Dyeing Reagents for Thin-Layer and Paper Chromatography

Contents

Introduction

The first collection of instructions for the preparation of staining reagents was published by K.G. Krebs, D. Heusser, and H. Wimmer in Egon Stahl's "Handbook of Thin-Layer Chromatography" in the sixties and lateron in a revised form repeatedly as a brochure by E. Merck Darmstadt, Germany until 1980 when the last unchanged edition was released.

About 20 years later two comprehensive books on staining reagents were published by H. Jork, W. Funk, W. Fischer, and H. Wimmer "Thin-Layer Chromatography – Reagents and Detection Methods, Vols. 1a and 1b" (VCH Weinheim, ISBN 3-527-27834-6 and ISBN 3-527-28205-X) - somehow as a replacement for the antiquated staining reagents brochure. Both volumes are recommended to any thin-layer chromatographer because they show for each reagent at least one approved example and furthermore they offer plenty of information not only on instructions for preparation and handling but also on reaction mechanisms, coloration of derivates, limits of detection etc. etc.

As still numerous chromatographers have been frequently asking for the cancelled dyeing reagents booklet its text was completely revised - mainly concerning ordering numbers, misprintings and other errors. For easy accessibility the resulting new list below is now being presented in the internet. An alphabetical index of compounds and compound classes for which a detection reagent is being sought is given at the beginning followed by the staining reagents listed in alphabetical order. Reagents for paper chromatography are additionally marked with "PC".

Staining of Thin-layer Chromatograms

Spraying: Dry the chromatogram to remove the solvent, then cool. Place it vertically into a spraying box or into a fume cupboard and protect the surroundings by covering with filter paper or the like. Apply the spray solution from about 30 cm until the layer is evenly wetted but not for so long that the chromatogram begins to run with liquid. In most cases the chromatogram is specially treated at this stage. For details please refer to the directions quoted for the particular reagent concerned. Unless otherwise stated, subsequent treatment should be taken as meaning drying at room temperature.

Dipping: In case of quantitative evaluation dipping of the chromatogram into the staining solution is becoming ever more usual with respect to precision and repeatability. In general less concentrated reagent solutions prepared with less polar solvents are common for dipping purposes. Care should be taken in the choice of solvent to ensure that neither the chromatographically separated substances nor their reaction products are soluble in the solvent of the dipping reagent.

Ready-to-use Spray Solutions

Merck sells a number of ready-to-use spray solutions in 100 ml glass bottles which can directly be connected to the rechargeable electro-pneumatically operated TLC sprayer (Ord. No. 1.08540):

Aniline phthalate	Ord. No. 1.01269.0100
Bromocresol green	Ord. No. 1.01994.0100
2',7'-Dichlorofluorescein	Ord. No. 1.09219.0100
4-(Dimethylamino)-benzaldehyde	Ord. No. 1.03722.0100
Dragendorff-reagent	Ord. No. 1.02035.0100
Molybdatophosphoric acid	Ord. No. 1.00480.0100
Ninhydrin	Ord. No. 1.06705.0100
Rhodamine B	Ord. No. 1.07602.0100

After-treatment

Frequently, optimum color development after reagent application by spraying or dipping is only obtained by heating. A plate heater or an adjustable drying oven is generally used for this purpose. Occasionally, in the case of fluorescing chromatogram zones an additional treatment with solutions of liquid paraffin, polyethylene glycols, and other viscous liquids can lead to a stabilization and also to a tremendous enhancement of the fluorescence intensity of compounds.

Paper Chromatograms

For the detection of paper chromatograms see:

I.M. Hais, K. Macek, Paper Chromatography, Publishing House Czechoslovak Academy of Sciences, Prague, and Academic Press, New York and London, 1963.

F. Cramer, Papier-Chromatographie, Verlag Chemie, Weinheim, 5th Ed., 1962.

See also the relevant chapters in handbooks on the subject, e.g.: E. Stahl, Thin-Layer Chromatography, Springer and Academic Press, New York and London, 2nd Ed., 1969.

K. Randerath, Thin-Layer Chromatography, Verlag Chemie and Academic Press, New York and London, 2nd Ed., 1966.

Index of Compounds and Compound Classes and Appropriate Detection Reagents

Zinc ions No. 121, 253

Reagents

1. Acetic anhydride - sulfuric acid for Δ^5-3-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.

After-treatment: Heat 10 min at 110°C. Characteristic fluorescence in long-wave UV light.

Literature:
C. Michalec, Biochim. et biophys. Acta **19**, 187 (1956).
R. Tscheche, J. Chromatog. **5**, 217 (1961).
K. Takeda, S. Hara, A. Wada, N. Matsumoto, J. Chromatog. **11**, 562 (1963).

Chemicals:
Acetic anhydride GR ACS, ISO, Ord. No. 1.00042
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

2. Alizarin for Cations.

Spray solution: Saturated ethanolic alizarin solution.

After-treatment: Place the moist chromatogram into a chamber saturated with ammonia vapours.

Literature:
G. de Vries, G.P. Schuetze, E. van Dalen, J. Chromatog. **13**, 119 (1964).

Chemicals:
Alizarin indicator (C.I. 58000)
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

3. Aluminium chloride for flavonoids.

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

Literature:
T.G. Gage, C.D. Douglas, S.H. Wender, Anal. Chem. **23**, 1582 (1951.

Chemicals:
Aluminium chloride hexahydrate extra pure Ph Eur, USP, Ord. No. 1.01084
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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

Spray solution I: 2% ethanolic 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 vapours.

Literature:
G. Gabel, K.H. Mueller, J. Schoknecht, Dtsch. Apoth. Ztg. **102**, 293 (1962).

Chemicals:
4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one GR, Ord. No. 1.07293
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Ammonia solution 25% GR, Ord. No. 1.05432

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.

After-treatment: Heat 15-20 min at 110°C. Sugars show brown spots.

Literature:
T.E. Timell, C.P.J. Glandemanns, Anal. Chem. **28**, 1916 (1956).

Chemicals:
o-Aminodiphenyl
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
Ethanol abs. GR, Ord. No. 1.00972

6. 4-Aminohippuric acid for reducing sugars.

Spray solution: 0.3% ethanolic 4-aminohippuric acid solution.

After-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, Ord. No. 1.00084
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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% phosphoric acid to the solution.

Literature:
L. Vigyáz-Vámos, Magyar Kém. Folyôrat **59**, 183 (1953).
S. Hirase, C. Araki, S. Nakanishi, Bull. Chem. Soc. (Japan) **26**, 183 (1953).

Chemicals:
2-Aminophenol, Ord. No. 8.00419
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

8. Ammonia for tetracyclines.

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

Literature:
M. Urx, J. Vondrácková, L. Kovarík, O. Horský, M. Herold, J. Chromatog. **11**, 62 (1963).

Chemicals:
Ammonia solution 25% GR, Ord. No. 1.05432

9. Ammonium cerium(IV) nitrate – N,N-dimethyl-1,4-phenylenediammonium dichloride for polyalcohols.

Solution a: 1% solution of ammonium cerium(IV) nitrate in 0.2 N nitric acid.
Solution b: Dissolve 1.5 g N,N-dimethyl-1,4-phenylenediammonium dichloride 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.

After-treatment: Heat 10 min at 105°C. Yellowish green spots on red background.

Literature:
E. Knappe, D. Peteri, J. Rohdewald, Z. anal. Chem. **199**, 270 (1964).

Chemicals:
Ammonium cerium(lV) nitrate GR ACS, Ord. No. 1.02276
N,N-dimethyl-1,4-phenylenediammonium dichloride GR, Ord. No. 1.03067
Methanol GR ACS, ISO, Ord. No. 1.06009
Acetic acid 96% GR, Ord. No. 1.00062
Nitric acid 65% GR ISO, Ord. No. 1.00456

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.

Literature:
M. Trop, M. Sprecher, A. Pinsky, J. Chromatog. **32**, 426 (1968).

Chemicals:
Ammonium cerium(lV) nitrate GR ACS, Ord. No. 1.02276
Nitric acid 65% GR, Ord. No. 1.00456

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

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

Literature:
I.M. Jakovljevic, L. D. Seay, R. W. Shaffer, J. Pharm. Sci. **53**, 553 (1964).

Chemicals:
Ammonium cerium(lV) sulfate dihydrate GR, Ord. No. 1.02273
ortho-Phosphoric acid. 85% GR ISO, Ord. No. 1.00573

12. Ammonium iron(III) sulfate for flavonoids.

Spray solution: 0.2% aqueous solution of ammonium iron(III) sulfate.

Literature:
E.A.H. Roberts, D.J. Wood, Biochem. J. **49**, 414 (1951).

Chemicals:
Ammonium iron(III) sulfate dodecahydrate GR ACS, ISO, Ord. No. 1.03776

13. Ammonium iron(III) sulfate vor Vinca alkaloids.

Spray solution: Dissolve 1 g ammonium iron(III) sulfate in 100 ml phosphoric acid (75 or 85%). Spray the reagent on to heated chromatogram (100°C).

Literature:
I.M. Jakovljevic, L.D. Seay, R.W. Shaffer, J. Pharm. Sci. **53**, 553 (1964).

Chemicals:
Ammonium iron(III) sulfate dodecahydrate GR ACS, ISO, Ord. No. 1.03776
ortho-Phosphoric acid. 85% GR ISO, Ord. No. 1.00573

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 = turquois spots on colourless background

Literature:
F. Jungnickel, J. Chromatog. **31**, 617 (1967).

Chemicals:
Ammonium heptamolybdate tetrahydrate GR ACS, ISO, Ord. No. 1.01182
Crystal violet (C.I. 42555) indicator ACS, Ord. No. 1.01408
Brilliant green (C:I: 42040), Ord. No. 1.01310
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Acetone GR ACS, ISO, Ord. No. 1.00014

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.

After-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.

Literature:
C.S. Hanes, F.A. Isherwood, Nature **164**, 1107 (1949).
T.H. Bevan, G.I. Gregory, T. Malkin, A.G. Poole, J. Chem. Soc. **1951**, 841.
S. Burrows, F.S.M. Grylls, J.S. Harrison, Nature **170**, 800 (1952).
C.W. Stanley, J. Chromatog. **16**, 467 (1964).

Chemicals:
Ammonium heptamolybdate tetrahydrate GR ACS, ISO, Ord. No. 1.01182
Hydrochloric acid 25% GR, Ord. No. 1.00316
Perchloric acid 70-72% GR ACS, Ord. No. 1.00519
Acetone GR ACS, ISO, Ord. No. 1.00014

16. Ammonium molybdate - tin(II) chloride for phosphoric acids.

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. Heat, if necessary, at 105°C for 3 - 5 minutes.

Literature:
H. Seiler, Helv. Chim. Acta **44**, 1753 (1961).

Chemicals:
Ammonium heptamolybdate tetrahydrate GR ACS, ISO, Ord. No. 1.01182
Tin(II) chloride dihydrate GR ACS, Ord. No. 1.07815
Hydrochloric acid 25% GR, Ord. No. 1.00316

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.

Literature:
M.H. Abraham, A.G. Davies, D.R. Llewellyn, E.M. Thain, Anal. Chim. Acta **17**, 499 (1957).

Chemicals:
Ammonium thiocyanate GR ACS, ISO, Ord. No. 1.01213
Iron(II) sulfate heptahydrate GR ACS, ISO, Ord. No. 1.039651.01213
Acetone GR ACS, ISO, Ord. No. 1.00014

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.

After-treatment: Heat 10 min at 85°C. Characteristic colours: 1,4-aldohexose oligosaccharides turn blue.

Literature:
R.W. Bailey, E.J. Bourne, J. Chromatog. **4**, 206 (1960).
J.L. Buchan, R.J. Savage, Analyst **77**, 401 (1952).
S. Schwimmer, A. Bevenne, Science **123**, 543 (1956).

Chemicals:
Aniline GR, Ord. No. 1.01261
Diphenylamine GR and redox indicator, Ord. No. 1.03086
Acetone GR ACS, ISO, Ord. No. 1.00014
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

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.

After-treatment: Heat the chromatogram 10 min at 105°C.

Literature:
I.L. Bryson, T.I. Mitchell, Nature **167**, 864 (1951).

Chemicals:
Aniline GR, Ord. No. 1.01261
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
1-Butanol GR ACS, ISO, Ord. No. 1.01990

20. Aniline phthalate.

100 ml ready to use spray solution for chromatography (c = ca. 3.2% in 2-propanol/methanol).

After-treatment: Heat the chromatogram 10 min. at 105°C.

Ord. No. 1.01269

21. Aniline phthalate for reducing sugars and anions of halogen oxy-acids.

Spray solution: Dissolve 0.93 g aniline and 1.66 g phthalic acid in 100 ml 1-butanol saturated with water.

After-treatment: Heat 10 min at 105°C.

Literature:
S.M. Partridge, Nature **164**, 443 (1965).
W. Peschke, J. Chromatog. **20**, 572 (1965).

Chemicals:
Aniline GR, Ord. No. 1.01261
Phthalic acid GR, Ord. No. 1.09611
1-Butanol GR ACS, ISO, Ord. No. 1.01990

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.

After-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.

After-treatment: Heat the sprayed chromatogram 5-10 min at 90-100°C.

Literature:
E. Stahl, U. Kaltenbach, J. Chromatog. **5**, 351 (1961).
B.P. Lisboa, J. Chromatog. **16**, 136 (1964).

Chemicals:
4-Methoxybenzaldehyde (anisaldehyde) Reag. Ph Eur, Ord. No. 1.59608
Acetic acid 96% GR, Ord. No. 1.00062
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 l-butanol and shake well after addition of 1 g sodium dithionite.

After-treatment: Heat 10 min at 130°C.

Literature:
R.C. Bean, G.G. Portwe, Anal. Chem. **31**, 1929 (1959).
L. Hough, J.K.N. Jones, W.H. Wadman, J. Chem. Soc. **1950**, 1702.

Chemicals:
p-Anisidinium chloride, Ord. No. 8.20103
Sodium dithionite LAB, Ord. No. 1.06507

Methanol GR ACS, ISO, Ord. No. 1.06009
1-Butanol GR ACS, ISO, Ord. No. 1.01990

24. p-Anisidine phthalate for reducing sugars.

Spray solution: 0.1 M solution of *p*-anisidine and phthalic acid in 96% ethanol.

