with 25%/solution ammonia solution or place the chromatogram in a chamber saturated with ammonia.


Chemicals:
- Dithizone (diphenylthiocarbazone) G R, art. no. 3092
- Carbon tetrachloride G R, art. no. 2222
- Ammonia solution min. 25%/solution (0.91) G R, art. no. 5432

129. Dragendorff reagent for polyethylene glycols, polyethylene glycol ethers and polyethylene glycol esters.

Solution a: Dissolve 1.7 g bismuth(III) nitrate in a mixture of 20 ml glacial acetic acid and 80 ml water, add a solution of 40 g potassium iodide in 100 ml water and 200 ml glacial acetic acid and make up to 1000 ml with water.

Solution b: 20%/solution aqueous barium chloride solution.

Spray solution: Mix 2 parts a with 1 part b before use.


Chemicals:
- Bismuth (III) nitrate basic G R, art. no. 1878
- Potassium iodide G R, art. no. 5043
- Barium chloride G R, art. no. 1719
- Acetic acid glacial min. 96%/solution (1.06) G R, art. no. 90062

130. Dragendorff reagent acc. to Bregoff-Delwische for quaternary bases.

Stock solution: Dissolve 8.0 g bismuth(III) nitrate in 20–25 ml 25%/solution nitric acid. Add this solution slowly with stirring to a slurry of 20 g potassium iodide and 1 ml 6 N hydrochloric acid and 5 ml water. Add water to the dark precipitate until an orange-red colour develops. The volume of the solution should be 95 ml. Any solid residue present is filtered off and the solution made up to 100 ml with water. The solution is stable for several weeks in the refrigerator when stored in an amber flask.

Spray solution: Mix in this order: 20 ml water, 5 ml 6 N hydrochloric acid, 2 ml stock solution and 6 ml 6 N sodium hydroxide solution. In case bismuth hydroxide is not completely dissolved by shaking, add several drops of 6 N hydrochloric acid.

Note: The spray solution is stable for about 10 days in the refrigerator.


Chemicals:
- Bismuth (III) nitrate basic G R, art. no. 1878
- Nitric acid min. 65%/solution (1.4) G R, art. no. 454
- Hydrochloric acid min. 25%/solution (1.125) G R, art. no. 316
- Potassium iodide G R, art. no. 3043
- Sodium hydroxide solution min. 10%/solution (1.109) G R, art. no. 5588

131. Dragendorff reagent acc. to Munier for alkaloids and other nitrogen-containing compounds.

Solution a: Dissolve 1.7 g bismuth(III) nitrate and 20 g tartaric acid in 80 ml water.

Solution b: Dissolve 16 g potassium iodide in 40 ml water.

Stock solution: Mix equal parts of a and b. Stock solution is stable for several months, if refrigerated.

Spray solution: Dissolve 10 g tartaric acid in 50 ml water and add 10 ml of the stock solution.

Note: For detecting vitamin B1 spray with the stock solution.


Chemicals:
- Bismuth (III) nitrate basic G R, art. no. 1878
- Potassium iodide G R, art. no. 3043
- Li(+)-Tartaric acid G R, art. no. 804

132. Dragendorff reagent acc. to Munier and Macheboeuf for alkaloids and other nitrogen-containing compounds.

Solution a: Dissolve 0.85 g bismuth(III) nitrate in 10 ml glacial acetic acid and 40 ml water.
134. Ethylenediamine – potassium hexacyanoferrate(III) for catechol amines (adrenaline, noradrenaline and acetyl derivatives).

**Spray solution:** Solution of 0.1 g potassium hexacyanoferrate(III) in 5 ml ethylenediamine, 45 ml ethanol and 50 ml water.

**Treatment:** Heat the chromatogram 10 min at 105° C. Inspection under UV light.


**Chemicals:**
- Ethylenediamine, art. no. 947
- Potassium hexacyanoferrate (III) G R, art. no. 4973
- Ethanol abs. G R, art. no. 972

135. Ethylenediamine – potassium hexacyanoferrate(III) for catechol amines (adrenaline, noradrenaline and acetyl derivatives).

**Spray solution:** Solution of 0.1 g potassium hexacyanoferrate(III) in 5 ml ethylenediamine, 45 ml ethanol and 50 ml water.

**Treatment:** Heat the chromatogram 10 min at 105° C. Inspection under UV light.


**Chemicals:**
- Ethylenediamine, art. no. 947
- Potassium hexacyanoferrate (III) G R, art. no. 4973
- Ethanol abs. G R, art. no. 972

136. Fast blue salt B for phenols and coupling amines (diazonium reagent).

**Spray solution I:** A freshly prepared 0.5% aqueous fast blue salt B solution.

**Spray solution II:** 0.1 N sodium hydroxide solution.

**Treatment:** Spray with I and then with II.


**Chemicals:**
- Fast blue salt B, art. no. 3191
- 0.1 N Sodium hydroxide solution Titrisol®, art. no. 9959

137. Fluorescein for lipids.

**Spray solution:** 0.01% ethanolic solution of fluorescein.

**Treatment:** Dry with warm air and handle subsequently with water vapour or spray slightly with water.

**Chemicals:**
- Fluorescein, art. no. 3990
- Ethanol abs. G R, art. no. 972

138. Fluorescein – ammonia for purines, pyrimidines and barbiturates.

**Spray solution:** 0.005% solution of fluorescein in 0.5 N ammonia solution.

Inspect the chromatogram in long- and short-wave UV light.

**Literature:** T. Wieland, L. Bauer, Angew. Chem. 63, 511 (1951).

**Chemicals:**
- Fluorescein, art. no. 3990
- Ammonia solution min. 25% (0.91) G R, art. no. 5432
139. Fluorescein — bromine for unsaturated compounds.

Spray solution: 0.1% ethanolic fluorescein solution.

Bromine solution: 5% bromine in carbon tetrachloride.

Procedure: After spraying with the fluorescein solution place the chromatogram into a chamber containing the bromine solution. Fluorescein is converted to eosin which shows no fluorescence in long-wave UV light. Compounds adding on to bromine prevent the formation of eosin and the fluorescence remains. Larger amounts of substance show yellow spots on reddish background.


Chemicals:
- Fluorescein, art. no. 3992
- Sodium carbonate G R, art. no. 6392
- Rhodamine B G R, art. no. 7599
- Ethanol abs. G R, art. no. 972

140. Fluorescein — hydrogen peroxide for hypnotics containing bromine.

Spray solution I: 0.1% fluorescein solution in 50% ethanol.

Spray solution II: Mix equal parts of 30% hydrogen peroxide and glacial acetic acid.

Procedure: Spray with I and then with II, heat finally 20 min at 90° C.

Note: Bromine formed by oxidation reacts with fluorescein under formation of eosin.


Chemicals:
- Fluorescein, art. no. 3990
- Acetic acid glacial min. 96% (1.106) G R, art. no. 90062
- Peroxydol® (30% by weight H₂O₂) G R, art. no. 7209
- Ethanol abs. G R, art. no. 972

141. Fluorescein — rhodamine B — sodium carbonate for chlorinated hydrocarbons and heterocyclic compounds.

Spray solution I: 0.5% ethanolic rhodamine B solution.

Spray solution II: 10% aqueous sodium carbonate solution.

Procedure: Using plates impregnated with fluorescein sodium spray the chromatograms after development first with I, dry and spray liberally with II. Inspect in daylight and in long-wave UV light.

Chemicals:
- Fluorescein sodium, art. no. 3992
- Sodium carbonate G R, art. no. 6392
- Rhodamine B G R, art. no. 7599
- Ethanol abs. G R, art. no. 972

142. Fluorescence indicators and luminescent substances as general visualisation reagents.

A. Spray reagents:
1. 2',7'-Dichlorofluorescein, spray reagent no. 89.
2. Fluorescein, spray reagent no. 137.
3. Methylumbelliferone, spray reagent no. 189.
4. Morin, spray reagent no. 195.
5. Rhodamine B, spray reagent no. 260, 261.

B. Additives to adsorbents:
6. Fluorescein sodium, 0.04% in water added to the adsorbent suspension, art. no. 3922.

7. Fluorescence indicator F₃₄₅ for thin layer chromatography (1–2% added to the adsorbent) for detection in short-wave UV light (λ max 254 nm), art. no. 9182.

The following adsorbents contain an additional fluorescence indicator for detection in long- and short-wave UV light (λ max 254 nm and λ max 366 nm):
- Silica gel HF₃₄₅, art. no. 7741
- Silica gel F₃₄₅, art. no. 7748
- Aluminium oxide F₃₄₅, art. no. 1104
- Aluminium oxide F₃₄₅, art. no. 1065

143. Folin Ciocalteu reagent for phenols.

Stock solution: Dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water, add 5 ml 85%/a phosphoric acid and 10 ml 37%/a hydrochloric acid and reflux the mixture for 10 hours. Add subsequently 15 g lithium sulfate, 5 ml water and 1 drop bromine, heat again 15 min and make up to 100 ml with water after cooling.

The solution shall not show green colouring.

Spray solution I: 20%/a aqueous sodium carbonate solution.

Spray solution II: Dilute freshly before use 1 part of the stock solution with 3 parts water.

Procedure: Spray with I, dry for a short while and spray with II.

144. Formaldehyde – hydrochloric acid for indoles and indole derivatives (Prochazka reagent).

**Spray solution:** Freshly prepared mixture of 10 ml formaldehyde solution (35\%), 10 ml hydrochloric acid (1.125) and 20 ml ethanol.

**Treatment:** Heat 5 min at 100° C. The yellow-orange-greenish fluorescence colours become stronger by blowing vapours of aqua regia over the layer.

**Literature:** Z. Prochážka, Chem. Listy 47, 1643 (1953).

**Chemicals:**
- Formaldehyde solution (35%) G R, art. no. 4003
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316
- Ethanol abs. G R, art. no. 972
- Nitric acid min. 65% (1.4) G R, art. no. 454

145. Formaldehyde – phosphoric acid for steroid alkaloids, steroid sapogenins and phenothiazine derivatives.

**Spray solution:** Dissolve 0.03 g paraformaldehyde in 100 ml 85% phosphoric acid with stirring at room temperature. The reagent is stable for several weeks.

**Literature:** K. Schreiber, O. Aurich, G. Osske, J. Chromatog. 12, 63 (1963).

**Chemicals:**
- Paraformaldehyde, art. no. 4005
- ortho-Phosphoric acid min. 85% (1.71) G R, art. no. 573

146. Formaldehyde – sulfuric acid for aromatic compounds.

**Spray solution:** Mixture of 0.2 ml 37% formaldehyde solution and 10 ml 97% sulfuric acid.

**Procedure:** Spray the chromatogram directly after taking out of the developing chamber. Varicoloured spots.


**Chemicals:**
- Formaldehyde solution min. 37%, art. no. 4003
- Sulfuric acid 95–97% (1.84) G R, art. no. 731

147. Furfural – sulfuric acid for carbamate esters.

**Spray solution I:** 1% solution of furfural in acetone.

**Spray solution II:** 10% solution of sulfuric acid in acetone.

**Procedure:** Spray with I and subsequently with II.


**Chemicals:**
- Furfural G R, art. no. 4013
- Sulfuric acid 95–97% (1.84) G R, art. no. 731
- Acetone G R, art. no. 14

148. Glucose – aniline for acids (Schweppe reagent).

**Spray solution:** Dissolve 2 g glucose in 20 ml water and also 2 ml aniline in 20 ml ethanol. Mix both solutions and make up to 100 ml with 1-butanol.

**Procedure:** After spraying heat the chromatogram 5–10 min at 125° C. Dark brown spots on white background.

**Literature:** H. Schweppe, Dissert. Münster 1954.

**Chemicals:**
- D-Glucose, art. no. 8342
- Aniline G R, art. no. 1261
- Ethanol abs. G R, art. no. 972
- 1-Butanol G R, art. no. 1990

149. Glucose – phosphoric acid for aromatic amines.

**Spray solution:** Dissolve 2 g glucose in 10 ml 85% phosphoric acid and 40 ml water. Add 30 ml ethanol and 30 ml 1-butanol.

**Treatment:** Heat for about 10 min at 45° C.


**Chemicals:**
- D-Glucose, art. no. 8342
- ortho-Phosphoric acid min. 85% (1.7) G R, art. no. 573
- Ethanol absol. G R, art. no. 972
- 1-Butanol G R, art. no. 1990

150. Glyoxalbis-(2-hydroxyanil) for cations.

**Spray solution:** Dissolve 1 g glyoxalbis-(2-hydroxyanil) and 3 g potassium hydroxide in 100 ml methanol.

**Procedure:** Spray the dried chromatogram and dry again with a stream of air at 30° C.

151. Hydrazine sulfate for piperonal, vanillin and ethyl vanillin.

Spray solution: Mix 90 ml of a saturated aqueous solution of hydrazine sulfate with 10 ml 4 N hydrochloric acid.

Note: Inspect the moist chromatogram in long-wave UV light before and after exposure to ammonia vapour.


Chemicals:
- Hydrazinium sulfate G R, art. no. 4603
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316
- Ammonia solution min. 25% (0.91) G R, art. no. 5432

152. Hydrochloric acid for glycols.

Spray solution: Mix 1 part 36% hydrochloric acid with 4 parts ethanol.

Procedure: Glycols appear as pink spots on heating to 90°C.

Note: To be used also as a general spray reagent for TLC.


Chemicals:
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
- Ethanol abs. G R, art. no. 972


Spray solution: 0.3% aqueous hydrogen peroxide solution.

Treatment: Irradiate the chromatogram with long-wave UV light until maximal blue fluorescence.


Chemicals:
- Perhydrol® (30% by weight H₂O₂) G R, art. no. 7209

154. 4-Hydroxybenzaldehyde — sulfurous acid for sapogenins and corticosteroids (Komarowsky reagent).

Solution a: 50% sulfurous acid.

Solution b: 2% methanolic solution of 4-hydroxybenzaldehyde.

Spray solution: Mix freshly before use 5 ml a with 50 ml b.

Treatment: Heat 3–4 min at 105°C or 10 min at 60°C. Yellow to pink spots.


155. Hydroxylamine — iron(III) chloride for lactones, esters, amides and anhydrides of carboxylic acids.

Solution a: Dissolve 20 g hydroxylamine hydrochloride in 50 ml water, make up to 200 ml with ethanol. Store the solution in the refrigerator.

Solution b: Dissolve 50 g potassium hydroxide in as little water as possible and make up to 500 ml with ethanol.

Spray solution 1: Mix equal parts of a and b and filter off the precipitated potassium chloride. Place the solution in the refrigerator (stable for about 2 weeks).

Spray solution 2: Dissolve 10 g finely powdered iron(III) chloride in 20 ml 36% hydrochloric acid. Shake with 200 ml diethyl ether until a homogeneous mixture is obtained. The solution II is stable for some time only well sealed.

Procedure: Spray with I, dry at room temperature and spray with II.


Chemicals:
- Hydroxylamine hydrochloride G R, art. no. 4616
- Potassium hydroxide G R, art. no. 5033
- Iron(III) chloride G R, art. no. 3943
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
- Ethanol abs. G R, art. no. 972
- Diethyl ether G R, art. no. 921

156. 8-Hydroxyquinoline for barium, strontium and calcium ions.

Spray solution: Dissolve 0.5 g 8-hydroxyquinoline in 100 ml 60% ethanol.

Treatment: Respray with 25% ammonia solution or place the chromatogram into a chamber with ammonia.

Inspect in long-wave UV light.


Chemicals:
- 8-Hydroxyquinoline G R, art. no. 7098
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25% (0.91) G R, art. no. 5432

157. 9-Hydroxyquinoline — hypobromite for arginine and other guanidine derivates (Sakaguchi reagent).

Spray solution 1: 0.1% solution of 8-hydroxyquinoline in acetone.

Spray solution 2: Mixture of 0.2 ml bromine and 100 ml 0.5 N sodium hydroxide solution.
Procedure: Spray with I and after drying with II. The spots show orange to red colour.

J. Kalousk, M. Kurác, J. Bilek, Českoslov. farm. 4, 188 (1955).

Chemicals:
- 8-Hydroxyquinoline G R, art. 7098
- Bromine G R, art. no. 1948
- 0.5 N Sodium hydroxide solution Titrisol®, art. no. 9957
- Acetone G R, art. no. 14

158. 8-Hydroxyquinoline – kojic acid for aluminium, magnesium, calcium, strontium and barium ions.

Spray solution I: Solution of 2.5 g 8-hydroxyquinoline and 0.5 g kojic acid in 500 ml 90% ethanol.

Spray solution II: 25% ammonia solution.

The spots fluoresce in long-wave UV light.


Chemicals:
- 8-Hydroxyquinoline G R, art. no. 7098
- Kojic acid, art. no. 5193
- Ethanol abs. G R, art. no. 972
- Ammonia solution min 25% (0.91) G R, art. no. 5432

159. Indanedione for carotenoid aldehydes.

Spray solution: Dissolve 0.5 g 2-diphenylacetyl-1,3-indanedione-1-hydrazone in 20 ml water, filter after short warming and add 0.3 ml 36% hydrochloric acid.

Treatment: Dry with cold air.


Chemicals:
- 2-Diphenylacetyl-1,3-indanedione-1-hydrazone
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317

160. Iodine as general detection reagent.

Place the chromatogram into a chamber in which some crystals of iodine have been placed. Iodine vapour is more quickly generated by gently warming the chamber. Many organic compounds show brown spots.

Modification: Place the chromatogram 5 min into a strong iodine atmosphere or spray with a 5% solution of iodine in chloroform. Excess iodine evaporates on standing in the air. After spraying with 1% aqueous starch solution the spots turn blue. The background also turns blue if there is too much iodine still on the layer (test on a corner or part of the covered layer).


Chemicals:
- Iodine G R, art. no. 4761
- Chloroform G R, art. no. 2445
- Starch soluble G R, art. no. 1252

161. Iodine azide for sulfur-containing amino acids, sulfides and penicillins.

Iodine azide solution: Freshly prepared solution of 3 g sodium azide in 100 ml 0.1 N iodine solution.

Dry iodine azide is explosive!

Iodine azide-starch reagent:

Spray solution I: Freshly prepared solution of 1 g sodium azide in 100 ml 0.005 N iodine solution.

Spray solution II: 1% aqueous starch solution.

Procedure: Spray with I and subsequently with II.

Literature: E. Chargaff, C. Levine, C. Green, J. Biol. Chem. 175, 67 (1948).
W. Awe, I. Reinecke, J. Thum, Naturwissenschafen 41, 528 (1954).

Chemicals:
- Sodium azide, art. no. 6688
- 0.1 N Iodine solution Titrisol®, art. no. 9910
- Starch soluble G R, art. no. 1252

162. Iodine – potassium iodide acidic for alkaloids.

Spray solution: Dissolve 1 g iodine and 10 g potassium iodide in 50 ml water and add 2 ml glacial acetic acid. Make up this solution to 100 ml with water.

Literature: F. Santavy, not published.

Chemicals:
- Iodine G R, art. no. 4761
- Potassium iodide G R, art. no. 5043
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

163. Iodine – potassium iodide for organic compounds.

Spray solution: Dissolve 0.2 g iodine and 0.4 g potassium iodide in 100 ml water.

164. Iodine – sulfanilic acid – N-(1-naphthyl)-ethylene diamine for hydroxylamines (Csaky reagent).

Solution a: 1.3% solution of iodine in acetic acid.
Solution b: 1% sulfanilic acid solution in 30% acetic acid.
Spray solution I: Prepare freshly before use a mixture of equal parts of a and b.
Spray solution II: 0.1% aqueous solution of N-(1-naphthyl)-ethylene diamine dihydrochloride.

Procedure: Spray with I and subsequently with II.


Chemicals:
- Iodine G R, art. no. 4761
- N-(1-Naphthyl)-ethylene diamine dihydrochloride G R, art. no. 6237
- Sulfanilic acid G R, art. no. 686
- Acetic acid glacial min. 96%/o (1.05) G R, art. no. 90062

165. Iodine – sulfanilic acid for organic compounds containing nitrogen, polyethylene glycols and polyethylene glycol derivatives.

Spray solution: Mix equal parts of 0.1 N iodine solution and 10% sulfanilic acid.

Chemicals:
- 0.1 N Iodine solution Titrisol®, art. no. 9910
- Sulfanilic acid 95–97%/o (1.84) G R, art. no. 731

166. Iron(III) chloride for phenols and hydroxamic acids.

Spray solution: 1–5% solution of iron(III) chloride in 0.5 N hydrochloric acid.
Note: Hydroxamic acids turn red, phenols blue or greenish.


Chemicals:
- Iron(III) chloride G R, art. no. 3943
- 0.5 N Hydrochloric acid Titrisol®, art. no. 9971


Spray solution: Dissolve 5 g iron(III) chloride and 2 g iodine in a mixture of 50 ml acetone and 50 ml 20%/o aqueous tartaric acid solution.


168. Iron(III) chloride – perchloric acid for indoles (Salkowsky reaction).

Spray solution: Mix 1 ml 0.5 M aqueous iron(III) chloride solution with 50 ml 35%/o perchloric acid.

Treatment: Heat 5 min at 60° C.
Blow vapours of aqua regia over the layer for intensification of the colours.


Chemicals:
- Iron(III) chloride G R, art. no. 3943
- Perchloric acid about 60%/o (1.35) G R, art. no. 518
- Hydrochloric acid min. 25%/o (1.125) G R, art. no. 316
- Nitric acid min. 65%/o (1.4) G R, art. no. 454

169. Iron(III) chloride – perchloric acid for phenothiazines.

Spray solution: Mix 5 ml 5%/o aqueous iron(III) chloride solution with 45 ml 20%/o perchloric acid and 50 ml 50%/o nitric acid.

Colour reaction.


Chemicals:
- Iron(III) chloride G R, art. no. 3943
- Perchloric acid about 20%/o (1.12) G R, art. no. 516
- Nitric acid min. 65%/o (1.4) G R, art. no. 454

170. Iron(III) chloride – potassium hexacyanoferrate(III) – arsenite for thyroid hormones and other iodine containing compounds.

Solution a: Dissolve 2.7 g iron(III) chloride in 100 ml 2 N hydrochloric acid.

Solution b: 3.5%/o aqueous potassium hexacyanoferrate(III) solution.

Solution c: Dissolve 5 g sodium metasulphite in 30 ml 1 N sodium hydroxide solution at 0° C and mix with 65 ml 2 N hydrochloric acid with stirring.

Spray solution: Mix 5 parts a, 5 parts b and 1 part c.
Dry the chromatogram with precaution at 50° C and spray, cover with a second glass plate and store in darkness for 15 min. Iodine containing compounds show light blue spots on yellowish background.

171. **Iron(III) chlorides — sulfoisalicylic acid for thiophosphate esters.**

*Spray solution I:* 0.1% solution of iron(III) chloride in 80% ethanol.

*Spray solution II:* 1% solution of sulfoisalicylic acid in 80% ethanol.

*Procedure:* Place the chromatogram 10 min into a bromine atmosphere and spray subsequently with I. Dry 15 min at room temperature and spray with II. White spots on violet background.


*Chemicals:*
- Iron(III) chloride G R, art. no. 3943
- Sulfoisalicylic acid G R, art. no. 691
- Bromine G R, art. no. 1948
- Ethanol abs. G R, art. no. 972

172. **Iron(III) chlorides — sulfuric acid for bile acids.**

*Spray solution:* Dissolve 2 g iron(III) chloride in 83 ml anhydrous 1-butanol and mix with 15 ml 97% sulfuric acid.

*Treatment:* After drying for 15 min at room temperature heat 25-30 min with conjugated bile acids, 45-50 min with free bile acids.


*Chemicals:*
- Iron(III) chloride G R, art. no. 3943
- 1-Butanol G R, art. no. 1990
- Sulfuric acid 95-97% (1.84) G R, art. no. 731

173. **Iron(III) chlorides — sulfuric acid for indol derivatives (Salkowsky reaction).**

*Spray solution:* Mix 3 ml 1.5 M aqueous iron(III) chloride solution with 100 ml water and add 60 ml 97% sulfuric acid.

*Treatment:* Heat 5 min at 60° C. Blow vapours of aqua regia over the layer for intensification of the colours.

*Literature:* P. E. Pilet, Rev. gén. bot. 64, 1 (1957).

*Chemicals:*
- Iron(III) chloride G R, art. no. 3943
- Sulfuric acid 95-97% (1.84) G R, art. no. 731
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316
- Nitric acid min. 65% (1.4) G R, art. no. 454

174. **Iron(II) thiocyanate for peroxides.**

*Solution a:* 4% aqueous iron(II) sulfate solution.

*Solution b:* 1.3% solution of ammonium thiocyanate in acetone.

*Spray solution:* Mix freshly before use 10 ml a and 15 ml b.

*Note:* Fast appearance of brown-red spots (iron(III) thiocyanate) shows the presence of peroxide compounds.

*Literature:* E. Stahl, Chemiker-Ztg. 82, 323 (1957).

*Chemicals:*
- Iron(II) sulfate G R, art. no. 3963
- Ammonium thiocyanate G R, art. no. 1213
- Acetone G R, art. no. 14

175. **Isatin — sulfuric acid for thiophene derivatives.**

*Spray solution:* Dissolve 0.4 g isatin in 100 ml 97% sulfuric acid.

*Treatment:* Heating to 120° C is occasionally needed.

*Variocoloured spots.*


*Chemicals:*
- Isatin G R, art. no. 4734
- Sulfuric acid 95-97% (1.84) G R, art. no. 731

176. **Isatin — zinc acetate for amino acids.**

*Spray solution:* Dissolve 1 g isatin and 1.5 g zinc acetate in 100 ml 95% isopropanol by warming to 80° C and add 1 ml glacial acetic acid after cooling.

*The reagent is stable stored in the refrigerator.*

*Treatment:* Heat 30 min at 80-85° C or better inspect the chromatogram after standing 20 hours at room temperature.


*Chemicals:*
- Isatin G R, art. no. 4734
- Zinc acetate G R, art. no. 8802
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- 2-Propanol G R, art. no. 9634

177. **isonicotinic acid hydrazide for Δ4-3-ketosteroids.**

*Spray solution:* Dissolve 1 g isonicotinic acid hydrazide (INH) and 1 ml glacial acetic acid in 100 ml ethanol.

*Procedure:* Dry after spraying at room temperature.

*Spots show yellow fluorescence in long-wave UV light.*
178. Kojic acid for metal ions.

**Spray solution:** Dissolve 0.1 kojic acid in 100 ml 60% ethanol.

**Note:** Inspect fluorescence under UV light.


**Chemicals:**
- Kojic acid [5-hydroxy-2-hydroxymethylpyrone-(4)], art. no. 5193
- Ethanol abs. G R, art. no. 972

179. Lead acetate basic for flavonoids.

**Spray solution:** 25% aqueous solution of basic lead acetate.

Fluorescing spots in long-wave UV light.


**Chemicals:**
- Lead(II) hydroxide acetate, art. no. 7414

180. Lead (IV) acetate for 1,2-diol groups.

**Spray solution:** 1% solution of lead (IV) acetate in benzene. (prepare freshly)

**Treatment:** Heat 5 min at 110°C.

White spots on brown background.

**Literature:** J. Wright, Chem. & Ind. (London) 1963, 1125.

**Chemicals:**
- Lead(IV) acetate, art. no. 7418
- Benzene G R, art. no. 1783

181. Lead(IV) acetate—rosaniline for 1,2-diol groups.

**Spray solution I:** Dissolve 3 g lead (II, IV) oxide in 100 ml acetic acid with occasionally stirring until complete solution.

**Spray solution II:** Dissolve 0.05 g rosaline base in a mixture of 10 parts glacial acetic acid and 90 parts acetone.

0.1% methanolic fuchsin solution may be used equally.

**Procedure:** Spray with I and after 4–5 min with II.

**Literature:** K. Sampson, F. Schild, R. J. Wicker, Chem. & Ind. (London) 1961, 82.

182. Leukomethylene blue for ubi-, plasto- and tocopherylquinones.

**Spray solution:** Add a suspension of 0.25 g zinc powder in 1 ml glacial acetic acid to 5 ml 0.02% solution of methylene blue in acetone.

**Literature:** T. W. Goodwin, Lab. Practice 1964, 295.

**Chemicals:**
- Zinc powder G R, art. no. 8789
- Methylene blue B, art. no. 1283
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Acetone G R, art. no. 14
- Methanol G R, art. no. 6009

183. Magnesium acetate for anthraquinone glycosides and their aglucones.

**Spray solution:** 0.5% methanolic magnesium acetate solution.

**Procedure:** After spraying dry 5 min at 90°C.

Orange to violet colour.


**Chemicals:**
- Magnesium acetate G R, art. no. 5819
- Methanol G R, art. no. 6009

184. Mercury(II) chloride—diphenylcarbazone for barbiturates.

**A. Solution a:** 2% ethanolic mercury(II) chloride solution.

**B. Solution b:** 0.2% ethanolic diphenylcarbazone solution.

**Spray solution:** Mix freshly before use equal parts of a and b.

Pink spots on violet background.

**Literature:** E. K. J. Christensen, T. Vos, T. Huizanga, Pharm. Weekblad 100, 517 (1965).

**B. Spray solution I:** 0.1% ethanolic diphenylcarbazone solution.

**Spray solution II:** 0.33% mercury(II) nitrate solution in 0.05 N nitric acid.

**Procedure:** Spray with I until the plate is faintly pink, then spray with II.

Pink spots on violet background, the latter is bleached by sunlight or UV light and the spots turn violet.

C. Spray solution I (Mercury(II) sulfate solution): Suspense 5 g mercury(II) oxide in 100 ml water and add 20 ml 97% sulfuric acid with stirring. After cooling fill up to 250 ml with water.

Spray solution II: 0.01% diphenylcarbazone solution in chloroform.

Procedure: Spray with I, dry and spray with II.


Chemicals:
Mercury(II) chloride G R, art. no. 4419
Diphenylcarbazone G R, art. no. 3087
Ethanol abs. G R, art. no. 972
Mercury(II) nitrate G R, art. no. 4435
Nitric acid min. 65% (1.4) G R, art. no. 454
Mercury(II) oxide yellow G R, art. no. 4461
Sulfuric acid 95-97% (1.84) G R, art. no. 731
Chloroform G R, art. no. 2445

185. Mercury(II) chloride — potassium iodide for steroid alkaloids (Meyer reagent). PC.

Spray solution I: Dissolve 13.35 g mercury(II) chloride and 49.8 g potassium iodide separately each in 20 ml water. Mix both solutions and fill up with water to 1 l. Before spraying add 1 part 17% hydrochloric acid to 10 parts of this solution.

Spray solution II: Dissolve 5 g zinc chloride in 80 ml water and add 15 ml 36% hydrochloric acid.

Spray solution III: 15% ammonia solution.

Procedure: After spraying with I, the steroid alkaloids appear as faint yellow spots. Rinse the chromatogram 10 min with water and, after removal of the water, spray with II and subsequently with III.

Note: The resulting dark brown spots are not stable for a prolonged period.


Chemicals:
Mercury(II) chloride G R, art. no. 4419
Potassium iodide G R, art. no. 5043
Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
Zinc chloride G R, art. no. 8816
Ammonia solution min. 25% (0.91) G R, art. no. 5432

186. Mercury(II) nitrate for barbiturates.

Spray solution: 1% aqueous mercury(I) nitrate solution.


Chemicals:
Mercury(II) nitrate G R, art. no. 4437

187. 4-Methoxy-8-nitroaniline diazotised for the identification of vitamin C.

Solution a: Dissolve 0.5 g 4-methoxy-2-nitroaniline in 125 ml glacial acetic acid. Dilute the solution to 250 ml with 10% sulfuric acid.

Solution b: 0.2% aqueous sodium nitrite solution.

Spray solution I: Mix equal parts of a and b.

Spray solution II: 2 N sodium hydroxide.

Procedure: Spray with I, and then with II.

Blue spots on orange background.


Chemicals:
4-Methoxy-2-nitroaniline, art. no. 6225
Acetic acid glacial min. 96% (1.106) G R, art. no. 90062
2 N Sodium hydroxide solution, art. no. 9136
Sulfuric acid 95-97% (1.84) G R, art. no. 731
Sodium nitrite G R, art. no. 6549

188. Methylene blue for sulfate esters of steroids.

Spray solution: Dissolve 0.025 g methylene blue in 100 ml 0.05 N sulfuric acid. Before use dilute 1 part of the spray solution with 1 part acetone.