After-treatment: Heat 10 min at 100°C.

Chemicals:
p-Anisidine, Ord. No. 8.00458
Phthalic acid GR, Ord. No. 1.09611
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

After-treatment: Heat 5-6 min at 110°C. Ketoses and oligosaccharides containing ketoses show yellow spots.

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

Chemicals:
Anthrone for synthesis, Ord. No. 8.01461
Acetic acid 96% GR, Ord. No. 1.00062
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

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

Chemicals:
Antimony(III) chloride GR, Ord. No. 1.07838
Chloroform GR ISO, Ord. No. 1.02445

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.

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

Literature:
E. Stahl, Chemiker-Ztg. **82**, 323 (1958).
K. Takeda, S. Hara, A. Wada, N. Matsumoto, J. Chromatog. **11**, 562 (1963).

Chemicals:
Antimony(III) chloride GR, Ord. No. 1.07838
Chloroform GR ISO, Ord. No. 1.02445
Carbon tetrachloride GR, Ord. No. 1.02222

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.

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

Literature:
H.P. Kaufmann, A.K. sen Gupta, Chem. Ber. **97**, 2652 (1964).

Chemicals:
Antimony(III) chloride GR, Ord. No. 1.07838
Acetic acid 96% GR, Ord. No. 1.00062
Chloroform GR ISO, Ord. No. 1.02445

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.

After-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. Colours from yellow to green.

Literature:
W.L. Anthony, W.T. Behr, J. Chromatog. **13**, 567 (1964).

Chemicals:
Antimony(III) chloride GR, Ord. No. 1.07838
1-Butanol GR ACS, ISO, Ord. No. 1.01990

Acetic acid 96% GR, Ord. No. 1.00062
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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.

After-treatment: Heat the chromatogram until the spots appear. Inspect in long-wave UV light.

Literature:
J.M. MacMahon, R.B. Davis, G. Kalnitzky, J. Am. Chem. Soc. 74, 4483 (1952).
E. Stahl, Chemiker-Ztg. **82**, 323 (1958).
R. Ikan, J. Kashman, E.D. Bergmann, J. Chromatog. **14**, 275 (1964).
H.G. Henkel, W. Ebing, J. Chromatog. **14**, 285 (1964).

Chemicals:
Antimony(V) chloride GR, Ord. No. 1.07837
Carbon tetrachloride GR, Ord. No. 1.02222
Chloroform GR ISO, Ord. No. 1.02445

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.

After-treatment: Place the chromatogram into a chamber saturated with ammonia vapours.

Literature:
G.P. Heisig, F.H. Pollard, Anal. Chim. Acta **16**, 234 (1957).

Chemicals:
Aurin tricarboxylic acid ammonium salt GR (reagent for aluminium) ACS, Ord. No. 1.00128
Ammonium acetate GR ACS, Ord. No. 1.01116
Ammonia solution 25% GR, Ord. No. 1.05432

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.
Caution: Benzidine is carcinogenic!

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

Chemicals:
Benzidine
Acetic acid 96% GR, Ord. No. 1.00062

33. Benzidine for terpene aldehydes, flavonoids, carbohydrates.

Spray solution: Dissolve 0.5 g benzidine in 20 ml glacial acetic acid and 80 ml ethanol. **Caution: Benzidine is carcinogenic!**

After-treatment: Heat 15 min at 100°C. Spraying with dilute hydrochloric acid after heating intensifies the colour of the spots of some substances.

Literature:
J.KN. Jones, J.B. Pridham, Biochem. J. **58**, 288 (1954).

Chemicals:
Benzidine
Acetic acid 96% GR, Ord. No. 1.00062
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid 25% GR, Ord. No. 1.00316

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. **Caution: Benzidine is carcinogenic!**

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 constant stirring.

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

Literature:
J. Sherma, L.V.S. Hood, J. Chromatog. **17**, 307 (1965).

Chemicals:
Benzidine
Sodium nitrite GR ACS, Ord. No. 1.06549
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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.
Caution: Benzidine is carcinogenic!

Procedure: Spray consecutively with I and II.

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

Chemicals:
Sodium peroxide granular GR ACS, Ord. No. 1.06563
Benzidine
Acetic acid 96% GR, Ord. No. 1.00062

36. Benzidine - trichloroacetic acid for sugars.

Spray solution: Dissolve 0.5 g bcnzidine in 10 ml glacial acetic acid, add 10 ml 40% aqueous trichloroacetic acid and fill up to 100 ml with ethanol.
Caution: Benzidine is carcinogenic!

After-treatment: Irradiate the chromatogram 1.5 min with UV light. Sugars show greyish-brown to deep reed-brown spots.

Literature:
J.S.D. Bacon, J. Edelmann, Biochem. J. **48**, 114 (1951).
G. Harris, I.C. Macwilliam, Chem. & Ind. (London) **1954**, 254.

Chemicals:
Benzidine
Trichloroacetic acid GR ACS, Ord. No. 1.00807
Acetic acid 96% GR, Ord. No. 1.00062
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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).
R. Strohecker, H.M. Henning, Vitaminbestimmungen, Verlag Chemie Weinheim 1963, p. 311.

Chemicals:
2,2'-Bipyridine GR, Ord. No. 1.03098
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

38. Bismuth chloride for sterols.

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

After-treatment: Heat at 110°C until maximal fluorescence of the spots in long-wave UV light.

Literature:
J.W. Copius-Peereboom, Thin Layer Chromatography, Ed. G.B. Marini-Bettolo, Elsevier Amsterdam, 1964, p. 199.

Chemicals:
Bismuth(III) chloride LAB, Ord. No. 1.12403
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

39. Boric acid - citric acid for quinolines.

Spray solution: Dissolve 0.5 g boric acid and 0.5 g citric acid in 20 ml methanol.

After-treatment: Heat at 100°C. Inspect in UV light.

Literature:
R. Neher, A. Wettstein, Helv. Chim. Acta **35**, 276 (1952).

Chemicals:
Boric acid cryst. GR ACS, ISO, Ord. No. 1.00165
Citric acid monohydrate GR ACS, ISO, Ord. No. 1.00244
Methanol GR ACS, ISO, Ord. No. 1.06009

40. Bromine - fluorescein - silver nitrate for insecticides.

Spray solution: Fill up 1 ml of a 0.25% solution of fluorescein in N,N-dimethyl-formamide 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 s 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.

Literature:
K.C. Walker, M. Beroza, J. Assoc. Off. Agr. Chemists **46**, 250 (1963).

Chemicals:
Bromine GR ISO, Ord. No. 1.01948

Fluorescein (C.I. 45350)
N,N-Dimethylformamide GR ISO, Ord. No. 1.03053
Ethylene glycol monophenyl ether for synthesis, Ord. No. 8.07291
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Acetone GR ACS, ISO, Ord. No. 1.00014

41. Bromocresol green.

100 ml ready to use spray solution for chromatography (c = 0.1% in 2-propanol).

Ord. No. 1.01994

42. Bromocresol green - indicator reagent.

Spray solution: Dissolve 0.04 g bromocresol green in 100 ml ethanol. Add sodium hydroxide solution (c = 0.1 mol/L) until blue colour appears.

Literature:
F. Bryant, B.T. 0verell, Biochim. et biophys. Acta **10**, 471 (1953).

Chemicals:
Bromocresol green indicator pH 3.8-5.4 ACS, Ord. No. 1.08121
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sodium hydroxide solution 0.1 mol/l Titrisol® Ord. No. 1.09959

43. Bromocresol green - bromophenol blue - potassium permanganate for organic acids.

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.

Literature:
J. Pásková, V.J. Munk, J. Chromatog. **4**, 241 (1960).

Chemicals:
Bromocresol green indicator pH 3.8-5.4 ACS, Ord. No. 1.08121
Bromophenol blue indicator pH 3.0-4.6 ACS, Ord. No. 1.08122
Potassium permanganate GR ACS, Ord. No. 1.05082
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 sodium hydroxide solution (c = 0.1 mol/L, glass electrode).

Procedure: Develop the chromatogram with the eluent di-iso-propyl ether - formic acid -water (90+7+3) and heat subsequently 10 min at 100°C. Spray after cooling to room temperature. Yellow spots on blue background.

Literature:
E. Knappe, D. Peteri, Z. anal. Chem. **188**, 184 (1962).

Chemicals:
Bromocresol purple indicator, Ord. No. 1.03025
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sodium hydroxide solution 0.1 mol/l Titrisol®, Ord. No. 1.09959

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.
Literature:
H. Seiler, T. Kaffenberger, Helv. Chim. Acta **44**, 1282 (1961).

Chemicals:
Bromocresol purple indicator, Ord. No. 1.03025
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

46. Bromocyan - 4-aminobenzoic acid (reagent acc. to Koenig) for tertiary pyridine compounds with at least one free α-position.

Primary treatment: Before spraying place the chromatogram for 1 hour into a chamber with a solution of bromocyan (**Caution, very poisonous!**). For preparation of the bromocyan solution add 10% aqueous solution of sodium cyanide to saturated bromine water, cooled in ice, until the colour of bromine has disappeared.

Spray solution: Dissolve 2 g 4-aminobenzoic acid in 75 ml 0.75 N hydrochloric acid and fill up the solution to 100 ml with ethanol.

Literature:
E. Kodicek, K.K. Reddi, Nature **168**, 475 (1951).

Chemicals:
Bromine GR ISO, Ord. No. 1.01948.
Sodium cyanide pure, Ord. No. 1.06437
4-Aminobenzoic acid extra pure USP, Ord. No. 1.00102
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

Variation: Mixture of equal parts of a 2% ethanolic 4-aminobenzoic acid solution and phosphate buffer (c = 0.1 mol/L, pH 7.0).

Procedure: After spraying dry the chromatogram 15 min at room temperature and place subsequently into a chamber with some crystals of bromocyan.

Literature:
E. Hodgson, E. Smith, F.E. Guthrie, J. Chromatog. **20**, 176 (1965).

Chemicals:
Bromocyan
4-Aminobenzoic acid extra pure USP, Ord. No. 1.00102
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Buffer Solution concentrated Titrisol pH 7.0 (phosphate), Ord. No. 1.09887

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 reagent No 303: Sulfanilic acid diazotised.

Procedure: Spray the chromatogram consecutively with I and II.

Literature:
J.W. Copius-Peereboom, H.W. Beekes, J. Chromatog. **14**, 417 (1964).

Chemicals:
Bromophenol blue indicator pH 3.0-4.6 ACS, Ord. No. 1.08122
Methyl red (C.I. 13020) indicator ACS, Ord. No. 1.06076
Potassium dihydrogen phosphate solution 1/15, mol/l, Ord. No. 1.04875
di-Sodium hydrogen phosphate solution 1/15 mol/l, Ord. No. 1.06587
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

48. Bromosuccinimide - fluorescein for lipids.

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.

Literature:
A. Popov, V. Gadeva, J. Chromatog. **16**, 256 (1964).
J. Micev, A. Popov, L. Nedelceva, J. Chromatog. **24**, 432 (1966).

Chemicals:
N-Bromosuccinimide, Ord. No. 8.01949
Fluorescein (C.I. 45350)
Acetic acid 96% GR, Ord. No. 1.00062
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

49. Bromosuccinimide - fluorescein for sulfur containing compounds.

Spray solution I: 0.035% solution of N-bromosuccinimide in 1,1,1-trichloro-ethane.

Spray solution II: Fill up 3 ml 0.33% solution of fluorescein in sodium hydroxide solution (c = 0.1 mol/L) to 100 ml with ethanol.

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

Literature:
J.W. Cook, J. Assoc. Off. Agr. Chemists **37**, 983 (1954).

Chemicals:
N-Bromosuccinimide for synthesis, Ord. No. 8.01949
Fluorescein (C.I. 45350)
Sodium hydroxide solution 0.1 mol/l Titrisol®, Ord. No. 1.09959
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1,1,1-Trichloroethane for synthesis, Ord. No. 8.18753

50. Bromothymol blue for lipoids.

Spray solution: Dissolve 0.04 g bromothymol blue in 100 ml sodium hydroxide solution (c = 0.01 mol/L).

Literature:
H. Jatzkewitz, E. Mehl, Hoppe-Seylers Z. physiol. Chem. **320**, 251 (1960).

Chemicals:
Bromothymol blue indicator ACS, Ord. No. 1.03026
Sodium hydroxide solution 0.01 mol/l Titrisol®, Ord. No. 1.09961

51. Cacotheline for vitamin C.

Spray solution: 2% aqueous cacotheline solution.

After-treatment: Heat at 110°C. Violet spots.

Literature:
B. Tegethoff, Z. Naturforsch. **8b**, 374 (1953).

Chemicals:
Cacotheline

52. Carbazole - sulfuric acid for sugars.

Spray solution: Dissolve 0.5 g carbazole in 95 ml ethanol and add 5 ml 97% sulfuric acid. Prepare freshly before use.

After-treatment: Heat 10 min at 120°C. Violet spots on blue background.

Chemicals:
Carbazole for synthesis, Ord. No. 8.20255
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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.

Literature:
J.F. Heremans, J.P. Vaerman, Clin. Chim. Acta **3**, 430 (1958).
D. Hamerman, Science **122**, 924 (1955).

Chemicals:
Carmine (C.I. 75470)
Aluminium chloride (anhydrous, sublimed), Ord. No. 8.01082
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Formaldehyde solution min. 37% GR, Ord. No. 1.04003

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 sodium hydroxide solution (c = 1 mol/L). 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 of 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.

After-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.

Literature:
C.H. Bowden, N.F. MacLagan, J.H. Wilkinson, Biochem. J. **53**, 93 (1955).

Chemicals:
Cerium(IV) sulfate tetrahydrate GR, Ord. No. 1.02274
Sodium metaarsenite
1,2-*Phenylenediamine for synthesis, Ord. No. 8.09721*
Sulfuric acid 0.5 mol/l Titrisol®, Ord. No. 1.09984
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Sodium hydroxide solution 1 mol/l Titrisol®, Ord. No. 1.09956
Acetone GR ACS, ISO, Ord. No. 1.00014

55. Cerium(IV) sulfate - nitric acid for polyphenyls.

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

After-treatment: Heat 15-20 min at 120°C. Inspect in long-wave UV light.

Literature:
F. Geiss, H. Schlitt, Euratom-Bericht EUR-I-19 d (Nov. 1961).

Chemicals:
Cerium(IV) sulfate tetrahydrate GR, Ord. No. 1.02274
Nitric acid 65% GR ISO, Ord. No. 1.00456

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

Spray solution: Slurry 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.

After-treatment: Heat some minutes at 110°C until the spots appear.

Note: The reagent dyes the alkaloids apomorphine, brucine, colchicine, papaverine and physostigmine. Organic iodine compounds also can be detected.

Literature:
O.-E. Schultz, D. Strauss, Arzneimittel-Forsch. **5**, 342 (1955).

Chemicals:
Cerium(IV) sulfate tetrahydrate GR, Ord. No. 1.02274
Trichloroacetic acid GR ACS, Ord. No. 1.00807
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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.

After-treatment: Heat 15 min at 120°C.

Note: Not applicable with aluminium oxide layers.

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

Chemicals:
Cerium(IV) sulfate tetrahydrate GR, Ord. No. 1.02274
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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. Gaenshirt, A. Malzacher, Arch. Pharm. **293**, 925 (1960).

Chemicals:
Hydrochloric acid 1 mol/L Titrisol®, Ord. No. 1.09970
Chloramine T trihydrate GR, Ord. No. 1.02426
Ammonia solution 25% GR, Ord. No. 1.05432

59. Chloramine T - trichloroacetic acid for digitalis glycosides.

Spray solution: Mix 10 ml of a freshly prepared 3% aqueous chloramine T 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 trihydrate GR, Ord. No. 1.02426
Trichloroacetic acid GR ACS, Ord. No. 1.00807
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 3-methyl-1-phenyl-3-pyrazoIon-5-one in pyridine and potassium cyanide solution (c = 1 mol/L).

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.

Literature:
Private communication G. Bohnstedt, Inst. f. Organ. Chemie, Universitaet des Saarlandes.

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082
Hydrochloric acid 25% GR, Ord. No. 1.00316
Pyridine GR ACS, Ord. No. 1.09728
Potassium cyanide GR ACS, ISO, Ord. No. 1.04967

61. Chlorine - tolidine for compounds convertible into chloramines.

Chlorination: Place the chromatogram into a 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.

Literature:
F. Reindl, W. Hoppe, Chem. Ber. **87**, 1103 (1954).

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082
Hydrochloric acid 25% GR, Ord. No. 1.00316
Acetic acid 96% GR, Ord. No. 1.00062
o-Tolidine
Potassium iodide GR ISO, Ord. No. 1.05043

62. Chlorine - tolidine (modif. act. to Greig and Leaback).

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

Spray solution II: Mix before use equal volumes of a saturated solution of *o*-tolidine in 2% acetic acid and 0.85% aqueous potassium iodide solution.

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

Literature:
C.C. Greig, D.H. Leaback, Nature **188**, 310 (1960).

Chemicals:
Acetic acid 96% GR, Ord. No. 1.00062
Potassium iodide GR ISO, Ord. No. 1.05043
Potassium hypochlorite
o-Tolidine

63. Chlorocyan - 4-aminobenzoic acid for tertiary pyridine compounds with at least one free α-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 slurry of chloramine T, 20 ml 1 N hydrochloric acid and 10 ml 10% aqueous potassium cyanide solution. **Caution, poisonous!** The spots will appear after a short time.

Literature:
E. Nuernberg, Dtsch. Apotheker-Ztg. **101**, 142 (1961).

Chemicals:
4-Aminobenzoic acid extra pure USP, Ord. No. 1.00102
Chloramine T trihydrate GR, Ord. No. 1.02426
Potassium cyanide GR ACS, ISO, Ord. No. 1.04967
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Methanol GR ACS, ISO, Ord. No. 1.06009

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 GR, Ord. No. 1.02427
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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: Sodium hydroxide solution (c = 3 mol/L).

Procedure: Spray subsequently with I and II.

Literature:
L. Maiwald, H. Maske, Hoppe-Seylers Z. physiol. Chem. **306**, 143 (1956).

Chemicals:
1-Chloro-2,4-dinitrobenzene GR, Ord. No. 1.02427
Sodium hydroxide solution min. 27% (1.3) GR, Ord. No. 1.05591
Methanol GR ACS, ISO, Ord. No. 1.06009

66. Chlorophenol red - indicator reagent.

Spray solution: 0.04% ethanolic solution of chlorophenol red. Adjust the solution with sodium hydroxide solution (c = 0.1 mol/L) to pH 7.0.

Literature:
A.R. Jones, E.J. Dowling, W.J. Skroba, Anal. Chem. **25**, 394 (1953).

Chemicals:
Chlorophenol red indicator, Ord. No. 1.03024
Sodium hydroxide solution 0.1 mol/L Titrisol®, Ord. No. 1.09959

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: After spraying heat 5-10 min at 130°C. Inspect in long-wave UV light.

Literature:
R. Tscheche, G. Wulf, Chem. Ber. **94**, 2019 (1961).
R. Tschesche, J. Chromatog. **5**, 217 (1961).
K. Takeda, S. Hara, A. Wada, N. Matsumoto, J. Chromatog. **11**, 562 (1963).

Chemicals:
Chlorosulfonic acid, Ord. No. 8.00220
Acetic acid 96% GR, Ord. No. 1.00062

68. Chromosulfuric acid as universal detectant 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.

Literature:
J. Bertetti, Ann. Chim. (Rome) **44**, 495 (1954).

Chemicals:
Potassium dichromate GR ACS, ISO, Ord. No. 1.04864
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

69. Chromotropic acid for methylenedioxyphenyl-type compounds (e.g. narcotine, hydrastine, sesamine and other compounds splitting off formaldehyde).

Solution a: 100% aqueous solution of chromotropic acid sodium salt.

Solution b: Add 5 parts 97% sulfuric acid to 3 parts water and cool to room temperature.

Spray solution: Prepare freshly before use a mixture of 1 part a and 5 parts b.

After-treatment: Heat 30 min at 105°C.

Literature:
M. Beroza, Agricult. and Food Chemistry **11**, 51 (1963).

Chemicals:
Chromotropic acid disodium salt dihydrate GR, Ord. No. 1.02498
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

70. Cinnamaldehyde - acetic anhydride - sulfuric acid for steroid sapogenins.

Spray solution I: 1% 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 for synthesis, Ord. No. 8.02505
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Acetic anhydride GR ACS, ISO, Ord. No. 1.00042
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

After-treatment: Place the plate into a hydrogen chloride atmosphere. Red spots.

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

Chemicals:
Cinnamaldehyde for synthesis, Ord. No. 8.02505
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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

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

After-treatment: Heat at 40-50°C. Blue spots. The reaction is not sensitive.

Literature:
R. Donner, K. Lohs, J. Chromatog. **17**, 349 (1965).

Chemicals:
Cobalt(II) chloride hexahydrate GR ACS, Ord. No. 1.02539
Acetone GR ACS, ISO, Ord. No. 1.00014

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 acetic acid (c = 2 mol/L).

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

Literature:
E. Beerstecher, Anal. Chem. **22**, 1200 (1950).
R.U. Magee, J.B. Headridge, Analyst **82**, 95 (1957).

Chemicals:
Lead(II) nitrate GR ACS, Ord. No. 1.07398
Cobalt(II) nitrate hexahydrate GR, Ord. No. 1.02536
Sodium nitrite GR ACS, Ord. No. 1.06549
Acetic acid 96% GR, Ord. No. 1.00062
Nitric acid 65% GR ISO, Ord. No. 1.00456

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

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

After-treatment: Dry and place into a chamber saturated with ammonia vapours.

Literature:
E.J. Shellard, J.V. Osisiogu, Lab. Practice **13**, 516 (1964).

Chemicals:
Cobalt(II) nitrate hexahydrate GR, Ord. No. 1.02536
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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 hexahydrate GR, Ord. No. 1.02536
Lithium hydroxide (about 98% LiOH) LAB, Ord. No. 1.05691
Methanol GR ACS, ISO, Ord. No. 1.06009
Methanol dried SeccoSolv, Ord. No. 1.06012

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 by placing the chromatogram into water vapours.

Literature:
E.S. Lane, J. Chromatog. **18**, 426 (1965).

Chemicals:
Cobalt(II) chloride hexahydrate GR ACS, Ord. No. 1.02539
Ammonium thiocyanate GR ACS, ISO, Ord. No. 1.01213

77. Copper acetate - potassium hexacyanoferrate(II) for the identification of higher fatty acids acc. to Kaufmann. PC.

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.

Procedure: 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.

Literature:
H.P. Kaufmann, W.H. Nietsch, Fette u. Seifen, Anstrichmittel **56**, 154 (1954).

Chemicals:
Copper(II) acetate monohydrate GR, Ord. No. 1.02711
Potassium hexacyanoferrate(II) trihydrate GR ACS, ISO, Ord. No. 1.04984

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

Dip solution 1: Dilute 10 ml saturated copper(II) acetate solution to 1 1 with water.

Dip solution II: 0.1% ethanolic rubeanic acid solution with 0.5 % ammonia.

Procedure: 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.

Literature:
P.E. Ballance, W.M. Crombie, Biochem. J. **69**, 632 (1958).

Chemicals:
Copper(II) acetate monohydrate GR, Ord. No. 1.02711
Rubeanic acid (dithiooxamide) GR, Ord. No. 1.00629
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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. Hranisavljevic-Jacovljevic, I. Pexjkovic-Tadic, A. Stojiljkovic, J. Chromatog. **12**, 70 (1963).

Chemicals:
Copper(II) chloride dihydrate GR ACS, Ord. No. 1.02733

80. Copper sulfate - benzidine for pyridine 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.
Caution: Benzidine is cancerogenic!

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

Chemicals:
Copper(II) sulfate pentahydrate GR ACS, ISO, Ord. No. 1.02790
Benzidine
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

Literature:
M. Frahm, A. Gottesleben, K. Soehring, Pharm. Acta Helv. **38**, 785 (1963).

Chemicals:
Copper(II) sulfate pentahydrate GR ACS, ISO, Ord. No. 1.02790
Potassium permanganate GR ACS, Ord. No. 1.05082
Quinine hydrochloride Ph Eur, Ord. No. 8.17037
Pyridine GR ACS, Ord. No. 1.09728

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 a change of colour.

Literature:
H. Reznik, K. Egger, Z. anal. Chem. **183**, 196 (1961).

Chemicals:
Copper(II) sulfate pentahydrate GR ACS, ISO, Ord. No. 1.02790
Sodium carbonate anhydrous GR ISO, Ord. No. 1.06392
tri-Sodium citrate dihydrate GR ACS, ISO, Ord. No. 1.06448

83. α-Cyclodextrin for straight-chain lipids.

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

Preparation: K. Freudenberg et al., Liebigs Ann. Chem. **558**, 1 (1947).
D. French et al., J. Am. Chem. Soc. **71**, 353 (1949).

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

Literature:
D.C. Malins, H.K. Mangold, J. Am. Oil Chemists Soc. **37**, 576 (1960).
H.K. Mangold, J.L. Gellermann, H. Schlenk, Federation Proc. **17**, 269 (1958).
H.K. Mangold, B.G. Lamp, H. Schlenk, J. Am. Chem. Soc. **77**, 6070 (1955).

Chemicals:
Iodine resublimed GR ACS, IAO, Ord. No. 1.04761

Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
α-Cyclodextrine, Ord. No. 1.02126

84. Cysteine - sulfuric acid for desoxyribonucleosides (modif. reagent acc. to Dische).

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.

Literature:
G. Buchanan, Nature **168**, 1091 (1951).

Chemicals:
L-Cysteine hydrochloride monohydrate, Ord. No. 1.02839
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Acetone GR ACS, ISO, Ord. No. 1.00014

85. 3,5-Diaminobenzoic acid - phosphoric acid for 2-deoxy-sugars.

Spray solution: Dissolve 1 g 3,5-diaminobenzoic acid in 25 ml 80% phosphoric acid and dilute with 60 ml water.

After-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.

Literature:
M. Pesez, Bull. soc. chim. biol. **32**, 701 (1950).

Chemicals:
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
3,5-Diaminobenzoic acid for synthesis, Ord. No. 8.20405

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.

Literature:
R. Wasicky, O. Frehden, Mikrochim. Acta **1**, 55 (1937).

Chemicals:
o-Dianisidine (3,3'-dimethoxybenzidine)
Acetic acid 96% GR, Ord. No. 1.00062
2,7-Diaminofluorene

87. Diazotisation and coupling with 1-naphthol for aromatic primary amines and sulfonamides (Bratton-Marshall reagent).

Spray solution I: Freshly prepared 1% sodium nitrite solution in hydrochloric acid (c = 1 mol/L).

Spray solution II: Freshly prepared 0.2% 1-naphthol solution in potassium hydroxide (c = 1 mol/L).

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

Note: Instead of l-naphthol a 0.4% methanolic solution of N-(l-naphthyl)ethylene diammonium dichloride may be used as coupling agent.

Literature:
A.C. Bratton, E.K. Marshall, J. Biol. Chem. **128**, 537 (1939).
A. Wankmueller, Naturwissenschaften **39**, 302 (1952).
G. Wagner, Arch. Pharm. **285**, 409 (1952).
T. Bican-Fister, V. Kajganovic, J. Chromatog. **11**, 492 (1963).

Chemicals:
1-Naphthol GR, Ord. No. 1.06223
Sodium nitrite GR ACS, Ord. No. 1.06549
N-(1-Naphthyl)ethylenediamine dihydrochloride GR, Ord. No. 1.06237
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Potassium hydroxide solution 1 mol/l Titrisol®, Ord. No. 1.09918
Methanol GR ACS, ISO, Ord. No. 1.06009

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

Spray solution: Freshly prepared 0.4% methanolic solution of 2,6-dibromo-quinone chlorimide.

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

Literature:
E. Nuernberg, Dtsch. Apotheker-Ztg. **101**, 268 (1961).

Chemicals:
2,6-Dibromoquinone chlorimide
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391

Ammonia solution 25% GR, Ord. No. 1.05432
Methanol GR ACS, ISO, Ord. No. 1.06009

89. 2',7'-Dichlorofluorescein.

100 ml ready to use spray solution for chromatography (c = 0.2% in 2-propanol).

Ord. No. 1.09219

90. 2',7'-Dichlorofluorescein fluorescence indicator for saturated and unsaturated lipids.