Note: The sulfate esters show differently coloured spots on blue background.

On development with chloroform the formed colour complexes migrate and leave white spots on blue background.


Chemicals:
Methylene blue B, art. no. 1283
Acetone G R, art. no. 14
Chloroform G R, art. no. 2445

189. Methylumbelliferone for heterocyclic compounds containing nitrogen (fluorescence indicator).

Spray solution: Dissolve 0.02 g 4-methylumbelliferone in 35 ml ethanol and fill up to 100 ml with water.

Treatment: Place the chromatogram into a chamber saturated with ammonia and inspect in long-wave UV light.


Chemicals:
4-Methylumbelliferone
Ethanol abs. G R, art. no. 972
Ammonia solution min. 25% (0.91) G R, art. no. 5432
190. **Methyl yellow for chlorinated insecticides.**  
*Spray solution:* Dissolve 0.1 g methyl yellow in 100 ml 75% ethanol.  
*Procedure:* After spraying dry the chromatogram at room temperature and irradiate with UV light without filter for 5 min. Red spots on yellow background.  
*Chemicals:*  
- 4-Dimethylaminoazobenzene (methyl yellow), art. no. 3055  
- Ethanol abs. G R, art. no. 972

191. **Millon’s reagent for phenols, phenol ethers and phenol ether glycosides.**  
*Spray solution:* Dissolve 5 g mercury in 10 g fuming nitric acid and add 10 ml water.  
*Treatment:* Heating at 100–110° C shows often change of colour.  
*Chemicals:*  
- Mercury G R, art. no. 4403  
- Nitric acid fuming 100% (1.32) G R, art. no. 455

192. **Molybdatophosphoric acid**  
*Spray reagent 3.5%/ for chromatography*  
Visualisation reagent ready for use in aerosol cans. Art. no. 531

193. **Molybdatophosphoric acid for reducing compounds, lipids, sterols and steroids.**  
A. *Spray solution:* 5–10% ethanolic molybdatophosphoric acid.  
*Treatment:* Heat at 120° C until maximal visualisation of the spots.  
*Note:* Treatment with ammonia vapour produces a colourless background.  
B. *Spray solution:* 20% solution of molybdatophosphoric acid in ethanol or ethylene glycol monomethylether (2-methoxyethanol).  
Antioxidants show blue spots after 1–2 min.  
*Chemicals:*  
- Molybdatophosphoric acid G R, art. no. 532  
- Ethanol abs. G R, art. no. 972  
- Ethylene glycol monomethylether G R, art. no. 858  
- Ammonia solution min. 25%/ (0.31) G R, art. no. 5432

194. **Molybdatophosphoric acid alkaline for estrogens.**  
*Spray solution I:* 8% methanolic solution of molybdatophosphoric acid.  
*Spray solution II:* 2.5% aqueous potassium hydroxide or 3% aqueous sodium hydroxide solution.  
*Procedure:* Spray with I and subsequently with II.  
*Note:* Instead of spraying with II place the chromatogram into a chamber saturated with ammonia.  
*Chemicals:*  
- Molybdatophosphoric acid G R, art. no. 532  
- Potassium hydroxide G R, art. no. 5033  
- Sodium hydroxide G R, art. no. 6498  
- Ammonia solution min. 25%/ (0.31) G R, art. no. 5432  
- Methanol G R, art. no. 6089

195. **Morin for aluminium ions.**  
*Spray solution:* 1% solution of morin in glacial acetic acid. Pronounced light green fluorescence in long-wave UV light.  
*Chemicals:*  
- Morin G R, art. no. 6098  
- Acetic acid glacial min. 96%/ (1.06) G R, art. no. 90062

196. **1.3-Naphthalenediol — phosphoric acid for sugars.**  
*Spray solution:* Mixture of 100 ml 0.2% ethanolic 1.3-naphthalenediol solution with 10 ml 85% phosphoric acid.  
*Treatment:* Heat 5–10 min at 100–105° C.  
*Chemicals:*  
- 1.3-Naphthalenediol G R, art. no. 6252  
- ortho-Phosphoric acid min. 85%/ (1.71) G R, art. no. 573  
- Ethanol abs. G R, art. no. 972

197. **1.3-Naphthalenediol — sulphuric acid for sugars.**  
*Solution a:* 0.2% ethanolic solution of 1.3-naphthalenediol.  
*Solution b:* 20% sulphuric acid.  
*Spray solution:* Prepare freshly before use a mixture of equal parts a and b.  
*Treatment:* Heat 5–10 min at 100–105° C.  
*Chemicals:*  
- 1.3-Naphthalenediol G R, art. no. 6252  
- Sulphuric acid 95–97%/ (1.84) G R, art. no. 731  
- Ethanol abs. G R, art. no. 972
198. 1,3-Naphthalenediol — trichloroacetic acid for sugars and uronic acids.

Solution a: 0.2% ethanolic 1,3-naphthalenediol solution.
Solution b: 20% aq. trichloroacetic acid solution.
Spray solution: Mix freshly before use equal parts of a and b.

Treatment: For ketoses heat 5–10 min at 100–105° C, for uronic acids 10–15 min in moist atmosphere (water bath) at 70–80° C.

Note: The presence of collidine and pyridine interferes with the color reaction. Instead of 1,3-naphthalenediol resorcinol, orcinol, phloroglucinol or 1-naphthol may be used. One part trichloroacetic acid may be replaced by 1/10 part phosphoric acid.


Chemicals:
- 1,3-Naphthalenediol G R, art. no. 6252
- Trichloroacetic acid G R, art. no. 807
- Ethanol abs. G R, art. no. 972
- 1-Naphthol G R, art. no. 6223
- Resorcinol G R, art. no. 7093
- Phloroglucinol G R, art. no. 7266
- 1.2-Naphthoquinone-4-sulfonic acid sodium salt in 5% aqueous sodium carbonate.

ortho-Phosphoric acid min. 85% (1.71) G R, art. no. 573

199. 1-Naphthol — hypobromite for arginine and other guanidine derivatives (Sakaguchi reagent).

Spray solution I: Solution of 0.1% 1-naphthol in 1N sodium hydroxide solution.

Spray solution II: Mixture of 100 ml 5% aqueous sodium hydroxide and 2 ml glacial acetic acid. Filter off from insoluble parts.

Procedure: Spray with I and then with II.

Note: For the detection of streptomycine it is recommended to spray with a mixture of 50 ml aqueous sodium hypochlorite solution (13% activated chlorine) and 50 ml ethanol instead of spraying with II.


Chemicals:
- 1-Naphthol G R, art. no. 6223
- Sodium hydroxide G R, art. no. 6498
- Bromine G R, art. no. 1948
- Sodium hypochlorite solution
- Ethanol abs. G R, art. no. 972

200. 1-Naphthol — sulfuric acid for sugars.

Spray solution: Mix 10.5 ml 15% ethanolic solution of 1-naphthol, 6.5 ml 97% sulfuric acid, 40.5 ml ethanol and 4 ml water.

Treatment: Heat 3–6 min at 100° C.


Chemicals:
- 1-Naphthol G R, art. no. 6252
- Sulfuric acid 95–97% (1.84) G R, art. no. 731
- Ethanol abs. G R, art. no. 972

201. Naphthoquinone-sulfonic acid sodium salt for amino acids (Folin reagent).

Spray solution: Prepare freshly a solution of 0.2 g 1,2-naphthoquinone-4-sulfonic acid sodium salt in 100 ml 5% aqueous sodium carbonate solution.

Procedure: Spray and dry the chromatogram at room temperature. No further treatment.

Amino acids show various colours.


Chemicals:
- 1,2-Naphthoquinone-4-sulfonic acid sodium salt G R, art. no. 6531
- Sodium carbonate 10-hydrate G R, art. no. 6391


Spray solution: Dissolve 0.5 g 1,2-naphthoquinone-4-sulfonic acid sodium salt in 95 ml water and add 5 ml glacial acetic acid. Filter off from insoluble parts.

Note: Inspect the colour of the spots after 30 min.


Chemicals:
- 1,2-Naphthoquinone-4-sulfonic acid sodium salt G R, art. no. 6531
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

203. Naphthoquinone-sulfonic acid — perchloric acid for sterols.

Spray solution: Dissolve 0.1 g 1,2-naphthoquinone-4-sulfonic acid in a mixture of 50 ml ethanol, 25 ml 60% perchloric acid, 25 ml 37% formaldehyde solution and 22.5 ml water.

Procedure: Heat at 70–80° C and inspect the development of the spots. First pink, after prolonged heating blue spots.


Chemicals:
- 1,2-Naphthoquinone-4-sulfonic acid sodium salt G R, art. no. 6531
- Perchloric acid 60% (1.35) G R, art. no. 518
- Formaldehyde solution min. 37% G R, art. no. 4003
- Ethanol abs. G R, art. no. 972
204. 1-Naphthylamine for 3,5-dinitrobenzoic acid esters and dinitrobenzamides.

Spray solution I: 0.5% ethanolic 1-naphthylamine solution.
Spray solution II: 10% methanolic potassium hydroxide solution.
Procedure: Spray with I and then with II.

Spots show redbrown colour.

Chemicals:
- 1-Naphthylamine G R, art. no. 6245
- Ethanol abs. G R, art. no. 972
- Methanol G R, art. no. 6009
- Potassium hydroxide G R, art. no. 5033

205. Nessler's reagent for alkaloids.

Spray solution: Nessler's reagent (s. spray reagent no. 284).
Note: Apomorphine, hydralazine and physostigmine show colour reaction.

Chemicals:
- Nessler's reagent

206. Ninhydrin

Spray reagent 0.1% for chromatography.

Visualization reagent ready for use in aerosol cans.
Art. no. 6738.

207. Ninhydrin for amino acids, amines and amino-sugars.

A. Spray solution: Dissolve 0.3 g ninhydrin in 100 ml 1-butanol and add 3 ml glacial acetic acid.
B. Spray solution: 0.2% ethanolic ninhydrin solution.

Treatment: Heat at 110°C until maximal visualization of the spots.
For panthothenic acid heat at 160°C.


Chemicals:
- Ninhydrin G R, art. no. 6762
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- 1-Butanol G R, art. no. 1990
- Ethanol abs. G R, art. no. 972

Stabilisation of ninhydrin spots.
Spray solution: Mix 1 ml saturated aqueous copper(II) nitrate solution with 0.2 ml 10% nitric acid and 100 ml ethanol.

Procedure: Spray the ninhydrin spots with the spray solution and place the chromatogram into a chamber with ammonia. The red copper complex is stable as long as no free hydrogen ions or strong complex forming compounds are present.

Chemicals:
- Copper(II) nitrate G R, art. no. 2752
- Nitric acid min. 65%/ (1.4) G R, art. no. 454
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25%/ (0.91) G R, art. no. 5432


Spray solution: Fill up to 500 ml with ethanol a solution of 1 g ninhydrin, 2.5 g cadmium acetate and 10 ml glacial acetic acid.

Treatment: Heat 20 min at 120°C.

This method is more suitable for detecting heterocyclic amines than procedure using reagent no. 207.

Alternative:
Dip solution: Dissolve 0.1 g cadmium acetate in 10 ml water, add 5 ml glacial acetic acid and 100 ml acetone and dissolve 1 g ninhydrin. This order of the reagents for the preparation of the dip solution must be observed. The solution is stable in the refrigerator.

Procedure: After dipping place the chromatogram for colour development 30 min into a chamber containing concentrated sulfuric acid.


Chemicals:
- Cadmium acetate G R, art. no. 2003
- Ninhydrin G R, art. no. 6762
- Acetic acid glacial min. 96%/ (1.05) G R, art. no. 90062
- Acetone G R, art. no. 14
- Ethanol abs. G R, art. no. 972
- Sulfuric acid 95–97%/ (1.84) G R, art. no. 731

209. Ninhydrin — copper(II) nitrate for amino acids (polychromatic detection).

Solution a: Dissolve 0.1 g ninhydrin in 50 ml ethanol and add 10 ml glacial acetic acid and 2 ml collidine.

Solution b: 1% ethanolic copper(II) nitrate solution.

Spray solution: Before use mix solution a and b in the proportion 50:3.

Stock solution: Dissolve by heating 2 g ninhydrin in 40 ml water. Add a solution of 0.08 g tin(II) chloride in 50 ml water and allow to stand. After filtration of the precipitate store in the refrigerator.

Spray solution: Add 50 ml water and 450 ml 2-propanol to 25 ml of the stock solution.


Chemicals:
- Ninhydrin G R, art. no. 6762
- Tin (II) chloride G R, art. no. 7815
- 2-Propanol G R, art. no. 9634

211. Nitric acid for alkaloids and amines.

Spray solution: Add 50 drops 65% nitric acid to 100 ml ethanol.

Note: Inspect in UV light.

The spray solution may be used in this or higher concentrations also in TLC for the identification of other organic compounds. Frequently fluorescent spots appear only after prolonged heating at 120°C.


Chemicals:
- Ethanol abs. G R, art. no. 972
- Nitric acid min. 65% (1.4) G R, art. no. 545

212. 4-Nitroaniline diazotised (acidic) for plasticisers.

Spray solution I: 0.5 N ethanolic potassium hydroxide solution.

Spray solution II: Dissolve 0.8 g 4-nitroaniline in 250 ml water, add 20 ml 25% hydrochloric acid and dropwise 5% aqueous sodium nitrite solution until the solution is colourless.

Procedure: Spray with I, dry 15 min at 60°C and spray with II. Yellow to orange spots.


Chemicals:
- 4-Nitroaniline, art. no. 6760
- Sodium nitrite G R, art. no. 6549
- Hydrochloric acid 25% (1.125) G R, art. no. 316
- Potassium hydroxide G R, art. no. 5033
- Ethanol abs. G R, art. no. 972

213. 4-Nitroaniline diazotised for phenols, phenol carboxylic acids, coupling amines and heterocyclic compounds.

Spray solution: Mix 10 ml 0.1% aqueous 4-nitroaniline solution with 10 ml 0.2% aqueous sodium nitrite solution and 20 ml 10% aqueous potassium carbonate solution.

Coloured products are formed.


Chemicals:
- 4-Nitroaniline, art. no. 6760
- Sodium nitrite G R, art. no. 6549
- Potassium carbonate G R, art. no. 4928

214. 4-Nitroaniline diazotised (buffered) for phenols.

Spray solution: Mix under cooling 5 ml 0.5% 4-nitroaniline solution in 2 N hydrochloric acid with 0.5 ml 5% aqueous sodium nitrate solution and add 15 ml 20% aqueous sodium acetate solution.


Chemicals:
- 4-Nitroaniline, art. no. 6760
- Sodium nitrate G R, art. no. 6549
- Sodium acetate G R, art. no. 6267
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316

215. 4-Nitrophenyldiazonium fluoroborate for phenols and coupling amines.

4-Nitrophenyldiazonium fluoroborate: Dissolve 14 g 4-nitroaniline in 30 ml 36% hydrochloric acid and 30 ml water by warming. After cooling at 5°C add a solution of 8 g sodium nitrite in 20 ml water and then 60 ml 40% hydrofluoboric acid. Filter off the yellow precipitate, wash with hydrofluoboric acid, ethanol and ether and dry in a vacuum desiccator.

Spray solution I: Prepare freshly a 1% 4-nitrophenyldiazonium fluoroborate solution in acetone.
218. 2-Nitroso-1-naphthol-4-sulfonic acid for thiophosphate esters and other sulfur compounds.

Spray solution: Dissolve 0.5 g palladium(II) chloride in 100 ml water containing a few drops 25% hydrochloric acid.


Chemicals:
- Palladium (II) chloride, art. no. 7110
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316


Spray solution: Dissolve 0.03 g paraformaldehyde in 100 ml 85% phosphoric acid under shaking. The reagent is stable for several weeks.


Chemicals:
- Paraformaldehyde, art. no. 4005
- Ortho-Phosphoric acid min. 85% (1.71) G R, art. no. 573

220. Periodic acid for steroids and bile acids.

A. Spray solution (for steroids): 20% aqueous periodic acid solution.
B. Spray solution (for bile acids): 60% aqueous periodic acid solution.

Treatment: Heat the chromatogram for about 10 min at 150° C until maximal visualisation of the spots.