A. Spray solution: 0.2 ethanolic solution of 2',7'-dichlorofluorescein.

B. Spray solution (for vitamin E): 0.01% ethanolic solution of 2',7'-dichloro-fluorescein.

Note: After drying with warm air it is sometimes advisable to place the chromatogram in a current of steam, or to spray it with water.
Inspect in long-wave UV light.

Literature:
D.C. Malins, H.K. Mangold, J. Am. Oil Chemists Soc. **37**, 576 (1960).
P.J. Dunphy, K.J. Whittle, J.F. Pennock, Chem. & Ind. (London) **1965**, 1217.

Chemicals:
2',7'-Dichlorofluorescein, Ord. No. 1.09676
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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, Ord. No. 1.09676
Aluminium chloride anhydrous, Ord. No. 1.01082
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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-Dichlorophenol-indophenol sodium salt dihydrate GR, Ord. No. 1.03028
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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

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

After-treatment: After brief warming the acids appear as red spots on light blue background.

Literature:
C. Passera, A. Pedrotti, G. Ferrari, J. Chromatog. **14**, 289 (1964).

Chemicals:
2,6-Dichlorophenol-indophenol sodium salt dihydrate GR, Ord. No. 1.03028
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

Literature:
Y.-T. Chen, F.A. Isherwood, L.W. Mapson, Biochem. J. **55**, 821 (1953).

Chemicals:
2,6-Dichlorophenol-indophenol sodium salt dihydrate GR, Ord. No. 1.03028
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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

Spray solution: Prepare freshly before use a 0.1 to l % solution of 2,6-dichloro-quinone 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.

Literature:
A. Seher, Fette u. Seifen, Anstrichmittel **61**, 345 (1959)
R.F. v. d. Heide, O. Wouters, Z. Lebensm.-Unters. u. Forsch. **115**; 129 (1962).
R. Segura-Cardona, K. Soehring, Med. Exp. **10**, 251 (1964).

Chemicals:
2,6-Dichloroquinone chlorimide GR, Ord. No. 1.03037
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
di-Sodium tetraborate 10-hydrate GR ACS, ISO, Ord. No. 1.06308

96. Dicobalt octacarbonyl for acetylene compounds.

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

Spray solution II: Hydrochloric acid (c = 1 mol/L).

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 chromato gram into a bromine atmosphere. The spots show yellow colours.

Literature:
K.E. Schulte, F. Ahrens, E. Sprenger, Pharm. Ztg. **108**, 1165 (1963).

Chemicals:
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Bromine GR ISO, Ord. No. 1.01948
Neatan®
Di-Cobalt octacarbonyl for synthesis, Ord. No. 8.20748
Petroleum benzine (100-140°C), Ord. No. 1.01770

97. Diethylamine - copper(II) sulfate for thiobarbiturates.

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 pentahydrate GR ACS, ISO, Ord. No. 1.02790
Methanol GR ACS, ISO, Ord. No. 1.06009
Diethylamine for synthesis, Ord. No. 8.03010

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 for synthesis, Ord. No. 8.00898
Sodium hydroxide solution min. 10% (1.11) GR, Ord. No. 1.05588
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

99. Dimedone - phosphoric acid for keto sugars.

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

After-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 GR, Ord. No. 1.06013
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

100. 4-Dimethylaminobenzaldehyde.

100 ml ready to use spray solution for chromatography (c = ca. 1.2% in 2-propanol).

Ord. No. 1.03722

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 5 g 85% phosphoric acid. After dissolution 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).
H. Kaiser, G. Hasenmayer, Arch. Pharm. **287**, 503 (1954).

Chemicals:
4-Dimethylaminobenzaldehyde GR ACS, Ord. No. 1.03058
Acetic acid 96% GR, Ord. No. 1.00062
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

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.

Spray solution II: Dissolve 1 g 4-dimethylaminobenzaldehyde in 30 ml ethanol. Add 30 ml 37% hydrochloric acid. If required dilute with 180 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.

Literature:
L.A. Elson, W.T.J. Morgan, Biochem. J. **27**, 1824 (1933).
R. Belcher, A.J. Mutten, C.M. Sabrook, Analyst **79**, 201 (1954).

Chemicals:
4-Dimethylaminobenzaldehyde GR ACS, Ord. No. 1.03058
Acetylacetone GR, Ord. No. 1.09600
Potassium hydroxide pellets GR, Ord. No. 1.05033
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1-Butanol GR ACS, ISO, Ord. No. 1.01990
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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% hydrochloric acid and 75 ml methanol.

After-treatment: In some cases it is necessary to warm the plate.

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

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

Literature:
R.A. Heacock, M.E. Mahon, J. Chromatog. **17**, 338 (1965).

Chemicals:
4-Dimethylaminobenzaldehyde GR ACS, Ord. No. 1.03058
Hydrochloric acid 25% GR, Ord. No. 1.00316
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Methanol GR ACS, ISO, Ord. No. 1.06009
Ethanol absolute GR ACS, ISO Ord. No. 1.00983

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% 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 GR ACS, Ord. No. 1.03058
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Nitric acid 65% GR ISO, Ord. No. 1.00456

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

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

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

Chemicals:
4-Dimethylaminobenzaldehyde GR ACS, Ord. No. 1.03058
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943

106. Dimethylaminobenzylidenerhodanine for silver, copper and mercury ions.

Spray solution: 1% ethanolic solution of 5-(4-dimethylaminobenzylidene)-rhodanine.

Treatment: Respray with 25% ammonia solution or place into a chamber saturated with ammonia vapours. Pink to violet spots.

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

Chemicals:
5-(4-Dimethylaminobenzylidene)rhodanine GR, Ord. No. 1.03059
Ethanol absolute GR ACS, ISO Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

107. Dimethylaminocinnamaldehyde for indoles.

Stock solution: Dissolve 2 g 4-dimethylaminocinnamaldehyde in a mixture of 100 ml hydrochloric acid (c = 6 mol/L) and 100 ml ethanol. Store the solution in the refrigerator.

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

After-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.

Literature:
J. Harley-Mason, A.A.P.G. Archer, Biochem. J. **69**, 60 (1958).

Chemicals:
4-(Dimethylamino)cinnamaldehyde for synthesis, Ord. No. 8.22034
Hydrochloric acid 25% GR, Ord. No. 1.00316
Nitric acid 65% GR ISO, Ord. No. 1.00456

108. N,N-Dimethyl-1,4-phenylenediammonium dichloride for bromine-containing hypnotics and chlorinated insecticides.

Spray solution: Dissolve 0.5 g N,N-dimethyl-1.4-phenylenediammonium dichloride 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.

Literature:
J. Baeumler, S. Rippstein, Helv. Chim. Acta **44**, 1162 (1961).

Chemicals:
N,N-Dimethyl-1,4-phenylenediammonium dichloride GR, Ord. No. 1.03067
Sodium rods, Ord. No. 1.06260
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

109. N,N-Dimethyl-1,4-phenylenediammonium dichloride for peroxides.

Spray solution: Dissolve 1.5 g N,N-dimethyl-1.4-diphenylenediammonium dichloride in a mixture of 128 ml methanol, 25 ml water and 1 ml glacial acetic acid. Peroxides show purple spots.

Literature:
E. Knappe, D. Peteri, Z. anal. Chem. **190**, 386 (1962).

Chemicals:
N,N-Dimethyl-1,4-phenylenediammonium dichloride GR, Ord. No. 1.03067
Acetic acid 96% GR, Ord. No. 1.00062
Methanol GR ACS, ISO, Ord. No. 1.06009

110. N,N-Dimethyl-1,4-phenylenediammonium dichloride - trichloroacetic acid for methyl-sugars.

Spray solution: Dissolve 0.4 g N,N-dimethyl-1,4-phenylenediammonium dichloride in 100 ml 2% aqueous trichloroacetic acid solution.

After-treatment: Heat 1-2 min at 120°C.

Note: The colour spots may be eluated for colorimetric determination.

Literature:
W.C. Schaefer, J.W. van Cleve, Anal. Chem. **28**, 1290 (1956).
L. Boggs, L.S. Cuendet, I. Ehrenthal, R. Koch, F. Smith, Nature **166**, 520 (1950).

Chemicals:
N,N-Dimethyl-1,4-phenylenediammonium dichloride GR, Ord. No. 1.03067
Trichloroacetic acid GR ACS, Ord. No. 1.00807

111. 1,3-Dinitrobenzene for 17-ketosteroids.

Solution a: 2% ethanolic solution of 1,3-dinitrobenzene.

Solution b: Methanolic potassium hydroxide solution (c = 2.5 mol/L).

Spray solution: Mix equal parts of a and b.

After-treatment: Heat 1-2 min at 80°C. Violet spots.

Literature:
T. Feher, Mikrochim. Acta **1965**, 105.
B.P. Lisboa, J. Chromatog. **16**, 136 (1964).
R. Neher, Steroid Chromatography, Elsevier 1964, Amsterdam, London, New York.

Chemicals:
1,3-Dinitrobenzene for synthesis, Ord. No. 8.22272
Potassium hydroxide pellets GR, Ord. No. 1.05033
Methanol GR ACS, ISO, Ord. No. 1.06009
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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-keto-steroids blue-violet and 20-ketosteroids brown.

Literature:
J. Barrolier, J. Heilmann, Z. physiol. Chem. **309**, 221 (1957).
O. Schindler, T. Reichstein, Helv. Chim. Acta **34**, 108 (1951).

Chemicals:
1,3-Dinitrobenzene for synthesis, Ord. No. 8.22272
Potassium hydroxide pellets GR, Ord. No. 1.05033
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 potassium hydroxide solution (c = 2 mol/L).

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.

Literature:
R. Tschesche, G. Grimmer, F. Seehofer, Chem. Ber. **86** 1235 (1953).
M.L. Lewbart, W. Wehrli, T. Reichstein, Helv. Chim. Acta **46**, 565 (1963).

Chemicals:
3,5-Dinitrobenzoic acid, Ord. No. 1.00138
Potassium hydroxide pellets GR, Ord. No. 1.05033
Methanol GR ACS, ISO, Ord. No. 1.06009

113. 3,5-Dinitrobenzoic acid for reducing sugars.

Spray solution: 1% solution of 3,5-dinitrobenzoic acid in sodium carbonate solution (c = 2 mol/L).

After-treatment: Dry 5-10 min at 100°C.

Literature:
F. Weygand, H. Hofmann, Chem. Ber. **83**, 405 (1950).

Chemicals:
3,5-Dinitrobenzoic acid, Ord. No. 1.00138
Sodium carbonate anhydrous GR ISO, Ord. No. 1.06392

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 1 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.

Literature:
G. Pataki, J. Chromatog. **16**, 541 (1964).

Chemicals:
1-Fluoro-2,4-dinitrobenzene GR, Ord. No. 1.02966
Sodium hydrogen carbonate GR ISO, Ord. No. 1.06329

Sodium hydroxide solution 1 mol/l Titrisol®, Ord. No. 1.09956
Methanol GR ACS, ISO, Ord. No. 1.06009
Diethyl ether GR ACS, Ord. No. 1.00921

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

A. Spray solution: 0,4% solution of 2,4-dinitrophenylhydrazine in hydrochloric acid (c = 2 mol/L).

B. Spray solution: Add 10 ml 37% hydrochloric acid to 1 g 2,4-dinitrophenylhydrazine in 1000 ml ethanol.

After-treatment: For distinction of the formed 2,4-dinitrophenylhydrazones (DNPH) spray consecutively with 0.2% solution of potassium hexacyanoferrate(III) in hydrochloric acid (c = 2 mol/L). 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.

Literature:
A. Mehlitz, K. Gierschner, T. Minas, Chemiker-Ztg. **87**, 573 (1963).

Chemicals:
2,4-Dinitrophenylhydrazine GR, Ord. No. 1.03081
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973

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

Spray solution: 0,5%, solution of 3,5-dinitrosalicylic acid (2-hydroxy-3,5-dinitrobenzoic acid) in 4% sodium hydroxide solution.

After-treatment: After brief pre-drying at room temperature heat 4-5 min at 100°C.

Literature:
A. Jeanes, C.S. Wise, R.J. Dimler, Anal. Chem. **23**, 415 (1951).

Chemicals:
2-Hydroxy-3,5-dinitrobenzoic acid, Ord. No. 8.00141
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498

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.

After-treatment: Heat 5-10 min at 100°C. Blue-grey spots.

Literature:
H. Jatzkewitz, Hoppe-Seylers Z. physiol. Chem. **320**, 251 (1960).

Chemicals:
Diphenylamine GR and redox indicator, Ord. No. 1.03086
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Acetic acid 96% GR, Ord. No. 1.00062

118. Diphenylamine - palladium(II) chloride for nitrosamines.

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.

After-treatment: After exposure to short-wave UV light the substances show violet spots.

Literature:
R. Preussmann, D. Daiber, H. Hengy, Nature **201**, 502 (1964).
R. Preussmann, G. Neurath, G. Wulf-Lorentzen, D. Daiber, H. Hengy, Z. anal. Chem. **202**, 187 (1964).

Chemicals:
Diphenylamine GR and redox indicator, Ord. No. 1.03086
Palladium(II) chloride anhydrous for synthesis, Ord. No. 8.07110
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sodium chloride GR ACS, ISO, Ord. No. 1.06404

119. Diphenylamine - zinc chloride for chlorinated insecticides.

Spray solution: Dissolve 0.5 g diphenylamine and 0.5 g zinc chloride in 100 ml acetone.

After-treatment: Heat 5 min at 200°C. Colour reaction.

Literature:
D. Kath, J. Chromatog. **15**, 269 (1964).

Chemicals:
Diphenylamine GR and redox indicator, Ord. No. 1.03086
Zinc chloride GR ACS, ISO, Ord. No. 1.08816
Acetone GR ACS, ISO, Ord. No. 1.00014

120. β-Aminoethyl diphenylborate for α- and γ-pyrones (Neu's reagent).

Spray solution: 1% methanolic β-aminoethyl diphenylborate [= 2-(Diphenylboryloxy)ethylamine] solution.

Procedure: Spray about 10 ml of the solution and inspect the fluorescence in long wave UV light.

Literature:
R. Neu, Naturwissenschaften **44**, 181 (1957).
E. Stahl, P.J. Schorn, Hoppe-Seylers Z. physiol. Chem. **325**, 263 (1961).

Chemicals:
2-(Diphenylboryloxy)ethylamine Reag. Ph Eur, Ord. No. 1.59626
Methanol GR ACS, ISO, Ord. No. 1.06009

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.

Literature:
F.W.M.H. Merkus, Pharm. Weekblad **98**, 947 (1963).

Chemicals:
Diphenylcarbazide GR and redox indicator, Ord. No. 1.03091
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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 ethanolic nitric acid (c = 0.05 mol/L).

Literature:
Y. Inouve, M. Noda, O. Hirayama, J. Am. Oil Chemists Soc. **32**, 132 (1955).

Chemicals:
Diphenylcarbazone, Ord. No. 1.03087
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Nitric acid 65% GR ISO, Ord. No. 1.00456

123. Diphenylcarbazone for cations.

Spray solution: Saturated solution of diphenylcarbazone in methanol.

Literature:
G.B. Heisig, F.H. Pollard, Anal. Chim. Acta **16**, 234 (1957).

Chemicals:
Diphenylcarbazone, Ord. No. 1.03087
Methanol GR ACS, ISO, Ord. No. 1.06009

124. Diphenylpicrylhydrazyl for essential oils.

Spray solution: Dissolve 0.06 g diphenylpicrylhydrazyl in 100 ml chloroform.

After-treatment: Heat 5-10 min at 110°C. Yellow spots on violet background.

Literature:
C. Bergstrom, C. Lagercrantz, Acta Chem. Scand. **18**, 560 (1964).

Chemicals:
2,2'-Diphenyl-1-picrylhydrazyl
Chloroform GR ISO, Ord. No. 1.02445

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.

Literature:
P.J. Stevens, J. Chromatog. **14**, 269 (1964).

Chemicals:
2,5-Diphenyl-3-(4-styrylphenyl)tetrazolium chloride
Methanol GR ACS, ISO, Ord. No. 1.06009
Sodium hydroxide solution min. 10% (1.11) GR, Ord. No. 1.05588

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 derivatives appear as red spots on yellow background.

Literature:
K.B. Augustinsson, M. Grahn, Acta Chem. Scand. **7**, 906 (1953).

Chemicals:
Dipicrylamine
Acetone GR ACS, ISO, Ord. No. 1.00014

127. Dipicrylamine for vitamin B_1 .

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% ammonia solution.

Literature:
K.B. Augustinsson, M. Grahn, Acta Chem. Scand. **7**, 906 (1953).

Chemicals:
Dipicrylamine
Magnesium carbonate
Methanol GR ACS, ISO, Ord. No. 1.06009
Ammonia solution 25% GR, Ord. No. 1.05432

128. Dithizone for ions of heavy metals.

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

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

Literature:
T. Barnabas, J. Barnabas, Naturwissenschaften **44**, 61 (1957).
F.W.H.M. Merkus, Pharm. Weekblad **98**, 955 (1963).

Chemicals:
Dithizone GR (1,5-diphenylthiocarbazone), Ord. No. 1.03092
Carbon tetrachloride GR, Ord. No. 1.02222
Ammonia solution 25% GR, Ord. No. 1.05432

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% aqueous barium chloride solution.

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

Literature:
K. Thoma, R. Rombach, E. Ullmann, Sci. Pharm. **32**, 216 (1964).

Chemicals:
Bismuth(III) nitrate basic GR, Ord. No. 1.01878
Potassium iodide GR ISO, Ord. No. 1.05043
Barium chloride dihydrate GR ACS, ISO, Ord. No. 1.01719
Acetic acid 96% GR, Ord. No. 1.00062

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

Stock solution: Dissolve 8.0 g bismuth(III) nitrate in 20-25 ml 25% 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 orangered 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 hydrochloric acid (c = 6 mol/L), 2 ml stock solution and 6 ml sodium hydroxide solution (c = 6 mol/L). In case bismuth hydroxide is not completely dissolved by shaking, add several drops of hydrochloric acid (c = 6 mol/L).

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

Literature:
H.M. Bregoff, E. Roberts, C.C. Delwiche, J. Biol. Chem. **205**, 565 (1953).

Chemicals:
Bismuth(III) nitrate basic GR, Ord. No. 1.01878
Nitric acid 65% GR ISO, Ord. No. 1.00456
Hydrochloric acid 25% GR, Ord. No. 1.00316
Potassium iodide GR ISO, Ord. No. 1.05043
Sodium hydroxide solution min. 10% (1.11) GR, Ord. No. 1.05588

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. The 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 B_1 spray with the stock solution.

Literature:
R. Munier, Bull. soc. chim. biol. **35**, 1225 (1953).

Chemicals:
Bismuth(III) nitrate basic GR, Ord. No. 1.01878
Potassium iodide GR ISO, Ord. No. 1.05043
L(+)Tartaric acid GR ACS, ISO, Ord. No. 1.00804

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.

Solution b: Dissolve 8 g potassium iodide in 20 ml water.

Stock solution: Mix equal parts of a and b. The mixture can be stored in a dark bottle for a long time.

Spray solution: Mix 1 ml stock solution with 2 ml glacial acetic acid and 10 ml water before use.

Literature:
R. Munier, M. Macheboeuf, Bull. soc. chim. biol. **33**, 846 (1951).
H. Jatzkewitz, Hoppe-Seylers Z. physiol. Chem. **292**, 99 (1953).

Chemicals:
Bismuth(III) nitrate GR, Ord. No. 1.01878
Potassium iodide GR ISO, Ord. No. 1.05043
Acetic acid 96% GR, Ord. No. 1.00062

133. Dragendorff reagent acc. to Thies and Reuther, modif. by Vagujfalvi, for alkaloids and other nitrogen-containing compounds.

Stock solution: Boil 2.6 g bismuth carbonate and 7 g sodium iodide with 25 ml glacial acetic acid for a few minutes. After 12 hours filter off the precipitated sodium acetate. Then mix 20 ml of the red-brown filtrate with 80 ml ethyl acetate and add 0.5 ml water. Store in a dark bottle.

Spray solution: Mix 10 ml stock solution with 100 ml glacial acetic acid and 240 ml ethyl acetate. After spraying of 5-10 ml alkaloids and some other compounds containing no nitrogen show orange spots.

After-treatment: A more sensitive detection is availably by subsequent spraying with sulfuric acid (c = 0.025-0.05 mol/L). The spots are bright orange to red on a grey background.

Literature:
H. Thies, F.W. Reuther, Naturwissenschaften **41**, 230 (1954).
D. Vagujfalvi, Planta Med. **8**, 34 (1960).
E. Tyihak, J. Chromatog. **14**, 125 (1964).

Chemicals:
Bismuth(III) carbonate
Sodium iodide extra pure BP, Ph Eur, USP, Ord. No. 1.06520
Acetic acid 96% GR, Ord. No. 1.00062
Ethyl acetate GR, Ord. No. 1.09623
Sulfuric acid 0.05 mol/l Titrisol®, Ord. No. 1.09984

133a Dragendorff reagent.

100 ml ready to use spray solution for chromatography (Solvent: ethyl acetate, acetic acid, water).

Ord. No. 1.02035

134. Ethylenediamine for catecholamines.

Spray solution: Mixture of equal parts of ethylenediamine with water or diluted sodium hydroxide solution.

Procedure: Heat the chromatogram 20 min at 50-60°C. Inspection in short- or long-wave UV light.

Literature:
R. Segura-Cardona, K. Soehring, Med. Exp. **10**, 251 (1964).

Chemicals:
Ethylenediamine for synthesis, Ord. No. 8.00947
Sodium hydroxide solution 2 mol/l, Ord. No. 1.09136

135. Ethylenediamine - potassium hexacyanoferrate(III) for catecholamines (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.

After-treatment: Heat the chromatogram 10 min at 105°C. Inspection under UV light.

Literature:
J.S. Stern, M.J. Franklin, J. Mayer, J. Chromatog. **30**, 637 (1967).

Chemicals:
Ethylenediamine for synthesis, Ord. No. 8.00947
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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: Sodium hydroxide solution (c = 0.1 mol/L).

Treatment: Spray with I and then with II.

Literature:
H. Jatzkewitz, U. Lenz, Hoppe-Seylers Z. physiol. Chem. **305**,53 (1956).

Chemicals:
Fast blue salt B, Ord. No. 1.03191
Sodium hydroxide solution 0.1 mol/l Titrisol®, Ord. No. 1.09959

137. Fluorescein for lipids.

Spray solution: 0.01% ethanolic solution of fluorescein.

After-treatment: Dry with warm air and handle subsequently with water vapour or spray lightly with water.

Chemicals:
Fluorescein (C.I. 45350)
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 (C.I. 45350)
Ammonia solution 25% GR, Ord. No. 1.05432

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 prevent the formation of eosin and the fluorescence remains. Larger amounts of substance show yellow spots on reddish background.

Literature:
F. Runge, A. Jumar, F. Koehler, J. prakt. Chem. **21**, 39 (1963

Chemicals:
Fluorescein (C.I. 45350)
Bromine GR ISO, Ord. No. 1.01948
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Carbon tetrachloride GR, Ord. No. 1.02222

Variation: With self-coated plates take 0.04% aqueous fluorescein sodium solution instead of water.
After-treatment: After development of the chromatogram blow bromine vapours over the dried plate.

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

Chemicals:
Fluorescein sodium (C.I. 45350) extra pure, Ord. No. 1.03992
Bromine GR ISO, Ord. No. 1.01948

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.

Literature:
H. Weichsel, Mikrochim. Acta **1965**, 325.

Chemicals:
Fluorescein (C.I. 45350)
Acetic acid 96% GR, Ord. No. 1.00062

Hydrogen peroxide 30% H_2O_2 (Perhydrol®) GR ISO, Ord. No. 1.07209
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 (C.I. 45350) extra pure, Ord. No. 1.03992
Sodium carbonate anhydrous GR ISO, Ord. No. 1.06392
Rhodamine B (C.I. 45170) GR, Ord. No. 1.07599
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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 reagents No. 260, 261.

B. Additives to adsorbents:

6. Fluorescein sodium 0.04% in water added to the adsorbent suspension, Ord. No. 1.03922.
7. Fluorescence indicator F_{254} for thin layer chromatography (1-2% added to the adsorbent) for detection in short-wave UV light (λ_{max} = 254 nm), Ord. No. 1.09182.

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 60 $HF_{254+366}$, Ord. No. 1.07741
Silica gel 60 $PF_{254+366}$, Ord. No. 1.07748
Aluminium oxide 60 $PF_{254+366}$ (Type E), Ord. No. 1.01104

143. Folin Ciocalteau reagent for phenols.

Stock solution: Dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water, add 5 ml 85% phosphoric acid and 10 ml 37% 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% 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.

Literature:
R.W. Keith, D. le Turneau, D. Mahlum, J. Chromatog. **1**, 534 (1958).

Chemicals:
Sodium molybdate dihydrate GR, Ord. No. 1.06521
Sodium tungstate dihydrate GR, Ord. No. 1.06673
Lithium sulfate monohydrate GR ACS, Ord. No. 1.05694
Sodium carbonate anhydrous GR ISO, Ord. No. 1.06392
Bromine GR ISO, Ord. No. 1.01948
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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.

After-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. Prochazka, Chem. Listy **47**, 1643 (1953).
E. Stahl, H. Kaldewey, Hoppe-Seylers Z. physiol. Chem. **323**, 182 (1961).

Chemicals:
Formaldehyde solution min. 37% GR, Ord. No. 1.04003
Hydrochloric acid 25% GR, Ord. No. 1.00316
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Nitric acid 65% GR ISO, Ord. No. 1.00456

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).
E.G.C. Clarke, Nature **181**, 1152 (1958).

Chemicals:
Paraformaldehyde extra pure DAC, BPC, USP, Ord. No. 1.04005
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

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. Variously coloured spots.

Literature:
N. Kucharczyk, J. Fohl, J. Vymetal, J. Chromatog. **11**, 55 (1963).

Chemicals:
Formaldehyde solution min. 37%, Ord. No. 1.04003
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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.

Literature:
A. Heyndrickx, M. Schauvliege, A. Blommel, J. pharm. Belg. **20**, 117 (1965).
I. Sunshine, Am. J. Clin. Pathol. **40**, 576 (1963).

Chemicals:
Furfural GR, Ord. No. 1.04013
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Acetone GR ACS, ISO, Ord. No. 1.00014

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. Muenster 1954.

Chemicals:
D-(+)-Glucose monohydrate, Ord. No. 1.08342
Aniline GR, Ord. No. 1.01261
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1-Butanol GR ACS, ISO, Ord. No. 1.01990

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.

After-treatment: Heat for about 10 min at 45°C.

Literature:
F. Micheel, H. Schweppe, Microchim. Acta **1954**, 53.

Chemicals:
D-(+)-Glucose monohydrate, Ord. No. 1.08342
ortho-Phosphoric acid min. 85% (1.7) GR, Ord. No. 1.00573
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1-Butanol GR ACS, ISO, Ord. No. 1.01990

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 50°C.

Literature:
H.G. Moeller, N. Zeller, J. Chromatog. **14**, 560 (1964).

Chemicals:
Glyoxalbis(2-hydroxyanil) GR, Ord. No. 1.04191
Potassium hydroxide pellets GR, Ord. No. 1.05033
Methanol GR ACS, ISO, Ord. No. 1.06009

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 hydrochloric acid (c =4 mol/L).

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

Literature:
K.G. Bergner, H. Sperlich, Dtsch. Lebensm.-Rundschau **47**, 134 (1951).

Chemicals:
Hydrazinium sulfate GR ACS, Ord. No. 1.04603
Hydrochloric acid 25% GR, Ord. No. 1.00316
Ammonia solution 25% GR, Ord. No. 1.05432

152. Hydrochloric acid for glycals.

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

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

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

Literature:
J.T. Edward, D.M. Waldron, J. Chem. Soc. **1952**, 3631.

Chemicals:
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

153. Hydrogen peroxide for aromatic acids.

Spray solution: 0.3% aqueous hydrogen peroxide solution.

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

Literature:
D.W. Grant, J. Chromatog. **10**, 511 (1963).

Chemicals:
Hydrogen peroxide 30% H_2O_2 (Perhydrol®) GR ISO, Ord. No. 1.07209

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

Solution a: 50% sulfuric acid.