Inspect also in long-wave UV light.


Chemicals:
- Periodic acid abt. 20% (1.12) G R, art. no. 516
- Periodic acid abt. 60% (1.53) G R, art. no. 518

221. Periodic acid — iron(III) chloride for indole derivatives.

Spray solution: Mix 100 ml 5% aqueous periodic acid solution with 2 ml 0.05 M iron(III) chloride solution.

Note: No reaction with isatin and other oxindole derivatives.


Chemicals:
- Periodic acid abt. 20% (1.12) G R, art. no. 516
- Iron (III) chloride G R, art. no. 3943

222. Phenol — sulfurous acid for sugars.

Spray solution: Dissolve 3 g phenol and 5 ml 97% sulfurous acid in 95 ml ethanol.

Treatment: Heat 10–15 min at 110° C.

Brown spots.

223. m-Phenylenediamine for reducing sugars.

Spray solution: Dissolve 3.6 g m-phenylenediamine dihydrochloride in 100 ml 70% ethanol.

Treatment: Heat briefly at 105°C.

Note: Intensely fluorescent colours in UV light.

Chemicals:
- Phenylhydrazine, art. no. 9721
- Hydrochloric acid, art. no. 972
- Hydrochloric acid fuming
- Phenylhydrazine, art. no. 972
- Phenylhydrazine HCl, art. no. 972

224. p-Phenylenediamine — phthalic acid for conjugated 3-ketosteroids.

Spray solution: Dissolve 0.9 g p-phenylenediamine and 1.6 g phthalic acid in 100 ml 1-butanol, saturated with water.

Treatment: Heat at 100—110°C. Yellow to orange spots.

Chemicals:
- Phenylhydrazine, art. no. 9721
- Phthalic acid, art. no. 9611
- 1-Butanol, art. no. 1990

225. o-Phenylenediamine — sulfuric acid for dehydroascorbic acid.

Spray solution: Dissolve 0.1 g o-phenylenediamine in a mixture of 50 ml 0.1 N sulfuric acid and 50 ml ethanol.

Treatment: Dissolve 3.6 g m-phenylenediamine dihydrochloride in 100 ml 70% ethanol.

Note: Intensely fluorescent spots in trichloroacetic acid solution.

Chemicals:
- O-Phenylenediamine, art. no. 9721
- 0.1 N Sulfuric acid Titrisol®, art. no. 9984
- Ethanol abs. G R, art. no. 972

226. o-Phenylenediamine — trichloroacetic acid for a-keto acids.

Spray solution: Dissolve 0.05 g o-phenylenediamine in 100 ml 10% aqueous trichloroacetic acid solution.

Procedure: Heat the chromatogram at 100°C for not more than 2 min. Green fluorescent spots in long-wave UV light.

Chemicals:
- o-Phenylenediamine, art. no. 9721
- Trichloroacetic acid G R, art. no. 972

227. Phenylfluorone for germanium.

Spray solution: 0.05% solution of phenylfluorone in a mixture of 3 parts ethanol and 1 part 37% hydrochloric acid.

Chemicals:
- Phenylfluorone, art. no. 7252
- Ethanol abs. G R, art. no. 972

228. Phenylhydrazine for dehydroascorbic acid.

Spray solution: Dissolve 0.3 g phenylhydrazine and 0.45 g sodium acetate in 10 ml water.

Chemicals:
- Phenylhydrazine G R, art. no. 7251
- Sodium acetate G R, art. no. 6268

229. Phosphoric acid for sterols and steroids.

A. Spray solution: Mix 85% phosphoric acid with water 1:1 (volume).

B. Spray solution: 15% methanolic phosphoric acid solution.

Procedure: Spray the layer thoroughly until transparent and heat 15—30 min at 120°C. The individual sterols or steroids require varying time for attainment of maximal colour intensity or fluorescence.

Note: All compounds of this class show fluorescence in long-wave UV light. Larger amounts of substance yield spots which are visible in daylight.

Chemicals:
- o-Phenylfluorone dibromide, art. no. 7252
- Phosphoric acid, art. no. 7252
- Methanol G R, art. no. 6009

230. Phosphoric acid — bromine for digitalis glycosides.

Spray solution I: 10% aqueous phosphoric acid solution.

Spray solution II: Mix 2 ml saturated aqueous potassium bromate solution, 2 ml saturated aqueous potassium bromide solution and 2 ml 25% hydrochloric acid.

Chemicals:
- o-Phenylenediamine, art. no. 9721
- Trichloroacetic acid G R, art. no. 807
**231. Picric acid for epoxides.**

*Procedure.* Spray with I and heat the plate 12 min at 120 °C. Digitalis glycosides of the series B, D and E show blue fluorescence in long-wave UV light. Heat the plate again at 120 °C and spray lightly with II. Glycosides of the series A show now orange, of the series C grey-green to grey-blue fluorescence in UV light.


*Chemicals:*
- Picric acid min. 85% (1.71) G R, art. no. 573
- Potassium bromide G R, art. no. 4912
- Potassium bromate G R, art. no. 4905
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316

**232. Picric acid for creatinine, glycochymidine (Jaffe reagent).**

*Spray solution I:* 1% ethanolic picric acid solution.

*Spray solution II:* 5% ethanolic potassium hydroxide solution.

*Procedure:* Spray with I, dry and spray with II.

Orange colour.


*Chemicals:*
- Picric acid moistened with about 50% water G R, art. no. 623
- Ethanol abs. G R, art. no. 972
- Diethyl ether G R, art. no. 921
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Ammonia solution min. 25% (0.91) G R, art. no. 5432

**233. Picric acid — perchloric acid for A1330-hydroxysteroids.**

*Spray solution:* Dissolve 0.1 g picric acid in a mixture of 36 ml glacial acetic acid and 6 ml 70% perchloric acid.

*Procedure:* Heat 3–5 min at 70–80 °C.

Yellow-red spots.


*Chemicals:*
- Picric acid moistened with about 50% water G R, art. no. 623
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062
- Perchloric acid abs. 70% (1.67) G R, art. no. 519

**234. Picryl chloride for hydroxylamines, hydrazines and pyridine derivatives.**

*Spray solution:* 0.5–1.5% ethanolic picryl chloride solution.

*Treatment:* Place the chromatogram into a chamber with ammonia.


*Chemicals:*
- Picryl chloride (2-chloro-1,3,5-trinitrobenzene)
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25% (0.91) G R, art. no. 5432

**235. Pinacryptol yellow for alkyl- and arylsulfonic acids.**

*Spray solution:* 0.05–0.1% aqueous pinacryptol yellow solution.

Yellow to orange fluorescence in long-wave UV light.


*Chemicals:*
- Pinacryptol yellow, art. no. 9723

**236. Potassium hexacyanoferrate(II) for iron(III)-ions.**

*Spray solution:* Freshly prepared 2% aqueous solution of potassium hexacyanoferrate (II).


*Chemicals:*
- Potassium hexacyanoferrate(II) G R, art. no. 4984

**237. Potassium hexacyanoferrate(II) — hydrogen peroxide for barbiturates.**

*Spray solution I:* Dissolve 0.1 g potassium hexacyanoferrate(II) in 100 ml water containing 0.5 ml 37% hydrochloric acid. Add to 10 ml of this solution 5 g ammonium chloride and make up to 100 ml with water.

*Spray solution II:* 30% hydrogen peroxide solution.

*Spray solution III:* 10% aqueous potassium carbonate solution.
240. Potassium hexacyanoferrate(III) — iron(III) chloride for reducing compounds, phenols, amines, thiosulfates, isothiocyanates.

**Solution a:** 1% aqueous potassium hexacyanoferrate(III) solution.

**Solution b:** 2% aqueous iron(III) chloride solution.

**Spray solution:** Mix freshly before use equal parts of a and b.

**Treatment:** Spray with 2 N hydrochloric acid for intensification of colours.


241. Potassium hexacyanoferrate(III) — phosphate buffer for adrenaline.

**Spray solution:** 0.44% solution of potassium hexacyanoferrate(III) in phosphate buffer solution, pH 7.8.

**Note:** Noradrenaline appears as brown red spots, adrenaline as light red and methyladrenaline as white spots on yellow-brown background.

**Literature:** S. Senoh, B. Witkop, J. Am. Chem. Soc. 81, 6222 (1959).

**Chemicals:**
- Potassium hexacyanoferrate(III) G R, art. no. 4973
- Iron(III) chloride G R, art. no. 3943
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316

242. Potassium hexacyanoferrate(III) — potassium hexacyanoferrate(II) for morphine.

**Spray solution:** Dissolve 57 mg potassium hexacyanoferrate(III) and 7.8 mg potassium hexacyanoferrate(II) in 100 ml water.


**Chemicals:**
- Potassium hexacyanoferrate(III) G R, art. no. 4973
- Potassium hexacyanoferrate(II) G R, art. no. 4973

243. Potassium hydroxide methanolic for coumarins, anthraquinone glycosides and their aglucones.

**Spray solution:** 5% methanolic potassium hydroxide solution. Inspect the chromatogram after drying in daylight and long-wave UV light.


**Chemicals:**
- Potassium hydroxide G R, art. no. 5033
- Methanol G R, art. no. 6009

244. Potassium iodide — hydrogen sulfide for heavy metal ions.

**Spray solution:** 2% aqueous potassium iodide solution.

**Procedure:** Dry the plate after spraying and place it into a chamber saturated with ammonia. After a few minutes place the plate into a second chamber with hydrogen sulfide.

**Caution:** Hydrogen sulfide is poisonous and explosive!
245. Potassium iodide – starch for peroxides.

Spray solution I: Add to a mixture of 40 ml glacial acetic acid and 10 ml 4% aqueous potassium iodide solution a little spatule of zinc powder.

Spray solution II: Freely prepared 1% aqueous starch solution.

Procedure: After filtrating of zinc powder, spray with II, dry 5 min at room temperature and spray with II until the layer is transparent. Peroxides show blue spots by formation of free iodine.


Chemicals:
- Potassium iodide G R, art. no. 5043
- Zinc powder G R, art. no. 8789
- Starch soluble G R, art. no. 1253
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

246. Potassium iodide plateate for alkaloids.

Spray solution: Add to 5 ml 5% hexachloroplatinic(IV) acid solution 45 ml 10% aqueous potassium iodide solution and 100 ml water. Prepare freshly before use.


Chemicals:
- Potassium iodide G R, art. no. 5043
- Hexachloroplatinic(IV) acid solution 10% G R, art. no. 7341

247. Potassium iodide plateate for alkaloids and other organic compounds containing nitrogen.

Spray solution: Add to 3 ml 10% hexachloroplatinic(IV) acid solution 97 ml water and 100 ml 6% aqueous potassium iodide solution. Prepare freshly before use.


Chemicals:
- Hexachloroplatinic(IV) acid solution 10% G R, art. no. 7341
- Potassium iodide G R, art. no. 5043

248. Potassium iodine plateate for ketosteroids. PC.

Spray solution: Add to 5 ml 5% hexachloroplatinic(IV) acid solution in 1 N hydrochloric acid 45 ml 10% aqueous potassium iodide solution and 100 ml water.

The reagent is stable for some time when stored in the dark.

Treatment: After spraying rinse out the excess reagent with water.


Chemicals:
- Potassium iodide G R, art. no. 5043
- Hexachloroplatinic(IV) acid, art. no. 7340
- 1 N Hydrochloric acid Titrisol® , art. no. 9970

249. Potassium permanganate alkaline for reducing compounds and aromatic polycarboxylic acids.

Spray solution: Add to 1% aqueous potassium permanganate solution an equal volume of 5% aqueous sodium carbonate solution.


Chemicals:
- Potassium permanganate G R, art. no. 5082
- Sodium carbonate anhydrous G R, art. no. 6392

250. Potassium permanganate alkaline for sugars and polyalcohols.

Spray solution: Dissolve 0.5 g potassium permanganate in 100 ml 1 N sodium hydroxide solution.

Treatment: After spraying heat the plate at 100° C.


Chemicals:
- Potassium permanganate G R, art. no. 5082
- 1 N Sodium hydroxide solution Titrisol®, art. no. 9956

251. Potassium permanganate neutral for easily oxidable compounds.

Spray solution: 0.05% aqueous potassium permanganate solution.

Chemicals:
- Potassium permanganate G R, art. no. 5082

252. Potassium permanganate – sulfuric acid (universal reagent).

Spray solution: Dissolve 0.5 g potassium permanganate in 15 ml 97% sulfuric acid.

Caution! Manganese heptoxide is explosive!

253. 1-(2-Pyridylazo)-2-naphthol (PAN) for lead, cadmium, cobalt, copper, manganese, nickel, zinc and uranyl ions.

**Spray solution:** 0.25% ethanolic solution of PAN.

**Treatment:** Place the plate into a chamber with ammonia.


**Chemicals:**
1-(2-Pyridylazo)-2-naphthol (PAN), art. no. 7531
Ethanol abs. G.R., art. no. 972
Ammonia solution min. 25% (0.91) G.R., art. no. 5432

254. 1-(2-Pyridylazo)-2-naphthol (PAN) — cobalt(II) nitrate for glucuronides of steroids.

**Spray solution I:** Mix freshly before use 1 part 0.4% ethanolic PAN solution and 4 parts methylene chloride (volume).

**Spray solution II:** Mix 8 ml 0.8% aqueous cobalt(II) nitrate solution with 4 ml 0.2 M acetate buffer solution (pH 4.6) and fill up to 100 ml with water.

**Procedure:** Spray with I until the layer is evenly yellow, dry and spray with II. Glucuronides show rapidly fading violet spots, the colours of which turn greenish on drying.


**Chemicals:**
1-(2-Pyridylazo)-2-naphthol (PAN), art. no. 7531
Cobalt(II) nitrate G.R., art. no. 2536
Standard acetate buffer solution pH 4.62 (0.2 M), art. no. 7827
Ethanol abs. G.R., art. no. 972
Dichloromethane (methylene chloride) art. no. 6050

255. Quercetin for cations of the hydrogen sulfide group, aluminium, magnesium, uranyl and tungstate ions.

**Spray solution:** 0.25% ethanolic quercetin solution.

**Treatment:** Spray with 25% ammonia solution or place into a chamber with ammonia. In long-wave UV light fluorescing spots.


**Chemicals:**
Quercetin, art. no. 7546
Ethanol abs. G.R., art. no. 972
Ammonia solution min. 25%/o (0.91) G.R., art. no. 5432

256. Quinalizarin for cations.

**Spray solution:** 0.05% solution of quinalizarin in 70% ethanol.

**Treatment:** Place the plate into a chamber saturated with ammonia vapour.


**Chemicals:**
1,2,3,8-Tetrahydroxyanthraquinone (quinalizarin) G.R., art. no. 8126
Ethanol abs. G.R., art. no. 972
Ammonia solution min. 25%/o (0.91) G.R., art. no. 5432

257. P-Quinone for ethanolamine.

**Spray solution:** Dissolve 0.5 g p-benzoquinone (p-quinone) in a mixture of 10 ml pyridine and 40 ml 1-butanol.

**Note:** After spraying red spots of ethanolamine will appear immediately. Choline shows no reaction.

**Chemicals:**
p-Benzoquinone, art. no. 2410
1-Butanol G.R., art. no. 1990
Pyridine G.R., art. no. 9728

258. Resorcinol — zinc chloride — sulfuric acid for plasticisers (especially phthalate esters).

**Spray solution I:** Add to a 20% ethanolic resorcinol solution some zinc powder.

**Spray solution II:** 4 N sulfuric acid.

**Spray solution III:** 40% aqueous potassium hydroxide solution.

**Procedure:** Spray with I, heat 10 min at 150° C, spray with II, heat 10 min at 120° C and spray with III. Orange spots on yellow background.

**Literature:** J. W. Copius-Peerboom, J. Chromatog. 4, 323 (1960).
D. Braun, Chimia (Switz.) 19, 77 (1965).

**Chemicals:**
Resorcinol G.R., art. no. 7593
Zinc chloride G.R., art. no. 8816
Ethanol abs. G.R., art. no. 972
Sulfuric acid 95–97%/o (1.84) G.R., art. no. 731
Potassium hydroxide G.R., art. no. 5033
259. Resorcy1 aldehyde — sulfuric acid for 16-dehydrosteroids.

Solution a: 0.5\% solution of resorcy1 aldehyde in acetic acid.
Solution b: 5\% sulfuric acid solution in glacial acetic acid.
Spray solution: Mix freshly before use equal parts of a and b.

Treatmen1: Heat at 100–110\°C until maximal visualisation of the spots.


Chemicals:

- Resorcy1 aldehyde
- Acetic acid glacial min. 96\% (1.04) G R, art. no. 90062
- Sulfuric acid 95–97\% (1.84) G R, art. no. 731

260. Rhodamine B

Spray reagent 0.1\% for chromatography

Visualisation reagent ready for use in aerosol cans. Art. no. 7596.

261. Rhodamine B, general spray reagent.

Spray solution: 0.025–0.25\% ethanolic solution of rhodamine B.
Inspect in long-wave UV light.


Chemicals:

- Rhodamine B G R, art. no. 7599
- Ethanol abs. G R, art. no. 972


Spray solution: Dissolve 0.001 g rhodamine 6 G in 100 ml acetone.
Inspect in long-wave UV light.


Chemicals:

- Rhodamine 6 G
- Acetone G R, art. no. 14

263. Rhodamine for carotenoid aldehydes.

Spray solution I: 1–5\% ethanolic solution of rhodamine.
Spray solution II: 25\% ammonia solution or 27\% aqueous sodium hydroxide solution.

Procedure: Spray with I, then with II and dry the chromatogram.

Literature: A. Winterstein, B. Heged1s, Chimia (Switz.) 14, 18 (1960).

264. Rhodizonic acid sodium salt for barium and strontium ions.

Spray solution I: 1\% aqueous solution of sodium rhodizonate.
Spray solution II: 25\% ammonia solution.


Chemicals:

- Rhodizonic acid sodium salt G R, art. no. 6595
- Ammonia solution min. 25\% (0.91) G R, art. no. 5432

265. Rubeanic acid for lead, cobalt, copper, manganese, nickel, mercury and bismuth ions.

Spray solution I: 0.5\% ethanolic solution of rubeanic acid.
Spray solution II: 25\% ammonia solution.

Procedure: Spray with I, dry briefly, then spray with II or place the chromatogram into a chamber with ammonia.


Chemicals:

- Rubeanic acid G R, art. no. 629
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25\% (0.91) G R, art. no. 5432

266. Silver nitrate for phenols.

Spray solution: Add with stirring 1 ml saturated aqueous silver nitrate solution to 20 ml acetone, then add water dropwise until the precipitated silver nitrate has just dissolved.
Light pink to deep green spots are yielded.


Chemicals:

- Silver nitrate G R, art. no. 1512
- Acetone G R, art. no. 14

267. Silver nitrate — ammonia for sugars and sugar alcohols (Dedonder reagent). PC.

Spray solution: Add with stirring 1 ml saturated aqueous silver nitrate solution to 20 ml acetone, then add water dropwise until the silver nitrate just dissolves.
Procedure: Spray the chromatogram liberally from both sides.

Treatment: Place the moist chromatogram 1 hour into a chamber saturated with ammonia (protected against light). Then heat the chromatogram at 80°C until the paper background has turned light brown, and remove the excess silver nitrate with 10%/s sodium thiosulphate solution. After rinsing for 2 hours under running water dry the chromatogram.


Chemicals:
- Silver nitrate G R, art. no. 1512
- Sodium thiosulphate G R, art. no. 6516
- Acetone G R, art. no. 14
- Ammonia solution min. 25%/s (0.91) G R, art. no. 5432

266. Silver nitrate – ammonia for reducing substances (Tollens or Zaffaroni reagent).

Solution a: 0.1 N silver nitrate solution.

Solution b: 5 N ammonia solution.

Spray solution: Mix a and b in the ratio 1:5 freshly before use.

Caution! Formation of explosive silver azide by prolonged standing.

Treatment: Heat 5–10 min at 105°C until the dark spots have become most intense.


Chemicals:
- 0.1 N Silver nitrate solution, art. no. 9081
- Ammonia solution min. 25%/s (0.91) G R, art. no. 5432

269. Silver nitrate – ammonia – fluorescein for halogen ions.

Spray solution I: Dissolve 1 g silver nitrate in 100 ml 0.5 N ammonia solution.

Spray solution II: 0.1%/s ethanolic fluorescein solution.

Procedure: Spray with I, dry briefly and spray with II.


Chemicals:
- Silver nitrate G R, art. no. 1512
- Fluorescein, art. no. 3990
- Ammonia solution min. 25%/s (0.91) G R, art. no. 5432
- Ethanol abs. G R, art. no. 972


Spray solution I: Mix freshly before use 50 ml 0.1 N silver nitrate solution with 50 ml 10%/s ammonia solution.

Longer standing may lead to formation of explosive silver azide.

Spray solution II: 10%/s aqueous sodium chloride solution.

Procedure: Spray with I, then dry and spray with II. Expose the chromatogram to daylight until the yellow-brown spots have attained maximum colour intensity.

Chemicals:
- 0.1 N Silver nitrate solution, art. no. 9081
- Ammonia solution abt. 10%/s (0.96) G R, art. no. 5423
- Sodium chloride G R, art. no. 6404


Solution a: 0.3%/s methanolic silver nitrate solution.

Solution b: Ammonia gas saturated methanol.

Solution c: Dissolve 7 g sodium in 100 ml methanol.

Spray solution: Mix freshly before use 20 ml a, 4 ml b and 8 ml c.

Treatment: Heat 10 min at 110°C.

Chemicals:
- Silver nitrate G R, art. no. 1512
- Sodium G R, art. no. 6261
- Methanol G R, art. no. 6009
- Ammonia solution abt. 10%/s (0.96) G R, art. no. 5423

272. Silver nitrate – bromophenol blue for purines (Wood reagent).

Spray solution: Dissolve 0.2 g bromophenol blue in 50 ml acetone and add 50 ml 2%/s aqueous silver nitrate solution. The reagent is stable for about one week.

Procedure: After development in acidic solvents dry the chromatogram and place into a chamber with ammonia. Then remove the excess ammonia by hot air and spray.


Chemicals:
- Bromophenol blue indicator, art. no. 8122
- Silver nitrate G R, art. no. 1512
- Acetone G R, art. no. 14


Solution a: 10%/s aqueous silver nitrate solution.

Solution b: 0.2%/s ethanolic fluorescein sodium solution.

Spray solution: Mix freshly before use 10 ml a and 50 ml b.

Yellow spots on salmon-pink background.


274. Silver nitrate — formaldehyde for chlorinated insecticides
(e.g. dieldrin, aldrin and lindane).

Spray solution I: 0.05 N silver nitrate solution.
Spray solution II: 35% formaldehyde solution.
Spray solution III: 2 N methanolic potassium hydroxide solution.
Spray solution IV: freshly prepared mixture of equal volumes of 30% hydrogen peroxide and 65% nitric acid.

Procedure: Spray with I, dry 30 min, spray with II and dry again 30 min. Spray with III and heat 30 min at 130°C. Spray with IV, allow the chromatogram to stand in the darkness for 12 hours, and expose to daylight. Dark green spots on light grey background.


Chemicals:
0.1 N Silver nitrate solution, art. no. 9081
Formaldehyde solution 35% G R, art. no. 4003
Potassium hydroxide G R, art. no. 5033
Methanol G R, art. no. 6009
Perhydrol® (30%/H2O2) G R, art. no. 7209
Nitric acid min. 65% (1.4) G R, art. no. 454

275. Silver nitrate — hydrogen peroxide for chlorinated hydrocarbons.

Spray solution: Dissolve 0.1 g silver nitrate in 1 ml water, add 10 ml ethylene glycol monophenyl ether, fill up to 200 ml with acetone and add 1 drop 30% hydrogen peroxide.

Treatment: Irradiate with unfiltered UV light. If long-wave UV light is used expose alumina layers about 50 min and silica gel layers up to 15 min. Dark spots are formed.


Chemicals:
Silver nitrate G R, art. no. 1512
Acetone G R, art. no. 14
Perhydrol® (30% by weight H2O2) G R, art. no. 7209
Ethylene glycol monophenyl ether G R, art. no. 7291

276. Silver nitrate — potassium dichromate for barbiturates.

Spray solution I: Add 25 ml saturated aqueous silver nitrate solution to a mixture of 50 ml acetone and 2 ml water.

Spray solution II: 0.3% aqueous potassium dichromate solution.
Spray solution III: 2% methanolic sodium hydroxide solution.

Procedure: Spray liberally with I and dry in the air. Then spray with II, dry, re-spray with III and re-dry again in the air. Then spray with III.


Chemicals:
Silver nitrate G R, art. no. 1512
Potassium dichromate G R, art. no. 4664
Sodium hydroxide G R, art. no. 6498
Acetone G R, art. no. 14
Methanol G R, art. no. 6009

277. Silver nitrate — potassium permanganate for reducing compounds.

Solution a: Mix freshly before use 1 part 0.1 N silver nitrate solution, 1 part 2 N ammonia solution and 2 parts 2 N sodium hydroxide solution.

Solution b: Dissolve 0.5 g potassium permanganate and 1 g sodium carbonate in 100 ml water.

Spray solution: Mix freshly before use equal parts of a and b.

Note: Reducing compounds show light yellow spots on green-blue background immediately after spraying.


Chemicals:
Potassium permanganate G R, art. no. 5082
Sodium carbonate 10-hydrate G R, art. no. 6391
0.1 N Silver nitrate solution, art. no. 9081
2 N Sodium hydroxide solution, art. no. 9136
Ammonia solution min. 25% (0.91) G R, art. no. 5432

278. Silver nitrate — sodium dichromate for purines, PC.

Dip solution I: 2% aqueous silver nitrate solution.
Dip solution II: 0.5% aqueous sodium dichromate solution.
Dip solution III: 0.5 N nitric acid.

Procedure: Dip into I, dry the chromatogram in the air 10 min and dip into II. Dip the chromatogram dyed red into III, thus discolouring the background, leaving the purines as red spots.


Chemicals:
Silver nitrate G R, art. no. 1512
Sodium dichromate G R, art. no. 6336
Nitric acid min. 65% (1.4) G R, art. no. 454
279. Silver nitrate — sodium hydroxide for sugars and polyalcohols.

*Spray solution I:* Fill up 1 ml saturated aqueous silver nitrate solution to 200 ml with acetone and add 5–10 ml water to dissolve the resulting precipitate.

*Spray solution II:* 0.5 N sodium hydroxide solution in aqueous methanol (dissolve 20 g sodium hydroxide in a minimum of water and fill up to 1 l with methanol).

Procedure: Spray with I and II and heat 1–2 min at 100°C.

Chemicals:
- Silver nitrate G R, art. no. 1512
- Sodium hydroxide G R, art. no. 6498
- Acetone G R, art. no. 14
- Methanol G R, art. no. 6009

280. Sodium dithionite for antimony, arsenic, mercury, silver and bismuth ions.

*Spray solution:* 0.1% aqueous sodium dithionite solution.


Chemicals:
- Sodium dithionite, art. no. 6507

281. Sodium hydroxide for Δ^4-3-ketosteroids.

*Spray solution:* 10% sodium hydroxide solution in 60% methanol.

*Treatment:* Heat 10 min at 80°C.

Δ^4-3-ketosteroids show yellow fluorescence in long-wave UV light.


Chemicals:
- Sodium hydroxide G R, art. no. 6498
- Methanol G R, art. no. 6009

282. Sodium meta-periodate — benzidine for compounds with 1,2-diol groups (sugars, polyalcohols).

*Spray solution I:* 0.5% aqueous sodium meta-periodate solution.

*Spray solution II:* Add 50 ml water, 20 ml acetone and 10 ml 0.2 N hydrochloric acid to a solution of 1.8 g benzidine in 50 ml ethanol.

Procedure: Spray with I and after 5 min with II.

White spots on blue background.


Chemicals:
- Sodium meta-periodate, art. no. 6597
- Benzidine G R, art. no. 1762

283. Sodium meta-periodate — benzidine — silver nitrate for substances with 1,2-diol groups (sugars, polyalcohols).

*Spray solution I:* 0.1% aqueous sodium meta-periodate solution.

*Spray solution II:* Add 70 ml water, 30 ml acetone and 1.5 ml 1 N hydrochloric acid to a solution of 2.8 g benzidine in 80 ml ethanol.

*Spray solution III:* Mix 1 ml aqueous saturated silver nitrate solution with stirring with 20 ml acetone and add water dropwise until the precipitated silver nitrate dissolves.

Procedure: Spray with I and dry the chromatogram at room temperature. Spray with II and place it into a chamber saturated with ammonia. Additionally you can spray with III, the white spots turn dark.


Chemicals:
- Sodium meta-periodate, art. no. 6597
- Benzidine G R, art. no. 1762
- Silver nitrate G R, art. no. 1512
- 1 N Hydrochloric acid Titrisol®, art. no. 9970
- Ethanol abs. G R, art. no. 972
- Acetone G R, art. no. 14

284. Sodium meta-periodate — Nessler's reagent for hydroxyamino acids (serine, threonine).

*Spray solution I:* 1% aqueous sodium meta-periodate solution.

*Spray solution II:* Nessler's reagent.

Make a paste with 10 g mercury(II) iodide and a little water and add 5 g potassium iodide. Add a solution of 20 g sodium hydroxide in 80 ml water. After complete solution fill up to 100 ml with water. Allow to stand for some days and decant after deposition of the precipitate.

Procedure: Spray with I, dry the chromatogram at room temperature and spray with II.


Chemicals:
- Sodium meta-periodate, art. no. 6597
- Mercury(II) iodide, art. no. 4420
- Potassium iodide G R, art. no. 5043
- Sodium hydroxide G R, art. no. 6498
285. Sodium meta-periodate — 4-nitroaniline for deoxy-sugars.

*Spray solution I:* Mix 1 part saturated aqueous sodium meta-periodate solution with 2 parts water.

*Spray solution II:* Mix 4 parts 1/4 ethanolic 4-nitroaniline solution with 1 part 37% hydrochloric acid.

*Procedure:* Spray with I, wait 10 min, then spray with II.

*Note:* Deoxy-sugars and glycols show yellow spots which fluoresce strongly in long-wave UV light. The colour changes to green by spraying with 5% methanolic sodium hydroxide solution.


*Chemicals:*
- Sodium meta-periodate, art. no. 6597
- 4-Nitroaniline, art. no. 6760
- Ethanol abs. G R, art. no. 972
- Hydrochloric acid fuming min. 37%/o (1.19) G R, art. no. 317
- Sodium hydroxide G R, art. no. 6498
- Methanol G R, art. no. 6009

286. Sodium nitrite — hydrochloric acid for indoles and thiazoles.

*Spray solution:* Freshly prepared solution of 1 g sodium nitrite in 100 ml 1 N hydrochloric acid.

Heat at 100° C.

*Note:* Indoles turn red and thiazole derivatives light green.

*Alternative:* Spray solution: 0.5% aqueous sodium nitrite solution.

*Treatment:* Place the chromatogram into a chamber with hydrogen chloride vapours.


*Chemicals:*
- Sodium nitrite G R, art. no. 6549
- 1 N Hydrochloric acid Titrisol®. art. no. 9970
- Hydrochloric acid fuming min. 37%/o (1.19) G R, art. no. 317

287. Sodium nitroprusside for compounds with SH-group (cysteine), with S-S-group (cystine) and arginine.

*Spray solution I:* Dissolve 1.5 g sodium nitroprusside in 5 ml 2 N hydrochloric acid. Filter after addition of 95 ml methanol and 10 ml 25% ammonia solution.

*Note:* SH-Compounds show red spots, arginine turns orange and later grey-blue.

*Spray solution II:* Dissolve 2 g sodium cyanide in 5 ml water and fill up to 100 ml with methanol.

*Note:* Spraying with II visualises compounds with -S-S-linkage as red spots on yellow background.

*Caution* when using this highly toxic reagent!

*Variation for -S-S-compounds:*

*Spray solution I:* Dissolve 5 g sodium cyanide and 5 g sodium carbonate in 100 ml 25% ethanol.

*Spray solution II:* Dissolve 2 g sodium nitroprusside in 100 ml 75% ethanol.

*Procedure:* Spray with I, dry briefly in the air and spray with II.

*Caution* when using this highly toxic reagent!