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

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

After-treatment: Heat 3-4 min at 105°C or 10 min at 60°C. Yellow to pink spots.

Literature:
P.J. Stevens, J. Chromatog. **14**, 269 (1964).

Chemicals:
4-Hydroxybenzaldehyde for synthesis, Ord. No. 8.04536
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Methanol GR ACS, ISO, Ord. No. 1.06009

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

Solution a: Dissolve 20 g hydroxylammonium chloride 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 I: 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 II: Dissolve 10 g finely powdered iron(III) chloride in 20 ml 36% hydrochloric acid. Shake with 200 ml diethyl ether until a homogenous 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.

Literature:
V.P. Whittaker, S. Wijesundera, Biochem. J. **51**, 348 (1952).

Chemicals:
Hydroxylammonium chloride GR ACS, ISO, Ord. No. 1.04616
Potassium hydroxide pellets GR, Ord. No. 1.05033
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Diethyl ether GR ACS, Ord. No. 1.00921

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 vapours. Inspect in long-wave UV light.

Literature:
W.A. Reeves, T.B. Crumler, Anal. Chem. **23**, 1576 (1952).
T.V. Arden et al., Nature **162**, 691 (1948).

Chemicals:
8-Hydroxyquinoline GR ACS, Ord. No. 1.07098
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

157. 8-Hydroxyquinoline - hypobromite for arginine and other guanidine derivates (Sakaguchi reagent).

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

Spray solution II: Mixture of 0.2 ml bromine and 100 ml sodium hydroxide solution (c = 0.5 mol/L).

Procedure: Spray with I and after drying with II. The spots show orange to red colour.

Literature:
J.B. Jepson, J. Smith, Nature **172**, 1100 (1953).
J. Kaloušek, M. Kutácek, J. Bílek, Ceskoslov. farm. **4**, 188 (1955).

Chemicals:
8-Hydroxyquinoline GR ACS, Ord. No. 1.07098
Bromine GR ISO, Ord. No. 1.01948
Sodium hydroxide solution 0.5 mol/l Titrisol®, Ord. No. 1.09957
Acetone GR ACS, ISO, Ord. No. 1.00014

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.

Literature:
F.H. Pollard, J.F.W. McOmie, I.I.M. Elbeih, J. Chem. Soc. **1951**, 466.

Chemicals:
8-Hydroxyquinoline GR ACS, Ord. No. 1.07098
Kojic acid [5-hydroxy-2-hydroxymethylpyrone-(4)]
Ethanol abs. GR, Ord. No. 1.00972
Ammonia solution 25% GR, Ord. No. 1.05432

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.

After-treatment: Dry with cold air.

Literature:
H. Thommen, O. Wiss, Z. Ernahrungswiss. **1963**, Suppl. 3, 18.

Chemicals:
2-Diphenylacetyl-1,3-indanedione-1-hydrazone
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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).

Literature:
G.C. Barret, Nature **194**, 1171 (1962).
A. Bettschart, H. Flueck, Pharm. Acta Helv. **31**, 260 (1956).
G. Brante, Nature **163**, 651 (1949).
R. Munier, M. Macheboeuf, Bull. Soc. chim. biol. **31**, 1144 (1949).
R. Munier, Bull. Soc. chim. France **19**, 852 (1952).

Chemicals:
Iodine resublimed GR ACS, ISO, Ord. No. 1.04761
Chloroform GR ISO, Ord. No. 1.02445
Starch soluble GR ISO, Ord. No. 1.01252

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 iodine solution (c = 0.05 mol I_2/L). Dry iodine azide is explosive!

Iodine azide-starch reagent:

Spray solution I: Freshly prepared solution of 1 g sodium azide in 100 ml iodine solution (c = 0.0025 mol/L).

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, Naturwissenschaften **41**, 528 (1954).

Chemicals:
Sodium azide extra pure, Ord. No. 1.06688
Iodine solution 0.05 mol I_2/l Titrisol®, Ord. No. 1.09910
Starch soluble GR ISO, Ord. No. 1.01252

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 resublimed GR ACS, ISO, Ord. No. 1.04761
Potassium iodide GR ISO, Ord. No. 1.05043
Acetic acid 96% GR, Ord. No. 1.00062

163. Iodine - potassium iodide for organic compounds.

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

Literature:
A. Zaffaroni, R.B. Burton, H. Kentmann, Science **111**, 6 (1950).
A. Bettschart, H. Flueck, Pharm. Acta Helv. **31**, 260 (1956).
J. Buechi, H. Schumacher, Pharm. Acta Helv. **32**, 194 (1957).

Chemicals:
Iodine resublimed GR ACS, ISO, Ord. No. 1.04761
Potassium iodide GR ISO, Ord. No. 1.05043

164. Iodine - sulfanilic acid - N-(1-naphthyl)ethylenediamine 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)ethylenediammonium dichloride.

Procedure: Spray with I and subsequently with II.

Literature:
J.M. Bremmer, Analyst **79**, 138 (1954).

Chemicals:
Iodine resublimed GR ACS, ISO, Ord. No. 1.04761
N-(1-Naphthyl)ethylenediamine dihydrochloride GR, Ord. No. 1.06237
Sulfanilic acid GR ACS, Ord. No. 1.00686
Acetic acid 96% GR, Ord. No. 1.00062

165. Iodine - sulfuric acid for organic compounds containing nitrogen, polyethylene glycols and polyethylene glycol derivatives.

Spray solution: Mix equal parts of iodine solution (c = 0.5 mol I_2/l) and 10% sulfuric acid.

Literature:
H. Feltkamp, F. Koch, J. Chromatog. **15**, 314 (1964).

Chemicals:
Iodine solution 0.05 mol I_2/l Titrisol®, Ord. No. 1.09910
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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

Spray solution: 1-5% solution of iron(III) chloride in hydrochloric acid (c = 0.5 mol/L.)

Note: Hydroxamic acids turn red, phenols blue or greenish.

Literature:
K. Fink, R.M. Fink, Proc. Soc. Expl. Bio. Med. **70**, 654 (1949).

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Hydrochloric acid 0.5 mol/l Titrisol® Ord. No. 1.09971

167. Iron(III) chloride - iodine for xanthine derivatives.

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

Literature:
J. Zarnak, S. Pfeiffer, Pharmazie **19**, 216 (1964).

Chemicals:
lron(III) chloride GR, Ord. No. 1.03943
L(+)Tartaric acid GR ACS, ISO, Ord. No. 1.00804

Iodine resublimed GR ACS, ISO, Ord. No. 1.04761
Acetone GR ACS, ISO, Ord. No. 1.00014

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

Spray solution: Mix 1 ml aqueous iron(III) chloride solution (c = 0.5 mol/L) with 50 ml 35% perchloric acid.

After-treatment: Heat 5 min at 60°C. Blow vapours of aqua regia over the layer for intensivation of the colours.

Literature:
S.A. Gordon, R.P. Weber, Plant. Physiol. **26**, 192 (1951).

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Perchloric acid 60% GR ACS, ISO, Ord. No. 1.00518
Hydrochloric acid 25% GR, Ord. No. 1.00316
Nitric acid 65% GR ISO, Ord. No. 1.00456

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

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

Literature:
A. Noirfalise, M.H. Grosjean, J. Chromatog. **16**, 236 (1964).

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Perchloric acid 60% GR ACS, ISO, Ord. No. 1.00518
Nitric acid 65% GR ISO, Ord. No. 1.00456

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 hydrochloric acid (c = 0.2 mol/L).

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

Solution c: Dissolve 5 g sodium metaarsenite in 30 ml sodium hydroxide solution (c = 1 mol/L) at 0°C and mix with 65 ml hydrochloric acid (c = 2 mol/L) with stirring.

Spray solution: Mix 5 parts a, 5 parts b and 1 part c.

Treatment: 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.

Literature:
E. Zappi, J. Chromatog. **31**, 241 (1967).

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Sodium metaarsenite
Sodium hydroxide solution 1 mol/l, Ord. No. 1.09956
Hydrochloric acid 25% GR, Ord. No. 1.00316

171. Iron(III) chloride - sulfosalicylic acid for thiophosphate esters.

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

Spray solution II: 1% solution of sulfosalicylic 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.

Literature:
M. Salamé, J. Chromatog. **16**, 476 (1964).

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
5-Sulfosalicylic acid dihydrate GR ACS, Ord. No. 1.00691
Bromine GR ISO, Ord. No. 1.01948
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

172. Iron(III) chloride - 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.

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

Literature:
W.L. Anthony, W.T. Beher, J. Chromatog. **13**, 567 (1964).

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
1-Butanol GR ACS, ISO, Ord. No. 1.01990
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

173. Iron(III) chloride - sulfuric acid for indole derivatives (Salkowsky reaction).

Spray solution: Mix 3 ml aqueous iron(III) chloride solution (c = 1.5 mol/L) with 100 ml water and add 60 ml 97% sulfuric acid.

After-treatment: Heat 5 min at 60°C. Blow vapours of aqua regia over the layer for intensivation of the colours.

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

Chemicals:
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Hydrochloric acid 25% GR, Ord. No. 1.00316
Nitric acid 65% GR ISO, Ord. No. 1.00456

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 appearence of brown-red spots (iron(III) thiocyanate) shows the presence of peroxide compounds.

Literature:
E. Stahl, Chemiker-Ztg. **82**, 323 (1957).
E. Knappe, D. Peteri, Z. anal. Chem. **190**, 386 (1962).

Chemicals:
Iron(II)sulfate heptahydrate GR ACS, ISO, Ord. No. 1.03965
Ammonium thiocyanate GR ACS, ISO, Ord. No. 1.01213
Acetone GR ACS, ISO, Ord. No. 1.00014

175. Isatin - sulfuric acid for thiophene derivatives.

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

After-treatment: Heating to 120°C is occasionally needed. Variously coloured spots.

Literature:
R.F. Curtis, G.T. Phillips, J. Chromatog. **9**, 366 (1962).

Chemicals:
Isatin for synthesis, Ord. No. 8.20709
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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 a refrigerator.

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

Literature:
J. Barrolier, J. Heilman, E. Watzke, Hoppe-Seylers Z. physiol. Chem. **304**, 21 (1956).

Chemicals:
Isatin for synthesis, Ord. No. 8.20709
Zinc acetate dihydrate GR, Ord. No. 1.08802
Acetic acid 96% GR, Ord. No. 1.00062
2-Propanol GR ACS, ISO, Ord. No. 1.09634

177. Isonicotinic acid hydrazide for Δ^4-3-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.

Literature:
B.P. Lisboa, Acta Endocrinol. **43**, 47 (1963).
B.P. Lisboa, J. Chromatog. **16**, 136 (1964).

Chemicals:
Isonicotinic acid hydrazide
Acetic acid 96% GR, Ord. No. 1.00062
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

178. Kojic acid for metal ions.

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

Note: Inspect fluorescence under UV light.

Literature:
F H. Pollard, J.F.W. McOmie, I.I.M. Elbeih, Nature **163**, 292 (1949).

Chemicals:
Kojic acid [5-hydroxy-2-hydroxymethylpyrone-(4)]
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

179. Lead acetate basic for flavonoids.

Spray solution: 25% aqueous solution of basic lead acetate. Fluoresceing spots in long-wave UV light.

Literature:
L. Hoerhammer, H. Wagner, K. Hein, J. Chromatog. **13**, 235 (1964).
R. Neu, P. Hagedorn, Naturwissenschaften **40**, 411 (1953).

Chemicals:
Lead(II) hydroxide acetate anhydrous, Ord. No. 1.07414

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

Spray solution: 1% solution of lead(IV) acetate in benzene. (Prepare freshly!)

After-treatment: Heat 5 min at 110°C. White spots on brown background.

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

Chemicals:
Lead(IV) acetate for synthesis, Ord. No. 8.07418
Benzene GR ACS, ISO, Ord. No. 1.01783

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 occasional stirring until completely dissolved.

Spray solution II: Dissolve 0.05 g rosaniline base in a mixture of 10 parts glacial acetic acid and 90 parts acetone. 0.1% methanolic fuchsine 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.
K.G. Bergner, H. Sperlich, Z. Lebensm.-Untersuch. u. Forsch. **97**, 253 (1953).

Chemicals:
Lead(II,IV) oxide extra pure, Ord. No. 1.06080
New Fuchsin (C.I. 42520), Ord. No. 1.04041
Acetic acid 96% GR, Ord. No. 1.00062
Acetone GR ACS, ISO, Ord. No. 1.00014
Methanol GR ACS, ISO, Ord. No. 1.06009

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 GR, Ord. No. 1.08789
Methylene blue B (C.I. 52015)
Acetic acid 96% GR, Ord. No. 1.00062
Acetone GR ACS, ISO, Ord. No. 1.00014

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.

Literature:
S. Shibita, M. Takido, O. Tanaka, J. Am. Chem. Soc. **72**, 2789 (1950).

Chemicals:
Magnesium acetate tetrahydrate GR ACS, Ord. No. 1.05819
Methanol GR ACS, ISO, Ord. No. 1.06009

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

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

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 nitric acid (c = 0.05 mol/L).

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.

Literature:
J. Lehmann, V. Karamustafauglu, Scand. J. Clin. & Lab. Invest. **14**, 554 (1962).

C. Spray solution I (Mercury[II]sulfate solution): Suspend 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.

Literature:
I. Sunshine, E. Rose, J. Le Beau, Clin. Chem. **9**, 312 (1963).

Chemicals:
Mercury(II) chloride sublimed GR ACS, Ord. No. 1.04419
Diphenylcarbazone Reag. Ph Eur, Ord. No. 1.59699
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Mercury(II) nitrate monohydrate GR ACS, Ord. No. 1.04439
Nitric acid 65% GR ISO, Ord. No. 1.00456
Mercury(II) oxide yellow GR ACS, Ord. No. 1.04461
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Chloroform GR ISO, Ord. No. 1.02445

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

Spray solution I: Dissolve 13.55 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 1. 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.

Literature:
R. Tschesche, R. Petersen, Chem. Ber. **87**, 269 (1953).

Chemicals:
Mercury(II) chloride sublimed GR ACS, Ord. No. 1.04419
Potassium iodide GR ISO, Ord. No. 1.05043
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Zinc chloride GR ACS, ISO, Ord. No. 1.08816
Ammonia solution 25% GR, Ord. No. 1.05432

186. Mercury(I) nitrate for barbiturates.

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

Literature:
J. Baeumler, Mitt. Gebiete Lebensm. u. Hygiene **48**, 135 (1957).
R. Deininger, Arzneimittel-Forsch. **5**, 472 (1955).

Chemicals:
Mercury(I) nitrate dihydrate GR ACS, Ord. No. 1.04437

187. 4-Methoxy-2-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: Sodium hydroxide (c = 2 mol/L).

Procedure: Spray with I, and then with II. Blue spots on orange background.

Literature:
N. Schmall, C.W. Pifer, E.G. Wollish, Anal. Chem. **25**, 1486 (1953).

Chemicals:
4-Methoxy-2-nitroaniline for synthesis, Ord. No. 8.06225
Acetic acid 96% GR, Ord. No. 1.00062
Sodium hydroxide solution 2 mol/l, Ord. No. 1.09136
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Sodium nitrite GR ACS, Ord. No. 1.06549

188. Methylene blue for sulfate esters of steroids.

Spray solution: Dissolve 0.025 g methylene blue in 100 ml sulfuric acid (c = 0.025 mol/L). 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.

Literature:
O. Crépy, O. Judas, B. Lachese, J. Chromatog. **16**, 340 (1964).

Chemicals:
Methylene blue B (C.I. 52015)
Acetone GR ACS, ISO, Ord. No. 1.00014
Chloroform GR ISO, Ord. No. 1.02445

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.

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

Literature:
I.M. Hais, K. Macek, Handbuch der Papierchromatographie I, p. 759, G. Fischer, Jena 1958.

Chemicals:
4-Methylumbelliferone
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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.

Literature:
L.F. Krzeminsky, W.A. Landmann, J. Chromatog. **10**, 525 (1963).

Chemicals:
4-Dimethylaminoazobenzene (C.I. 11020) indicator, Ord. No. 1.03055
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

After-treatment: Heating at 100-110°C often produces colour changes.

Literature:
E. Stahl, P.J. Schorn, Hoppe-Seylers Z. physiol. Chem. **325**,263 (1961).

Chemicals:
Mercury GR, Ord. No. 1.04403
Nitric acid fuming 100% (1.52) GR, Ord. No. 1.00455

192. Molybdatophosphoric acid (Phosphomolybdic acid).

100 ml ready to use spray solution for chromatography (c = 8% in 2-propanol).

Ord. No. 1.00480

193. Molybdatophosphoric acid for reducing compounds, lipids, sterols and steroids.

A. Spray solution: 5-10% ethanolic molybdatophosphoric acid.

After-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.
Literature:
D. Kritschevsky, M.C. Kirk, Arch. Biochem. Biophys. **35**, 346 (1952).
A. Seher, Fette u. Seifen, Anstrichmittel **61**, 345 (1959).

Chemicals:
Molybdatophosphoric acid hydrate GR ACS, Ord. No. 1.00532
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ethylene glycol monomethyl ether for synthesis, Ord. No. 8.00858
Ammonia solution 25% GR, Ord. No. 1.05432

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.

Literature:
B. Hoffmann, J. Chromatog. **34**, 269 (1968).

Chemicals:
Molybdatophosphoric acid hydrate GR ACS, Ord. No. 1.00532
Potassium hydroxyde pellets GR, Ord. No. 1.05033
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Ammonia solution 25% GR, Ord. No. 1.05432
Methanol GR ACS, ISO, Ord. No. 1.06009

195. Morin for aluminium ions,

Spray solution: 1% solution of morin in glacial acetic acid. Pronounced light green fluorescence in long-wave UV light.

Literature:
T.V. Toribara, R.E. Sherman, Anal. Chem. **25**, 1954 (1953).

Chemicals:
Morin dihydrate (C.I. 75660) GR, Ord. No. 1.06098
Acetic acid 96% GR, Ord. No. 1.00062

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.

After-treatment: Heat 5-10 min at 100-105°C.

Literature:
V. Prey, H. Berbaek, M. Kausz, Mikrochim. Acta **1961**, 968.

Chemicals:
1,3-Naphthalenediol
ortho-Phosphoric acid. 85% GR ISO, Ord. No. 1.00573
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

197. 1,3-Naphthalenediol - sulfuric acid for sugars.

Solution a: 0.2 % ethanolic solution of 1,3-naphthalenediol.

Solution b: 20% sulfuric acid.

Spray solution: Prepare freshly before use a mixture of equal parts a and b.

After-treatment: Heat 5-10 min at 100-105°C.

Literature:
M. Lato, E. Brunelli, G. Ciuffini, J. Chromatog. **34**, 26 (1968).

Chemicals:
1,3-Naphthalenediol
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

198. 1,3-Naphthalenediol- trichloroacetic acid for sugars and uronic acids.

Solution a: 0.2% ethanolic 1,3-naphthalenediol solution.

Solution b: 20% aqueous trichloroacetic acid solution.

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

After-treatment: For ketoses heat 5-10 min at 100-105°C, for uronic acids 10-15 min in a moist atmoshphere (water bath) at 70-80°C.

Note: The presence of collidine and pyridine interferes with the colour reaction. Instead of 1,3-naphthalenediol resorcinol, orcinol (3,5-dihydroxytoluene), phloroglucinol or 1-naphthol may be used. One part trichloroacetic may be replaced by 1/10 part phosphoric acid.

Literature:
S.M. Partridge, Biochem. J. **42**, 238 (1948).

Chemicals:
1,3-Naphthalenediol
Trichloroacetic acid GR ACS, Ord. No. 1.00807
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1-Naphthol GR, Ord. No. 1.06223
3,5-Dihydroxytoluene monohydrate for synthesis, Ord. No. 8.20933
Phloroglucinol (1,3,5-trihydroxybenzene) GR, Ord. No. 1.07069
Resorcinol GR, Ord. No. 1.07593
ortho-Phosphoric acid. 85% GR ISO, Ord. No. 1.00573

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

Spray solution I: Solution of 0.1% 1-naphthol in sodium hydroxide solution (c = 1 mol/L).

Spray solution II: Mixture of 100 ml 5% aqueous sodium hydroxide and 2 ml. bromine.

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.

Literature:
R. Acher, C. Cracker, Biochem. biophys. Acta **9**, 704 (1952).

Chemicals:
1-Naphthol GR, Ord. No. 1.06223
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Bromine GR ISO, Ord. No. 1.01948
Sodium hypochlorite solution, Ord. No. 1.05614
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

After-treatment: Heat 3-6 min at 100°C.

Literature:
H. Jacin, A.R. Mishkin, J. Chromatog. **18**, 170 (1965).

Chemicals:
1-Naphthol GR, Ord. No. 1.06223
Sulfuric acid. min. 95-97% (1.84) GR, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

201. 1,2-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.

Literature:
D. Mueting, Naturwissenschaften **39**, 303 (1952).
K.V. Giri et al., Naturwissenschaften **39**, 548 (1952).

Chemicals:
1,2-Naphthoquinone-4-sulfonic acid sodium salt GR, Ord. No. 1.06531
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391

202. 1,2-Naphthoquinone-sulfonic acid sodium salt for aromatic amines.

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.

Literature:
R.B. Smyth, G.G. McKeown, J. Chromatog. **16**, 454 (1964).

Chemicals:
1,2-Naphthoquinone-4-sulfonic acid sodium salt GR, Ord. No. 1.06531
Acetic acid 96% GR, Ord. No. 1.00062

203. 1,2-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.

Literature:
E. Richter, J. Chromatog. **18**, 164 (1965).
C.W.M. Adams, Nature **192**, 331 (1961).

Chemicals:
1,2-Naphthoquinone-4-sulfonic acid sodium salt GR, Ord. No. 1.06531
Perchloric acid 60% GR ACS, ISO, Ord. No. 1.00518
Formaldehyde solution min. 37% GR, Ord. No. 1.04003
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

204. l-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 red-brown colour.

Literature:
R.G. Rice, G.J. Keller, J.G. Kirchner, Anal. Chem. **23**, 194 (1951).

Chemicals:
1-Naphthylamine GR, Ord. No. 1.06245
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Methanol GR ACS, ISO, Ord. No. 1.06009
Potassium hydroxide pellets GR, Ord. No. 1.05033

205. Nessler's reagent for alkaloids.

Spray solution: Nessler's reagent (s. spray reagent No. 284).

Note: Apomorphine, hydrastinine and physostigmine show colour reaction.

Literature:
O.E. Schultz, D. Strauss, Arzneimittel-Forsch. **5**, 342 (1955).

Chemicals:
Nessler's reagent

206. Ninhydrin

100 ml ready to use spray solution for chromatography (c = ca. 0.2% in 2-propanol).

Ord. No. 1.06705

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.

After-treatment: Heat at 110°C until maximal visualization of the spots. For pantothenic acid heat at 160°C.

Literature:
R.A. Famy, A. Niederwieser, G. Pataki, M. Brenner, Helv. Chim. Acta **44**, 2022 (1961).
A.R. Patton, P. Chism, Anal. Chem. **23**, 1683 (1951).

Chemicals:
Ninhydrin GR, Ord. No. 1.06762
Acetic acid 96% GR, Ord. No. 1.00062
1-Butanol GR ACS, ISO, Ord. No. 1.01990
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

Literature:
E. Kawerau, T. Wieland, Nature **168**, 77 (1951).

Chemicals:
Copper(II) nitrate trihydrate extra pure, Ord. No. 1.02752
Nitric acid 65% GR ISO, Ord. No. 1.00456
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

208. Ninhydrin - cadmium acetate for amino acids and amines.

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.

After-treatment: Heat 20 min at 120°C. This method is more suitable for detecting heterocyclic amines than the 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.

Literature:
J. Barrolier, J. Heilmann, E. Watzke, Hoppe-Seylers Z. physiol. Chem. **309**, 219 (1957).

Chemicals:
Cadmium acetate dihydrate GR, Ord. No. 1.02003
Ninhydrin GR, Ord. No. 1.06762
Acetic acid 96% GR, Ord. No. 1.00062
Acetone GR ACS, ISO, Ord. No. 1.00014
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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 2,4,6-trimethylpyridine.

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

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

After-treatment: Heat the chromatogram until the colour development is just beginning. In transmitted light the gradual intensification of colours on the warm plate can be observed. Some amino acids show first small points of colours only, they should be marked with a sharp pencil. In this way one can often detect individual spots which later merge into each other. Some amino acids show characteristic colours. They differ amongst themselves also in the speed with which coloured products are formed.

Literature:
M. Brenner, A. Niederwieser, Experientia **16**, 378 (1960).

Chemicals:
Ninhydrin GR, Ord. No. 1.06762

Copper(II) nitrate trihydrate extra pure, Ord. No. 1.02752
Acetic acid 96% GR, Ord. No. 1.00062
2,4,6-Trimethylpyridine GR, Ord. No. 1.02635
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

210. Ninhydrin - tin(II) chloride for amines.

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.

Literature:
R.J. Block, Anal. Chem. **22**, 1327 (1950).

Chemicals:
Ninhydrin GR, Ord. No. 1.06762
Tin(II) chloride dihydrate GR ACS, Ord. No. 1.07815
2-Propanol GR ACS, ISO, Ord. No. 1.09634

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 concentration also in TLC for the identification of other organic compounds. Frequently fluorescent spots appear only after prolonged heating at 120°C.

Literature:
H. Schmid, J. Kebrle, P. Karrer, Helv. Chim. Acta **35**, 1864 (1952).

Chemicals:
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Nitric acid 65% GR ISO, Ord. No. 1.00546

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

Spray solution I: Potassium hydroxide solution in ethanol (c = 0.5 mol/L).

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.

Literature:
J.W. Copius-Peereboom, J. Chromatog. **4**, 323 (1960).
D. Braun, Chimia **19**, 77 (1965).

Chemicals:
4-Nitroaniline, Ord. No. 1.06760
Sodium nitrite GR ACS, Ord. No. 1.06549
Hydrochloric acid 25% GR, Ord. No. 1.00316
Potassium hydroxide pellets GR, Ord. No. 1.05033
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

Literature:
A. Sturm, H.W. Scheja, J. Chromatog. **16**, 194 (1964).

Chemicals:
4-Nitroaniline, Ord. No. 1.06760
Sodium nitrite GR ACS, Ord. No. 1.06549
Potassium carbonate GR ACS, ISO, Ord. No. 1.04928

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

Spray solution: Mix under cooling 5 ml 0.5% 4-nitroaniline solution in hydrochloric acid (c = 2 mol/L) with 0.5 ml 5% aqueous sodium nitrite solution and add 15 ml 20% aqueous sodium acetate solution.

Literature:
H.G. Bray, W.V. Thorpe, K. White, Biochem. J. **46**, 271 (1950).
T. Swain, Biochem. J. **53**, 200 (1953).
C.F. van Sumere, G. Wolf, H. Teuchy, J. Kint, J. Chromatog. **20**, 48 (1965).

Chemicals:
4-Nitroaniline, Ord. No. 1.06760
Sodium nitrite GR ACS, Ord. No. 1.06549
Sodium acetate GR, Ord. No. 1.06267
Hydrochloric acid 25% GR, Ord. No. 1.00316

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

4-Nitrophenyldiazonium fluoborate: 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 (tetrafluoroboric 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 fluoborate solution in acetone.

Spray solution II: 0.1% methanolic potassium hydroxide solution.

Procedure: Spray with I, then with II.

Literature:
J.H. Freeman, Anal. Chem. **24**, 955 (1952).
H. Seeboth, H. Goersch, Chem. Techn. **15**, 294 (1963).

Chemicals:
4-Nitroaniline, Ord. No. 1.06760
Sodium nitrite GR ACS, Ord. No. 1.06549
Tetrafluoroboric acid, Ord. No. 1.00171
Potassium hydroxide 0.5 mol/l in methanol, Ord. No. 1.09351
Acetone GR ACS, ISO, Ord. No. 1.00014
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Diethyl ether GR ACS, Ord. No. 1.00921
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

216. 2-Nitroso-1-naphthol-4-sulfonic acid for iron ions.

Spray solution: 0.05% solution of 2-nitroso-1-naphthol-4-sulfonic acid in 70% ethanol.

After-treatment: Respray with 25% ammonia solution or place the chromatogram into a chamber with ammonia vapours. Green spots.

Literature:
G.B. Heisig, F.H. Pollard, Anal. Chim. Acta **16**, 234 (1957).