*Variation for thiolaetones:*

*Spray solution I:* 1 N sodium hydroxide solution.

*Spray solution II:* Dissolve 2 g sodium nitroprusside in 100 ml 75% ethanol.

*Procedure:* Spray with I, dry briefly in the air and spray with II.


*Chemicals:*
- Sodium nitroprusside G R, art. no. 6541
- Sodium carbonate G R, art. no. 6391
- Hydrochloric acid min. 25%/o (1.125) G R, art. no. 316
- Ammonia solution min. 25%/o (0.91) G R, art. no. 5432
- Methanol G R, art. no. 6009
- Ethanol abs. G R, art. no. 972
- Sodium cyanide, art. no. 6437
- 1 N Sodium hydroxide solution Titrisol®, art. no. 9956

288. Sodium nitroprusside — acetaldehyde for secondary aliphatic and alicyclic amines.

*Spray solution:* Dissolve 5 g sodium nitroprusside in 100 ml 10%/o aqueous acetaldehyde solution. Before use mix 1 part of this solution with 1 part 2%/o aqueous sodium carbonate solution.


K. Mack, J. Hacaperková, B. Kakáč, Pharmazie 11, 533 (1956).

*Chemicals:*
- Sodium nitroprusside, G R, art. no. 6541
- Sodium carbonate G R, art. no. 6391
- Acetaldehyde, art. no. 4
290. Sodium nitroprusside — ammonia for hemlock alkaloids.

Spray solution I: 10% aqueous sodium nitroprusside solution.
Spray solution II: 10% ammonia solution.
Procedure: Spray with I and then with II.
Note: γ-Coniceine turns red.
Chemicals:
- Sodium nitroprusside G R, art. no. 6541
- Ammonia solution abt. 10% (0.96) G R, art. no. 5423

291. Sodium nitroprusside — hydrogen peroxide for guanidine, urea, thiourea and derivatives, creatine and creatinine.

Spray reagent: Mix 2 ml 5% aqueous sodium nitroprusside, 1 ml 10% aqueous sodium hydroxide and 5 ml 3% aqueous hydrogen peroxide and dilute with 15 ml water. The solution can be stored several days in the refrigerator.

Chemicals:
- Sodium nitroprusside G R, art. no. 6541
- Sodium hydroxide 10% solution (1.109) G R, art. no. 5588
- Hydrogen peroxide (30% by weight H₂O₂), art. no. 7209

292. Sodium nitroprusside — potassium hexacyanoferrate(III) for aliphatic nitrogen compounds, cyanamide, guanidine, urea, thiourea and derivatives, creatine and creatinine.

Spray solution: Mix in the ratio 1:1:1:3 10% aqueous sodium hydroxide solution, 10% aqueous sodium nitroprusside solution, 10% aqueous potassium hexacyanoferrate(III) solution and water. The mixture is allowed to stand for at least 20 min at room temperature before use. Stable for several weeks when stored in the refrigerator. Before use mix with an equal part of acetone.
296. **Sodium pentacyanoamino ferrate (II) for urea, thiourea and guanidines (Fearon reagent).**

*Sodium pentacyanoamino ferrate (II):* Dissolve 10 g sodium nitroprusside in 40 ml 25% ammonia solution. Allow the solution to stand at 0°C until all nitroso iron(III) cyanide is decomposed. This is the case if several drops of the mixture added to a solution of creatinine in 1 N sodium carbonate solution produce no longer any red colour. Then filter and add ethanol to the clear filtrate until no further precipitate is formed. Filter off the resulting precipitate, wash with absolute ethanol and dry over sulfuric acid in a vacuum desiccator. The salt is stable when stored protected from light and moisture.

**Spray solution:** Add to 5 ml 10% sodium hydroxide 15 ml 1% aqueous sodium pentacyanoamino ferrate(II) solution and 1 drop Perhydrol®. Stable for about 24 hours.

**Literature:** P. H. List, Hoppe-Seyler's Z. physiol. Chem. 305, 17 (1956).

**Chemicals:**
- Sodium nitroprusside G R, art. no. 6541
- Sodium carbonate G R, art. no. 6392
- Ethanol abs. G R, art. no. 972
- Ammonia solution min. 25% (0.91) G R, art. no. 5432
- Perhydrol® (30% by weight H₂O₂) G R, art. no. 7209
- Creatinine, art. no. 5208
- Sulfuric acid 95–97% (84) G R, art. no. 731

297. **Sodium sulfide solution for ions of the hydrogen sulfide group.**

**Spray solution:** Freshly prepared 0.5% aqueous sodium sulfide solution.

**Literature:** F. W. H. M. Merkus, Pharm. Weekblad 98, 947 (1963).

**Chemicals:**
- Sodium sulfide G R, art. no. 6639

298. **Sodium tetraphenylboron (Kalignost®) for alkaloids.**

**Spray solution I:** 1% sodium tetraphenylboron solution in ethyl methyl ketone, saturated with water.

**Spray solution II:** 0.015% methanolic solution of fisetin or quercetin.

**Procedure:** Spray with I, dry at room temperature, then spray with II and dry again at room temperature. Orange to red spots which fluoresce in long-wave UV light.

**Literature:** R. Neu, J. Chromatogr. 11, 364 (1963).

299. **Sodium tetraphenylboron (Kalignost®) — rhodamine B for potassium ions.**

**Spray solution I:** 0.1 N sodium hydroxide solution.

**Spray solution II:** 1% ethanolic Kalignost® solution.

**Spray solution III:** 0.5% ethanolic rhodamine B solution.

**Procedure:** Spray with I, dry, spray with II, and then with III. Intense dark blue fluorescence in long-wave UV light. Larger amounts of potassium appear in daylight as light red spots on dark red background.

**Chemicals:**
- Kalignost® (sodium tetraphenylboron) G R, art. no. 6669
- Quercetin, art. no. 7546
- Fisetin
- Methanol G R, art. no. 6009
- Ethyl methyl ketone G R, art. no. 9708

300. **Sodium thiosulfate — copper (II) acetate for antimony ions. PC.**

**Spray solution I:** Saturated aqueous sodium thiosulfate solution.

**Spray solution II:** Dissolve 0.4 g copper(II) acetate in a mixture of 2 ml glacial acetic acid and 48 ml water.

**Procedure:** Spray with I, heat briefly, rinse out excess sodium thiosulfate with water and spray with II.


**Chemicals:**
- Copper(II) acetate G R, art. no. 2711
- Sodium thiosulfate G R, art. no. 6516
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

301. **Starch for amylases.**

**Spray solution I:** 2% aqueous starch solution.

**Spray solution II:** 0.01 N iodine solution.

**Procedure:** Spray with I, then place the chromatogram into a moist chamber at 40–50 °C for 1 hour. After drying at room temperature spray with II.

**Note:** Amylases will appear as white spots on violet or brown background.

**Literature:** K. Wallenfels, E. v. Pechmann, Angew. Chem. 63, 44 (1951).
302. Sulfanilamide diazotised for phenols, coupling amines and heterocycles (Pauly reagent acc. to Kutacek).

Spray solution I: Dissolve 3 g sulfanilamide in 200 ml water, 6 ml 36% hydrochloric acid and 14 ml 1-butanol. Add freshly before use to 20 ml 0.3 g sodium nitrite.

Spray solution II: 10% aqueous sodium carbonate solution.

Procedure: Spray with I, and after 5–10 min with II.


Chemicals:
- Sulfanilamide, art. no. 8035
- Sodium nitrite G R, art. no. 6549
- Sodium carbonate 10-hydrate G R, art. no. 6391
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
- 1-Butanol G R, art. no. 1990

303. Sulfanilic acid diazotised for phenols, coupling amines and heterocycles (Pauly reagent).

Spray solution: Dissolve 4.5 g sulfanilic acid in 45 ml 12 N hydrochloric acid with warming and fill up the solution to 500 ml with water. Cool 10 ml of the diluted solution with ice and add 10 ml of cold 4.5% aqueous sodium nitrite solution. Allow to stand for 15 min at 0°C (it is stable for 1–3 days at this temperature) and add freshly before use equal parts of 10% aqueous sodium carbonate solution.


Chemicals:
- Sulfanilic acid G R, art. no. 686
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
- Sodium nitrite G R, art. no. 6549
- Sodium carbonate 10-hydrate G R, art. no. 6391

304. Sulfanilic acid – 1-naphthylamine for nitrosamines.

Solution a: 1% sulfanilic acid solution in 30% aqueous acetic acid.

Solution b: 0.1% 1-naphthylamine solution in 30% aqueous acetic acid.

Spray solution: Mix freshly before use equal parts of a and b.

Procedure: Irradiate the chromatogram for about 3 min with short-wave UV light, then spray with the spray solution.

Note: Aliphatic nitrosamines show red-violet spots, aromatic nitrosamines turn green to blue.


Chemicals:
- Sulfanilic acid G R, art. no. 686
- 1-Naphthylamine G R, art. no. 6245
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

305. Sulfuric acid as general visualisation reagent (in particular for sterols, steroids, bile acids and gibberellins).

Spray solutions:
A: Mix equal parts of 95% sulfuric acid and methanol with cooling.
B: 5% ethanolic solution of 95% sulfuric acid.
C: 15% solution of 95% sulfuric acid in 1-butanol.
D: 5% solution of 95% sulfuric acid in acetic anhydride.
E: Mix equal parts of 95% sulfuric acid and glacial acetic acid.

Procedure: Spray the chromatogram with one of these reagents, allow to dry for 15 min in the air and heat to 110°C until maximal visualisation of the spots.

Note: Cholesterol and vitamin A, their esters and many isoprenoid lipids show characteristic colours after spraying with spray solution A and subsequently heating: cholesterol and esters turn red, then red-violet and brown; vitamin A and esters turn blue. Most compounds may be subsequently charred, yielding black spots. Heating with sulfuric acid on layers impregnated with silver nitrate may be followed by oxidation to CO₂.


Chemicals:
- Sulfuric acid 95–97% (1.84) G R, art. no. 731
- Ethanol abs. G R, art. no. 972
- 1-Butanol G R, art. no. 1990
- Acetic anhydride G R, art. no. 42
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

306. Sulfuric acid – hypochlorite for digitalis glycosides.

Spray solution: Mix 10 ml 2 N sulfuric acid and 3 ml sodium hypochlorite solution.

Treatment: Heat 10–15 min at 125°C.
307. Tetracyanoethylene for aromatic hydrocarbons, phenols and heterocyclic compounds.

Spray solution: 10% solution of tetracyanoethylene in benzene.

Procedure: Spray directly after development of the chromatogram.

Note: Aromatic hydrocarbons show various colours, some of them for a short time only. Janák recommends warming at 100°C.


Chemicals:
- Benzene G R, art. no. 1783
- Tetracyanoethylene, art. no. 8240

308. Tetranitrodiphenyl for cardiac glycosides.

Spray solution I: Saturated solution of 2,2',4,4'-tetranitrodiphenyl in benzene.

Spray solution II: 10% potassium hydroxide solution in 50% aqueous methanol.

Procedure: Spray with I, dry at room temperature and spray with II.

Blue spots.


Chemicals:
- Potassium hydroxide G R, art. no. 5033
- Benzene G R, art. no. 1783
- 2,2',4,4'-Tetranitrodiphenyl
- Methanol G R, art. no. 6009

309. Tetraphenyldiboroxide for flavones. PC.

Prepare tetraphenyldiboroxide according to the directions by R. Neu from 3 g sodium tetraphenyloboron (Kaligrost®, 8.5 ml 2 N hydrochloric acid and 8.5 ml water. For details see R. Neu, Chem. Ber. 87, 802 (1954).

Dip solution I: Saturated solution of tetraphenyldiboroxide in petroleum benzine.

Dip solution II: 1-2% aqueous solution of a quaternary ammonium base (e.g. Laudacit®).

Procedure: Dip into I, dry briefly at room temperature and then dip into II. Subsequently dry at room temperature.


Chemicals:
- Kaligrost® (Sodium tetraphenyloboron) G R, art. no. 6669
- Hydrochloric acid min. 25% (1.123) G R, art. no. 316
- Petroleum benzine G R boiling range 40-60°C G R, art. no. 1775

310. Tetrazolium blue for corticosteroids and other reducing compounds.

Spray solution: Mix freshly before use equal parts of 0.5% methanolic tetrazolium blue solution and 6 N sodium hydroxide solution in water or water-methanol mixture.

Violets spots at room temperature or after short warming.


Chemicals:
- Tetrazolium blue, art. no. 8103
- Sodium hydroxide G R, art. no. 6498
- Methanol G R, art. no. 6009

311. Thiobarbituric acid for sorbic acid.

Spray solution: Saturated aqueous solution of thiobarbituric acid. Sorbic acid show red spots.


Chemicals:
- 2-Thiobarbituric acid, art. no. 8180

312. Thymol – sulfuric acid for sugars.

Spray solution: Dissolve 0.5 g thymol in 95 ml ethanol and add 5 ml 97% sulfuric acid with precaution.

Treatment: Heat 15–20 min at 120°C.

Sugars show pink spots.

313. Thymol blue for dimethylamino acids.

Spray solution: Dissolve 0.04 g thymol blue in a mixture of 25 ml 1-butanol, 25 ml ethanol and 50 ml 0.01 N sulfuric acid.

Yellow spots on red background.


Chemicals:
- Thymol blue indicator, art. no. 8176
- Ethanol abs. G R, art. no. 972
- 1-Butanol G R, art. no. 1990
- 0.01 N Sulfuric acid Titrisol®, art. no. 9982

314. Tin(II) chloride – hydrochloric acid – 4-dimethylaminobenzaldehyde for aromatic compounds containing nitro groups.

Spray solution I: Prepare freshly before use a mixture of 3 ml 15% aqueous tin(II) chloride and 15 ml 37% hydrochloric acid and dilute with 180 ml water.

Spray solution II: Dissolve 1 g 4-dimethylaminobenzaldehyde in a mixture of 30 ml ethanol, 3 ml 37% hydrochloric acid and 180 ml 1-butanol.

Treatment: Spray with I, dry at room temperature and spray with II.

Yellow spots after re-drying at room temperature.


Chemicals:
- Tin(II) chloride G R, art. no. 7815
- 4-Dimethylaminobenzaldehyde G R, art. no. 3058
- Ethanol abs. G R, art. no. 972
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317
- 1-Butanol G R, art. no. 1990

315. Tin(II) chloride – potassium iodide for gold ions.

Spray solution: Dissolve 5.6 g tin(II) chloride in 10 ml 37% hydrochloric acid. After dilution with water to 100 ml, add 0.2 g potassium iodide to the solution. Black spots.


Chemicals:
- Tin(II) chloride G R, art. no. 7815
- Potassium iodide G R, art. no. 3043
- Hydrochloric acid fuming min. 37% (1.19) G R, art. no. 317

316. Tin(IV) chloride for triterpenes, sterols, steroids, phenols and polyphenols.

Spray solution: Add 10 ml tin(IV) chloride to 160 ml of a mixture of equal volumes of chloroform and glacial acetic acid.

Treatment: Heat the layer 5-10 min at 100°C and inspect subsequently in daylight and in long-wave UV light.


Chemicals:
- Tin(IV) chloride fuming, art. no. 7811
- Chloroform G R, art. no. 2445
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062

317. Titan yellow for cadmium ions.

Spray solution: 0.1% aqueous titan yellow solution.

Treatment: Spray either with 25% ammonia solution or place the chromatogram sprayed with titan yellow solution into a chamber with ammonia.


Chemicals:
- Titan yellow G R, art. no. 1307
- Ammonia solution min. 25% (0.91) G R, art. no. 5432

318. p-Toluenesulfonic acid for steroids, flavonoids and catechins.

Spray solution: 20% solution of p-toluenesulfonic acid in chloroform.

Treatment: After spraying heat a few minutes at 100°C. Inspect the spots in long-wave UV light.


Chemicals:
- p-Toluenesulfonic acid G R, art. no. 9613
- Chloroform G R, art. no. 2445

319. Toluidine blue for acidic polysaccharides. PC.

Fixing solution: 20 ml 35% formaldehyde solution in 80 ml ethanol.

Spray solution: Dissolve 0.04 g toluidine blue in 80 ml acetone and 20 ml water.

Dip solution: 5% acetic acid solution.

Procedure: Place the chromatogram 15 min into the fixing solution. After drying, spray with the spray solution and rinse the excess dye first with dip solution, then with water.

320. Trichloroacetic acid for steroids, digitalis glycosides, Veratrum alkaloids and vitamin D.
A. Spray solution: 25% solution of trichloroacetic acid in chloroform.
B. Spray solution (for vitamin D): 1% trichloroacetic acid solution in chloroform.
C. Spray solution (for digitalis glycosides): Dissolve 3.3 g trichloroacetic acid in 10 ml chloroform and add 1-2 drops hydrogen peroxide.
Treatment: Heat 5-10 min at 120° C.
Inspect the spots in daylight and in long-wave UV light.
Chemicals:
Trichloroacetic acid G R, art. no. 807
Chloroform G R, art. no. 2445
Perhydro® (30%/ by weight H₂O₃) G R, art. no. 7209

321. Trifluoroacetic acid for steroids.
Spray solution: 1% trifluoroacetic acid in chloroform.
Treatment: Heat 5 min at 120° C.
Chemicals:
Trifluoroacetic acid, art. no. 8260
Chloroform G R, art. no. 2445

322. 2.4.6-Trinitrobenzoic acid for cardiac glycosides.
Spray solution 1: 0.1% solution of 2.4.6-trinitrobenzoic acid in a mixture of water and dimethylformamide.
Spray solution II: 5% aqueous sodium carbonate solution.
Spray solution III: 5% aqueous sodium dithionite phosphate solution.
Procedures: Spray with I, then with II, heat 4-5 min at 90-110° C, cool and spray finally with III.
Cardiac glycosides show orange-red spots.

323. 2.3.5-Triphenyltetrazolium chloride (TTC) for reducing sugars, corticosteroids and other reducing compounds.
Spray solution: Mix freshly before use one part 4% methanolic TTC solution with one part 1 N sodium hydroxide solution.
Treatment: Heat 5-10 min at 100° C.
Reducing compounds show red spots.
Tetrazolium blue is more sensitive.
Chemicals:
2.3.5-Triphenyltetrazolium chloride, art. no. 8380
Methanol G R, art. no. 6009
1 N Sodium hydroxide solution Titrisol®, art. no. 9956

324. Tungstophosphoric acid for reducing compounds, lipids, steroids and steroids.
Spray solution: 20% ethanolic solution of tungstophosphoric acid.
Treatment: Heat at 120° C until maximal visualisation of the spots.
Chemicals:
Tungstophosphoric acid G R, art. no. 583
Ethanol abs. G R, art. no. 972

325. Urea — hydrochloric acid for sugars.
Spray solution: Dissolve 5 g urea in 20 ml 2 N hydrochloric acid and add 100 ml ethanol.
Treatment: Heating at 100° C promotes reaction.
Ketoses and oligosaccharides containing ketoses turn blue.
Chemicals:
Urea G R, art. no. 8487
Hydrochloric acid min. 25%/ (1.125) G R, art. no. 316
Ethanol abs. G R, art. no. 972
326. **Vanillin — hydrochloric acid for catechins.**

*Spray solution:* Dissolve 0.5 g vanillin in 50 ml 37% hydrochloric acid.

*Treatment:* Dry the chromatogram at room temperature. Catechols show red spots.


*Chemicals:*
- Vanillin, art. no. 8510
- Hydrochloric acid fuming min. 37%/v (1.9) G R, art. no. 317

327. **Vanillin — phosphoric acid for steroids.**

*Spray solution:* Dissolve 1 g vanillin in 100 ml 50% aqueous phosphoric acid.

*Treatment:* Heat 10–20 min at 120° C.


*Chemicals:*
- Vanillin, art. no. 8510
- ortho-Phosphoric acid min. 85%/v (1.71) G R, art. no. 573

328. **Vanillin — potassium hydroxide for amino acids (ornithine, lysine, proline) and amines.**

*Spray solution I: 2%* vanillin solution in 2-propanol.

*Spray solution II: 1%* ethanolic potassium hydroxide solution.

*Procedure:* Spray with I and heat the chromatogram 10 min at 110° C. Ornithine then fluoresces intensively green-yellow in long-wave UV light, lysine only weakly green-yellow. After spraying with II, heat again in the same manner. Ornithine first shows a salmon colour and then fades, while proline, hydroxyproline, pipercolic acid and sarcosine turn red after several hours. Glycine turns brown-green, the other amino acids faintly brown.


*Chemicals:*
- Vanillin, art. no. 8510
- Potassium hydroxide G R, art. no. 5033
- Ethanol abs. G R, art. no. 972
- 2-Propanol G R, art. no. 9634

329. **Vanillin — sulfuric acid for higher alcohols, phenols, steroids and essential oils.**

*A. Spray reagent:* Dissolve 1 g vanillin in 100 ml 97%/v sulfuric acid.

*Treatment:* Heat the chromatogram at 120° C until the spots attain maximum colour intensity.


*B. Spray reagent:* Dissolve 0.5 g vanillin in 100 ml of a mixture of 97% sulfuric acid and ethanol (40+10).

*Treatment:* Heat the chromatogram at 120° C until the spots attain maximum colour intensity.


*Chemicals:*
- Vanillin, art. no. 8510
- Sulfuric acid 95—97%/v (1.84) G R, art. no. 731
- Ethanol abs. G R, art. no. 972

330. **Violuric acid for alkali and alkaline earth metal ions.**

*Spray solution: 1.5%* aqueous violuric acid solution. To effect solution it must not be heated above 60° C.

*Treatment:* Heat 20 min at 120° C.


*Chemicals:*
- Violuric acid, art. no. 9616

331. **Xanthydrol for tryptophan and other indole derivatives.**

*Spray solution:* Dissolve 0.1 g xanthydrol in 90 ml ethanol and add 10 ml 37%/v hydrochloric acid freshly before use.

*Treatment:* Heat at 110° C until maximal visualisation of the spots.


*Chemicals:*
- Xanthydrol abt. 10%/v in methanol, art. no. 8696
- Ethanol abs. G R, art. no. 972
- Hydrochloric acid fuming min. 37%/v (1.19) G R, art. no. 317

332. **Zinc chloride for steroid sapogenins and steroids.**

*Spray solution:* Dissolve 30 g zinc chloride in 100 ml methanol and filter off from the insoluble.

*Treatment:* Heat 1 hour at 105° C and cover the layer immediately with a glass plate for protection against the influence of moisture. The spots fluoresce in long-wave UV light.


*Chemicals:*
- Zinc chloride G R, art. no. 8816
- Methanol G R, art. no. 6009
333. Zinc uranyl acetate for sodium ions.

Spray solution: Dissolve 10 g uranyl acetate in 6 ml 30% acetic acid and fill up to 50 ml with water. Mix 30 g zinc acetate with 3 ml 30% acetic acid and fill up to 50 ml with water. Mix equal volumes of both solutions, allow to stand for one day and filter off.

Note: Inspect in UV light.


Chemicals:
- Uranyl acetate G R, art. no. 8473
- Zinc acetate G R, art. no. 8802
- Acetic acid glacial min. 96% (1.06) G R, art. no. 90062


Spray solution: Dissolve 0.05 g zirconyl chloride and 0.05 g alizarin sulfonic acid sodium salt in 100 ml 2 N hydrochloric acid.


Chemicals:
- Zirconium(IV) oxide chloride G R, art. no. 8917
- Alizarin sulfonic acid sodium salt G R, art. no. 6279
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316

335. Zirconyl chloride – citric acid for glycosides. PC.

Spray solution I: 2% methanolic zirconium(IV) oxide chloride solution.

Spray solution II: 5% aqueous citric acid solution.

Procedure: Glycosides are first hydrolysed on the chromatogram which has been placed into a covered beaker with boiling 25% hydrochloric acid. After drying, spray with I, dry again and spray vigorously with II.


Chemicals:
- Zirconium(IV) oxide chloride G R, art. no. 8917
- Citric acid G R, art. no. 244
- Hydrochloric acid min. 25% (1.125) G R, art. no. 316
- Methanol G R, art. no. 6009

Compounds or Compound Classes and Reagents for their Detection

<p>| Acetylene compounds | No. 96 |
| Acids, aromatic | No. 153, 249 |
| Acids, organic | No. 42, 43, 64, 66, 77, 78, 91, 93, 122, 148 |
| Adrenaline and derivatives | No. 95, 135, 238, 241 |
| Alcohols, higher | No. 329 |
| Aldehydes | No. 86, 115 |
| Alkali chlorides | No. 92 |
| Alkali ions | No. 330 |
| Alkaline earth metal ions | No. 330 |
| Alkaloids | No. 56, 76, 131, 132, 133, 162, 205, 211, 246, 247, 298 |
| Aluminium ions | No. 31, 158, 195, 255 |
| Amides | No. 69, 155 |
| Amines | No. 76, 102, 207, 208, 211, 240, 288 |
| Amines, aromatic | No. 87, 136, 149, 202, 213, 215, 302, 303 |
| Amines, quaternary | No. 130 |
| Amino acids | No. 114, 176, 201, 207, 208, 209, 210, 328 |
| Amino acids, sulfur containing | No. 161, 287 |
| Amino-sugars | No. 102, 207 |
| Ammonium ions | No. 73 |
| Amylase | No. 301 |
| Anhydrides | No. 155 |
| Anthroquinone glycosides | No. 183, 243 |
| Antimony ions | No. 280, 300 |
| Antioxidants | No. 95 |
| Arginine | No. 157, 199, 287 |
| Arsenic ions | No. 280 |
| Azulenes | No. 101 |
| Barbiturates | No. 74, 75, 81, 138, 184, 186, 237, 276 |
| Barium ions | No. 156, 158, 264 |
| Bile acids | No. 29, 172, 220, 305 |
| Bismuth ions | No. 265, 280 |
| Cadmium ions | No. 253, 317 |
| Caffeine | No. 58 |
| Calcium ions | No. 121, 156, 158 |</p>
<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>No. References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamate esters</td>
<td>No. 147, 112, 308, 322</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>No. 27</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>No. 159, 263</td>
</tr>
<tr>
<td>Carotenoid aldehydes</td>
<td>No. 134, 135</td>
</tr>
<tr>
<td>Catechins</td>
<td>No. 2, 123, 150, 256</td>
</tr>
<tr>
<td>Catechol amines</td>
<td>No. 61, 62</td>
</tr>
<tr>
<td>Cations, inorganic</td>
<td>No. 1</td>
</tr>
<tr>
<td>Chloramines</td>
<td>No. 126</td>
</tr>
<tr>
<td>Cholesterol, -esters</td>
<td>No. 31, 35</td>
</tr>
<tr>
<td>Choline</td>
<td>No. 253, 263</td>
</tr>
<tr>
<td>Cobalt ions</td>
<td>No. 106, 121, 253, 265</td>
</tr>
<tr>
<td>Copper ions</td>
<td>No. 125, 154, 310, 323</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>No. 82, 243</td>
</tr>
<tr>
<td>Coumarines</td>
<td>No. 232, 290, 292</td>
</tr>
<tr>
<td>Creatine</td>
<td>No. 290, 292</td>
</tr>
<tr>
<td>Creatinine</td>
<td>No. 95, 292</td>
</tr>
<tr>
<td>Cyanamide</td>
<td>No. 287</td>
</tr>
<tr>
<td>Cysteine, Cystine</td>
<td>No. 225, 228</td>
</tr>
<tr>
<td>Dehydroascorbic acid</td>
<td>No. 85, 285, 295</td>
</tr>
<tr>
<td>Deoxy-sugars</td>
<td>No. 84</td>
</tr>
<tr>
<td>Deoxyribonucleosides</td>
<td>No. 44</td>
</tr>
<tr>
<td>Dicarboxylic acids</td>
<td>No. 59, 230, 306, 320</td>
</tr>
<tr>
<td>Digitalis glycosides</td>
<td>No. 313</td>
</tr>
<tr>
<td>Dimethyl amino acids</td>
<td>No. 98, 204</td>
</tr>
<tr>
<td>3,5-Dinitrobenzoic acid esters</td>
<td>No. 180, 181, 282, 283</td>
</tr>
<tr>
<td>1,2-Diols</td>
<td>No. 28</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>No. 231</td>
</tr>
<tr>
<td>Epoxides</td>
<td>No. 105</td>
</tr>
<tr>
<td>Ergot alkaloids</td>
<td>No. 124, 329</td>
</tr>
<tr>
<td>Essential oils</td>
<td>No. 155</td>
</tr>
<tr>
<td>Esters</td>
<td>No. 194</td>
</tr>
<tr>
<td>Estrogens</td>
<td>No. 257</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>No. 151</td>
</tr>
<tr>
<td>Epoxides</td>
<td>No. 3, 26, 33, 82, 179, 309, 318</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>No. 334</td>
</tr>
<tr>
<td>Germanium ions</td>
<td>No. 227</td>
</tr>
<tr>
<td>Gibberellins</td>
<td>No. 305</td>
</tr>
<tr>
<td>Glycals</td>
<td>No. 152</td>
</tr>
<tr>
<td>Glycogenamidine</td>
<td>No. 147, 112, 230, 306, 320, 322, 335</td>
</tr>
<tr>
<td>Glycoalcohols</td>
<td>No. 315</td>
</tr>
<tr>
<td>Glycosides</td>
<td>No. 157, 199, 290, 292, 296</td>
</tr>
<tr>
<td>Gold ions</td>
<td>No. 45, 269</td>
</tr>
<tr>
<td>Halogen ions</td>
<td>No. 21</td>
</tr>
<tr>
<td>Halogen oxyacids</td>
<td>No. 128, 244</td>
</tr>
<tr>
<td>Heavy metal ions</td>
<td>No. 289</td>
</tr>
<tr>
<td>Hemlock alkaloids</td>
<td>No. 141, 189, 213, 302, 303, 307</td>
</tr>
<tr>
<td>Heterocyclic compounds</td>
<td>No. 69</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>No. 234</td>
</tr>
<tr>
<td>Hydrocarbons, aromatic</td>
<td>No. 146, 307</td>
</tr>
<tr>
<td>Hydrocarbons, chlorinated</td>
<td>No. 141, 275</td>
</tr>
<tr>
<td>Hydrocarbons see also sugars</td>
<td>No. 166</td>
</tr>
<tr>
<td>Hydroxamic acids</td>
<td>No. 284</td>
</tr>
<tr>
<td>Hydroxylamine</td>
<td>No. 164, 234</td>
</tr>
<tr>
<td>α-Hydroxy acids</td>
<td>No. 10</td>
</tr>
<tr>
<td>Hypnotics, bromine-containing</td>
<td>No. 108, 140</td>
</tr>
<tr>
<td>Indole and derivatives</td>
<td>No. 60, 71, 104, 107, 144, 168, 173, 221, 286, 331</td>
</tr>
<tr>
<td>Insecticides</td>
<td>No. 40, 108, 119, 190, 274</td>
</tr>
<tr>
<td>Iodine-containing compounds</td>
<td>No. 54, 170</td>
</tr>
<tr>
<td>Iron ions</td>
<td>No. 216, 236</td>
</tr>
<tr>
<td>Isotiocyanates</td>
<td>No. 240</td>
</tr>
<tr>
<td>Keto acids</td>
<td>No. 10, 93, 226</td>
</tr>
<tr>
<td>Ketones</td>
<td>Nr. 86</td>
</tr>
<tr>
<td>Ketones</td>
<td>No. 25, 99, 115</td>
</tr>
<tr>
<td>Ketones</td>
<td>No. 11, 177, 224, 248, 281</td>
</tr>
<tr>
<td>Lactones</td>
<td>No. 155</td>
</tr>
<tr>
<td>Lead ions</td>
<td>No. 121, 253, 265</td>
</tr>
<tr>
<td>Lipids</td>
<td>No. 48, 83, 90, 137, 193, 262, 324</td>
</tr>
<tr>
<td>Lipids</td>
<td>No. 50</td>
</tr>
<tr>
<td>Lithium ions</td>
<td>No. 31</td>
</tr>
<tr>
<td>Lysine</td>
<td>No. 328</td>
</tr>
<tr>
<td>Magnesium ions</td>
<td>No. 158, 255</td>
</tr>
<tr>
<td>Manganese ions</td>
<td>No. 35, 121, 253, 265</td>
</tr>
</tbody>
</table>
Mercaptans
Mercury ions
Metal ions
Methyl ketones
Methyl-sugars
Morphine

Narcotine
Nickel ions
Nicotinic acid
Nicotinamide
Nitro compounds, aromatic
Nitrogen compounds
Nitrogen compounds, aliphatic
Nitrosamines

Oils
Organic compounds
Ornithine
Oximes

Penicillins
Peroxides
Persulfates
Phenols

Phenol carboxylic esters
Phenol ethers
Phenothiazines
Phosphate esters
Phosphoric acids
Phthalate esters
Piperonal
Plasticisers
Plastoquinones
Polyalcohols
Polyethylene glycols and derivatives
Polyphenols
Polyphenyls
Polysaccharides
Potassium ions
Proazulenas
Proline

Purines
Pyridine compounds
Pyrimidines
Pyrones

Quinolines

Reducing compounds

Resins

Sapogenins
Serine
Sesamine
Silver ions
Sodium ions
Sorbic acid
Steroids

Steroid alkaloids
Steroid glucuronides
Steroid glycosides
Steroid sapogenins
Steroid sulfates
Sterols

Strontium ions
Sugars

Sugar phosphates
Sugars, reducing

Sulfides
Sulfonamides
Sulfonic acids
Sulfur-containing compounds

Terpenes
Terpene aldehydes
Tetraacyclines
Thiazoles
Thioacids

No. 10
No. 10
No. 178
No. 294
No. 110
No. 242

No. 69
No. 253, 265
No. 65
No. 65
No. 314
No. 131, 132, 133, 165, 189, 247
No. 292
No. 118, 304

No. 30
No. 68, 163
No. 328
No. 79

No. 161
No. 17, 109, 174, 245
No. 32

No. 4, 34, 37, 47, 88, 136, 143, 166, 191, 213, 214, 215, 240, 266, 302, 307, 316, 329

No. 213
No. 191
No. 145, 169
No. 72
No. 14, 16
No. 258

No. 151
No. 212, 258
No. 182
No. 9, 250, 279, 282, 283
No. 129, 165
No. 316
No. 55
No. 33, 319
No. 73, 299
No. 101
No. 328

No. 138, 272, 278
No. 46, 63, 80, 234
No. 138
No. 120
No. 39

No. 37, 193, 240, 249, 268, 277, 310, 323, 324
No. 30
No. 27, 154
No. 284
No. 69
No. 106, 121, 208
No. 333
No. 311

No. 1, 22, 27, 28, 67, 193, 220, 229, 233, 259, 282, 283, 305, 316, 318, 320, 321, 324, 327, 329, 332
No. 37, 145, 185, 219
No. 254
No. 27
No. 30, 57, 70, 145, 219, 332
No. 188
No. 1, 38, 67, 193, 203, 229, 305, 316, 324
No. 156, 158, 264
No. 5, 7, 19, 22, 33, 36, 52, 196, 197, 198, 200, 217, 222, 250, 267, 271, 312, 325

No. 15
No. 6, 18, 21, 23, 24, 113, 116, 223, 323
No. 161
No. 60, 87, 293
No. 233, 273
No. 49, 168, 218, 287

No. 22, 27, 30
No. 33
No. 8
No. 286
No. 270
<table>
<thead>
<tr>
<th>Solvents and reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
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<td>4013</td>
</tr>
</tbody>
</table>
8342 D(+)-Glucose (monohydrate)  
for biochemistry and microbiology  
500 g, 1 K, 2½ K, 50 K  

4191 Glyoxal bis-(2-hydroxyanil) GR  
25 g  

7341 Hexachloroplatinic (IV) acid solution 10%  
(abt. 3.9% Pt) GR  
5 ml, 25 ml  

4603 Hydrazinium sulfate GR  
100 g, 500 g  

316 Hydrochloric acid min. 25% (abt. 1.125) GR  
1 L, 2½ L, 60 K  

317 Hydrochloric acid fuming min. 37%  
(abt. 1.19) GR  
1 L, 2½ L, 35 K, 65 K  

9970 1 N Hydrochloric acid Titrisol®  
ampoule  

9971 0.5 N Hydrochloric acid Titrisol®  
ampoule  

9973 0.1 N Hydrochloric acid Titrisol®  
Hydroxychloric acid see Tetrahydrochloric acid  
Hydrogen peroxide GR see Peroxidol®  

800141 2-Hydroxy-3,5-dinitrobenzoic acid  
(monohydrate) for synthesis  
25 g, 100 g  

4616 Hydroxylammonium chloride GR  
100 g, 250 g, 1 K  

7098 8-Hydroxyquinoline GR  
50 g, 250 g  

4761 Iodine resublimed GR  
100 g, 500 g  

9099 0.1 N Iodine solution  
1 L  

3943 Iron(III) chloride GR  
250 g, 1 K, 50 K  

3965 Iron(II) sulfate GR  
500 g, 1 K, 5 K  

3954 Iron(II) sulfide fused small lumps  
1 K, 5 K  

807418 Lead(IV) acetate for synthesis  
100 g, 3.5 K  

7414 Lead(II) hydroxide acetate anhydrous,  
for Home analysis of glucose  
1 K, 5 K, 50 K  

7398 Lead(II) nitrate GR  
1 K  

6080 Lead(II, IV) oxide  
1 K, 50 K  

5691 Lithium hydroxide abt. 98% LiOH (LAB)  
100 g, 250 g, 1 K  

5694 Lithium sulfate GR  
250 g  

5819 Magnesium acetate GR  
250 g, 1 K  

4403 Mercury GR  
250 g, 1 K  

4419 Mercury(II) chloride GR  
50 g, 250 g, 1 K  

4420 Mercury(II) iodide extra pure DAB 6  
100 g, 1 K, 20 K  

4437 Mercury(II) nitrate GR  
50 g, 250 g  

4439 Mercury(II) nitrate 1-hydrate GR  
50 g, 250 g  

4461 Mercury(II) oxide yellow GR  
50 g, 250 g, 1 K  

6009 Methanol GR  
1 L, 2½ L, 20 K  

6012 Methanol dried (max. 0.01% H₂O) GR  
1 L, 2½ L  

822314 4-Methoxybenzaldehyde for synthesis  
250 ml, 1 L  

806225 4-Methoxy-2-nitroaniline for synthesis  
250 g  

1283 Methylene blue for microscopy  
25 g, 100 g  

Methylene chloride see Dichloromethane  

6076 Methyl red indicator  
25 g, 100 g  

3055 Methyl yellow indicator  
10 g, 50 g  

532 Molybdatephosphoric acid GR  
25 g, 100 g  

531 Molybdatephosphoric acid spray reagent 3.5%  
for chromatography  

spray can (abt. 260 ml)  

6098 Morin GR  
5 g  

6252 1.3-Naphthalenediol GR  
1 g, 5 g  

6223 1-Naphthol GR  
50 g, 250 g  

6531 1.2-Naphthoquinone-4-sulfonic acid sodium  
salt GR  
5 g, 25 g  

Naphthoresorcinol see 1.3-Naphthalenediol  

6245 1-Naphthylamine GR  
100 g, 500 g  

6237 N-(1-Naphthyl)-ethylene diammonium  
dichloride GR  
5 g, 25 g  

6746 Neat® for thin layer chromatography  
1 L  

6762 Ninhydrin GR  
10 g, 100 g, 1 K  

6758 Ninhydrin spray reagent 0.1% for  
chromatography  

spray can (abt. 260 ml)  

456 Nitric acid min. 65% (about 1.40)  
tested by the dibizone method GR  
1 L, 2½ L, 30 K  

455 Nitric acid fuming 100% (abt. 1.52) GR  
1 L, 30 K  

6760 4-Nitroaniline for determination of phenol  
50 g  

Orcinol cryst. see 3.5-Dihydroxytoluene  
(monohydrate) for synthesis  

Orthophosphoric acid see ortho-Phosphoric acid  

807110 Palladium(II) chloride (59% Pd) anhydrous  
1 g, 5 g, 10 g  

4005 Paraformaldehyde extra pure  
1 K, 5 K, 50 K  

518 Perchloric acid abt. 60% (abt. 1.53) GR  
1 L, 2½ L, 35 K  

519 Perchloric acid abt. 70% (abt. 1.67) GR  
1 L, 2½ L, 35 K  

7209 Perhydrol® (30% H₂O₂) GR  
250 ml, 1 L  

1770 Petroleum benzene boiling range 100–140°C  
extra pure  
1 L, 5 L, 40 K  

1775 Petroleum benzene GR boiling range 40–60°C  
1 L, 5 L, 18 K  

206 Phenol GR  
250 g, 1 K  

7293 1-Phenyl-2,3-dimethyl-4-amino- 
5-pyrazolone GR  
10 g, 100 g  

809721 1.2-Phenylenediamine for synthesis  
100 g, 250 g, 1 K  

7244 1.3-Phenylenediammonium dichloride GR  
50 g  

822297 1.4-Phenylenediammonium dichloride  
for synthesis  
250 g  

7252 Phenylfluorone  
1 g, 5 g  

7251 Phenylhydrazine GR  
100 ml, 1 L  

114
Phloretin (dihydrone) GR ................................ 25 g, 100 g
Phosphomolybdic acid see Molybdate-
phosphoric acid

8073 25 g, 100 g

4875 1/15 M Potassium dihydrogen phosphate
solution 1 L

4984 Potassium hexacyanoferrate(II) GR ........... 500 g
4973 Potassium hexacyanoferrate(III) GR ........... 250 g, 1 K, 50 K
5033 Potassium hydroxide pellets GR ................... 500 g, 1 K, 5 K, 50 K
9918 1 N Potassium hydroxide solution Titrisol®
ampoule
9351 0.5 M Potassium hydroxide solution
in methanol ........................................... 1 L
5043 Potassium iodide neutral GR ....................... 250 g, 1 K, 2 1/2 K
5082 Potassium permanganate GR ...................... 250 g, 1 K
9121 0.1 N Potassium permanganate solution ....... 1 L
9634 2-Propanol GR .................................... 1 L, 2 1/2 L, 20 K
9728 Pyridine GR ........................................ 500 ml, 1 L, 2 1/2 L
7531 1-(2-Pyridylazo)-2-naphthol (PAN) metal (pM)
indicator ............................................. 1 g, 5 g
7546 Quercetin cryst. (LAB) ............................... 10 g
7546 Quinazolin see 1,2,5,8-Tetrahydroxyanthra-
quinone
p-Quinone see p-Benzoquinone
7593 Resorcinol GR ..................................... 100 g, 250 g
7599 Rhodamine B GR and for microscopy ....... 25 g, 100 g
7596 Rhodamine B spray reagent (0.1%) ....... spray can (abt. 260 ml)
6595 Rhodizonic acid disodium salt GR .......... 5 g
629 Rubenic acid GR .................................... 20 g, 100 g
1512 Silver nitrate GR .................................. 25 g, 100 g, 250 g, 1 K
9990 0.1 N Silver nitrate solution Titrisol® ....... 1 ampoule
6261 Sodium GR ........................................ 250 g, 1 K
6268 Sodium acetate anhydrous GR ................. 250 g, 1 K, 5 K, 50 K
6688 Sodium azide extra pure ......................... 10 g, 250 g, 1 K, 50 K
6391 Sodium carbonate 10-hydrate cryst. GR .... 1 K, 5 K, 50 K

Sodium carbonate anhydrous GR ................. 500 g, 1 K, 5 K, 50 K
6404 Sodium chloride cryst. GR .......................... 500 g, 1 K, 5 K, 50 K
6448 tri-Sodium citrate 2-hydrate GR ............... 500 g, 1 K, 5 K
6336 Sodium dichromate GR ............................ 250 g, 1 K
6346 Sodium dihydrogen phosphate 1-hydrate GR 500 g, 1 K
6507 Sodium dithionate (LAB) ........................ 500 g, 2 1/2 K
6329 Sodium hydrogen carbonate GR ............... 500 g, 1 K, 5 K, 50 K
6587 1/15 M di-Sodium hydrogen phosphate solution ........... 1 L
6495 Sodium hydroxide pellets GR .................... 500 g, 1 K, 5 K, 50 K
5588 Sodium hydroxide solution 10% (1.11) GR .... 1 L
5591 Sodium hydroxide solution min. 27% (1.30) GR 2 1/2 L, 60 K
9961 0.01 N Sodium hydroxide solution Titrisol®
ampoule
9956 1 N Sodium hydroxide solution Titrisol®
ampoule
9957 0.5 N Sodium hydroxide solution Titrisol®
ampoule
9959 0.1 N Sodium hydroxide solution Titrisol®
ampoule
6523 Sodium iodide GR ................................ 100 g, 250 g, 1 K
6287 Sodium meta-arsenate GR ....................... 250 g
6597 Sodium meta-periodate .......................... 50 g, 250 g, 1 K
6521 Sodium molybdate GR ............................. 100 g, 250 g, 1 K
6549 Sodium nitride cryst. GR ......................... 500 g
6541 Sodium nitroprusside GR ......................... 25 g, 100 g, 500 g
6551 Sodium peroxide powder GR ..................... 100 g
6688 Sodium peroxide GR .............................. 250 g, 1 K
6308 di-Sodium tetraborate 10-hydrate cryst. GR .... 500 g, 1 K, 50 K
6516 Sodium thiosulfate 5-hydrate GR ............... 500 g, 1 K, 5 K
6673 Sodium tungstate GR ............................. 100 g, 250 g, 1 K, 50 K

Standard acetate buffer solution
see Acetate buffer solution pH 4.66

1252 Starch soluble GR .................................. 100 g, 250 g, 1 K
8035 Sulfanilamide extra pure ....................... 1 K, 50 K
686 Sulfanilic acid GR .................................. 100 g, 250 g
7996 Sulfadiazine (sulfur paraaffin grained) ........ 500 g
691 5-Sulfosalicylic acid (dihydrate) GR .......... 100 g, 250 g, 1 K, 50 K
731 Sulfuric acid 95–97% (abt. 1.84) GR .... 1 L, 2 1/2 L, 40 K
9981 1 N Sulfuric acid Titrisol® ....................... ampoule
9984 0.1 N Sulfuric acid Titrisol® .................... ampoule
9982 0.01 N Sulfuric acid Titrisol® .................... ampoule
804 L(+)Tartaric acid GR ............................. 250 g, 1 K, 5 K, 50 K
808240 Tetracyanoethylene for synthesis ....... 10 g
171 Tetrafluoroboric acid
(fluroboric acid) about 35% ........................ 2 1/2 L, 60 K
1.2.5.8-Tetrahydroxyanthraquinone (quinalizarin) GR ............................... 50 g
8103 Tetrazolium blue for microscopy ................................................. 1 g, 5 g
8180 2-Thiobarbituric acid reagent for sorbic acid ................................. 25 g
8167 Thymol cryst. extra pure ............................................................ 100 g, 1 K
8176 Thymol blue indicator .................................................................. 5 g, 25 g
7815 Tin(II) chloride GR ....................................................................... 100 g, 250 g, 1 K
7811 Tin(IV) chloride fuming GR ......................................................... 100 ml
1307 Titan yellow GR .......................................................................... 50 g
9613 4-Toluenesulfonic acid (monohydrate) GR ......................................... 100 g, 500 g
1273 Toluidine blue O for microscopy ................................................... 50 g
8072 Trichloroacetic acid GR ................................................................. 100 g, 250 g, 1 K
808260 Trifluoroacetic acid for synthesis ................................................ 25 ml, 100 ml, 1 l
2635 2,4,6-Trimethylpyridine GR ........................................................... 50 ml, 250 ml
8380 2,3,5-Triphenyltetrazolium chloride ............................................. 10 g, 100 g
583 Tungstophosphoric acid GR ............................................................. 100 g, 250 g
8473 Uranyl acetate GR ........................................................................ 25 g, 100 g, 500 g
8487 Urea GR ......................................................................................... 500 g, 1 K, 5 K, 50 K
8510 Vanillin ........................................................................................ 100 g, 1 K
8696 Xanthydrol abt. 10% in methanol .................................................... 10 ml
8789 Zinc powder GR ........................................................................... 500 g, 1 K
8802 Zinc acetate GR .............................................................................. 250 g, 1 K
8816 Zinc chloride dry GR ....................................................................... 250 g, 1 K
8917 Zirconium(IV) oxide chloride GR .................................................. 25 g, 100 g