Chemicals:
2-Nitroso-1-naphthol-4-sulfonic acid
Ammonia solution 25% GR, Ord. No. 1.05432
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

217. Orcinol - iron(III) chloride - sulfuric acid for sugars.

Solution a: Dissolve 1 g iron(III) chloride in 100 ml 10% sulfuric acid.

Solution b: 6% ethanolic orcinol (3,5-dihydroxytoluene) solution.

Spray solution: Mix freshly before use 10 ml a and 1 ml b.

After-treatment: Heat 10-15 min at 100°C.

Chemicals:
3,5-Dihydroxytoluene monohydrate for synthesis, Ord. No. 8.20933
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

218. Palladium(II) chloride 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.

Literature:
J. Baeumler, S. Rippstein, Helv. Chim. Acta **44**, 1162 (1961).

Chemicals:
Palladium (II) chloride anhydrous, Ord. No. 8.07110
Hydrochloric acid 25% GR, Ord. No. 1.00316

219. Paraformaldehyde - phosphoric acid for Solanum steroid alkaloids and steroid sapogenins.

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

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

Chemicals:
Paraformaldehyde extra pure DAC, BPC, USP, Ord. No. 1.04005
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

220. Perchloric acid for steroids and bile acids.

A. Spray solution (for steroids): 20% aqueous perchloric acid solution.

B. Spray solution (for bile acids): 60% aqueous perchloric acid solution.

After-treatment: Heat the chromatogram for about 10 min at 150°C until maximal visualisation of the spots. Inspect also in long-wave UV light.

Literature:
H. Metz, Naturwissenschaften **48**, 569 (1961).
S. Hara, M. Takeuchi, J. Chromatog. **11**, 565 (1963).

Chemicals:
Perchloric acid 60% GR ACS, ISO, Ord. No. 1.00518

221. Perchloric acid - iron(III) chloride for indole derivatives.

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

Note: No reaction with isatin and other oxindole derivatives.

Literature:
T.A. Bennet-Clark, M.S. Tambiah, N.P. Kefford, Nature **169**, 452 (1951).

Chemicals:
Perchloric acid 60% GR ACS, ISO, Ord. No. 1.00518
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943

222. Phenol - sulfuric acid for sugars.

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

After-treatment: Heat 10-15 min at 110°C. Brown spots.

Literature:
S. Adachi, J. Chromatog. **17**, 295 (1965).

Chemicals:
Phenol GR ACS, Ord. No. 1.00206
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

223. *m*-Phenylenediamine for reducing sugars.

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

After-treatment: Heat briefly at 105°C.

Note: Intensely fluorescent colours in UV light.

Literature:
S.S. Chernick, I.L. Chaikoff, S. Abraham, J. Biol. Chem. **193**, 793 (1951).

Chemicals:
m-Phenylenediamine dihydrochloride
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

After-treatment: Heat at 100-110°C. Yellow to orange spots.

Literature:
B.P. Lisboa, Acta Endocrinol. **43**, 47 (1963).
B.P. Lisboa, J. Chromatog. **16**, 136 (1964).

Chemicals:
p-Phenylenediamimonium dichloride for synthesis, Ord. No. 8.22297
Phthalic acid GR, Ord. No. 1.09611
1-Butanol GR ACS, ISO, Ord. No. 1.01990

225. 1,2-Phenylenediamine - sulfuric acid for dehydroascorbic acid.

Spray solution: Dissolve 0.1 g 1,2-phenylenediamine in a mixture of 50 ml sulfuric acid (c = 0.05 mol/L) and 50 ml ethanol.

Literature:
S. Ogawa, J. Pharm. Soc. Japan **73**, 59 (1953).

Chemicals:
1,2-Phenylenediamine, Ord. No. 8.09721
Sulfuric acid 0.05 mol/l, Ord. No. 1.09984
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

226. 1,2-Phenylenediamine - trichloroacetic acid for α-keto acids.

Spray solution: Dissolve 0.05 g 1,2-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.

Literature:
T. Wieland, F. Fischer, Naturwissenschaften **36**, 219 (1949).
O. Wiss, Hoppe-Seylers Z. physiol. Chem. **293**, 106 (1953).

Chemicals:
1,2-Phenylenediamine, Ord. No. 8.09721
Trichloroacetic acid GR ACS, Ord. No. 1.00807

227. Phenylfluorone for germanium.

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

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

Chemicals:
Phenylfluorone
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

228. Phenylhydrazine for dehydroascorbic acid.

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

Chemicals:
Phenylhydrazine GR, Ord. No. 1.07251
Sodium acetate anhydrous GR ACS, Ord. No. 1.06268

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 heating times 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.

Literature:
R. Neher, A. Wettstein, Helv. Chim. Acta **34**, 2278 (1951).

Chemicals:
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
Methanol GR ACS, ISO, Ord. No. 1.06009

230. Phosphoric acid - bromine for digitalis glycosides.

Spray solution I: 10% aqueous phosphoric acid solution.

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

Literature:
L. Fauconnet, M. Waldesbuehl, Pharm. Acta Helv. **38**, 423 (1963).

Chemicals:
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573
Potassium bromide GR ACS, Ord. No. 1.04905
Potassium bromate GR ACS, ISO, Ord. No. 1.04912
Hydrochloric acid 25% GR, Ord. No. 1.00316

231. Picric acid for epoxides.

Spray solution: 0.05 M ethanolic picric acid solution.

After-treatment: Place the sprayed chromatogram 30 min into a chamber with ether/ethanol/glacial acetic acid (80+20+1) and subsequently 1-2 min into a chamber with ammonia vapours. Orange spots on yellow background.

Literature:
J.A. Fioriti, R.J. Sims, J. Chromatog. **32**, 761 (1968).

Chemicals:
Picric acid desensitized (cont. about 30% water) GR, Ord. No. 1.00623
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Diethyl ether GR ACS, Ord. No. 1.00921
Acetic acid 96% GR, Ord. No. 1.00062
Ammonia solution 25% GR, Ord. No. 1.05432

232. Picric acid - alkali for creatinine, glycocyamidine (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.

Literature:
R. Williams, Biochem. Inst. Stud. IV, University of Texas, Publ., Austin/Texas No. 5109, 205 (1951).

Chemicals:
Picric acid desensitized (cont. about 30% water) GR, Ord. No. 1.00623
Potassium hydroxide pellets GR, Ord. No. 1.05033
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

233. Picric acid - perchloric acid for $\Delta^5$3β-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.

Literature:
W.R. Eberlein, J. Clin. Endocrinol. **25**, 288 (1965).

Chemicals:
Picric acid desensitized (cont. about 30% water) GR, Ord. No. 1.00623
Acetic acid 96% GR, Ord. No. 1.00062
Perchloric acid 70-72% GR ACS, ISO, Ord. No. 1.00519

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

Spray solution: 0.5-1.5% ethanolic picryl chloride solution.

After-treatment: Place the chromatogram into a chamber with ammonia.

Literature:
W.F.J. Cuthbertson, D.M. Ireland, W. Wolff, Biochem. J. **55**, 669 (1953).
J.M. Bremner, Analyst **79**, 198 (1954).

Chemicals:
Picryl chloride (2-chloro-1,3,5-trinitrobenzene)
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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.

Literature:
J. Borecky, J. Chromatog. **2**, 612 (1959).

Chemicals:
Pinacryptol yellow LAB, Ord. No. 1.09723

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

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

Literature:
F.H. Burstall, G.R. Davies, R.P. Linstead, R.A. Wells, J. Chem. Soc. **1950**, 516.

Chemicals:
Potassium hexacyanoferrate(II) trihydrate GR ACS, ISO, Ord. No. 1.04984

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.

Treatment: Spray with I and dry at 100°C. After cooling spray with II and heat 30 min at 150°C. Spray with III for intensivation of the yellow and red spots. This reaction may be applied after detection with mercury(I) nitrate.

Literature:
H. Weichsel, Mikrochim. Acta **1965**, 325.

Chemicals:
Potassium hexacyanoferrate(II) trihydrate GR ACS, ISO, Ord. No. 1.04984
Ammonium chloride GR, Ord. No. 1.01145
Potassium carbonate GR ACS, ISO, Ord. No. 1.04928
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Hydrogen peroxide 30% H_2O_2 (Perhydrol®) GR ISO, Ord. No. 1.07209

238. Potassium hexacyanoferrate(III) for adrenaline and derivatives.

Spray solution: Dissolve 0.1 g potassium hexacyanoferrate(III) in 100 ml 0.5% sodium hydroxide solution. Spots show red colour.

Literature:
A.H. Beckett, M.A. Beaven, A.E. Robinson, J. Pharm. Pharmacol. **12**, 203 T (1960).

Chemicals:
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Sodium hydroxide solution min. 10% (1.11) GR, Ord. No. 1.05588

239. Potassium hexacyanoferrate(III) for vitamin B_1 (thiochrome reaction).

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

Solution b: 15% aqueous sodium hydroxide solution.

Spray solution: Mix 1.5 ml a with 20 ml water and add 10 ml b. After drying inspect in long-wave UV light.

Literature:
D. Siliprandi, N. Siliprandi, Biochim. et biophys. Acta **14**, 52 (1954).

Chemicals:
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Sodium hydroxide solution min. 27% (1.30) GR, Ord. No. 1.05591

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.

After-treatment: Spray with hydrochloric acid (c = 2 mol/L) for intensivation of colours.

Literature:
G.M. Barton, R.S. Evans, J.A.F. Gardner, Nature **170**, 249 (1952).
M. Gillio-Tos, S.A. Previtera, A. Vimercati, J. Chromatog. **13**, 571 (1964).
H. Wagner, L. Hoerhammer, H. Nufer, Arzneimittel-Forsch. **15**, 453 (1965).

Chemicals:
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Iron(III) chloride hexahydrate GR, Ord. No. 1.03943
Hydrochloric acid 25% GR, Ord. No. 1.00316

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) GR ACS, ISO, Ord. No. 1.04973
di-Sodium hydrogen phosphate solution 1/15 mol/l, Ord. No. 1.06587

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.

Literature:
H.J. Kupferberg, A. Burghalter, E.L. Way, J. Chromatog. **16**, 558 (1964).

Chemicals:
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Potassium hexacyanoferrate(II) trihydrate GR ACS, ISO, Ord. No. 1.04984

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 in long-wave UV light.

Literature:
Z. Ledinova, I.M. Hais, Ceskolov. farm. **9**, 401 (1960).
L. Hoerhammer, H. Wagner, G. Bittner, Arzneimittel-Forsch. **13**, 537 (1963).

Chemicals:
Potassium hydroxide pellets GR, Ord. No. 1.05033
Methanol GR ACS, ISO, Ord. No. 1.06009

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 vapours. After a few minutes place the plate into a second chamber with hydrogen sulfide gas. **Caution! Hydrogen sulfide is poisonous and explosive!**

Literature:
H. Seiler, M. Seiler, Helv. Chim. Acta **43**, 1939 (1960).

Chemicals:
Potassium iodide GR ISO, Ord. No. 1.05043
Ammonia solution 25% GR, Ord. No. 1.05432
Iron(II) sulfide fused sticks, Ord. No. 1.03956
Hydrochloric acid 25% GR, Ord. No. 1.00316

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 spatula-tipful of zinc powder.

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

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

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

Chemicals:
Potassium iodide GR ISO, Ord. No. 1.05043
Zinc powder GR, Ord. No. 1.08789
Starch soluble extra pure, Ord. No. 1.01253
Acetic acid 96% GR, Ord. No. 1.00062

246. Potassium iodine platinate 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.

Literature:
J. Smith, Chromatographic and Electrophoretic Techniques, W. Heinemann, London 1969, Vol. I, p. 519.

Chemicals:
Potassium iodide GR ISO, Ord. No. 1.05043
Hexachloroplatinic(IV) acid solution about 10% GR, Ord. No. 1.07341

247. Potassium iodine platinate 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.

Literature:
R. Munier, Bull soc. chim. France **19**, 852 (1952).
R. Hilz, F.F. Castano, G.A. Lightbourne, J. Lab. Clin. Med. **54**, 632 (1959).

Chemicals:
Hexachloroplatinic(IV) acid solution about 10% GR, Ord. No. 1.07341
Potassium iodide GR ISO, Ord. No. 1.05043

248. Potassium iodine platinate for ketosteroids. PC.

Spray solution: Add to 5 ml 5% hexachloroplatinic(IV)acid solution in hydrochloric acid (c = 1 mol/L) 45 ml 10% aqueous potassium iodide solution and 100 ml water. The reagent is stable for some time when stored in the dark.

After-treatment: After spraying rinse out the excess reagent with water.

Literature:
R.T. Burton, A. Zaffaroni, E.H. Keutmann, J. Clin. Endocrinol. **8**, 618 (1958).

Chemicals:
Potassium iodide GR ISO, Ord. No. 1.05043
Hexachloroplatinic(IV) acid hexahydrate, Ord. No. 8.07340
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970

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.

Literature:
O.B. Maximov, L.S. Panthinkhina, J. Chromatog, **20**, 150 (1965).
I.M. Hais, K. Macek, Papierchromatographie I, G. Fischer, Jena 1958, p. 735.

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082
Sodium carbonate anhydrous GR ISO, Ord. No. 1.06392

250. Potassium permanganate alkaline for sugars and polyalcohols.

Spray solution: Dissolve 0.5 g potassium permanganate in 100 ml sodium hydroxide solution (c = 1 mol/L).

After-treatment: After spraying heat the plate at 100°C.

Literature:
G.W. Hay, B.A. Lewis, F. Smith, J. Chromatog. **11**, 479 (1963).

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082
Sodium hydroxide solution 1 mol/l Titrisol®, Ord. No. 1.09956

251. Potassium permanganate neutral for easily oxidisable compounds.

Spray solution: 0.05% aqueous potassium permanganate solution.

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082

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**!

Literature:
H. Ertel, L. Horner, J. Chromatog. **7**, 268 (1962).

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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.

After-treatment: Place the plate into a chamber with ammonia vapours.

Literature:
H. Seiler, M. Seiler, Helv. Chim. Acta **44**, 939 (1961).
F.W.H.M. Merkus, Pharm. Weekblad **98**, 947 (1963).

Chemicals:
1-(2-Pyridylazo)-2-naphthol (PAN) metal indicator, Ord. No. 1.07531
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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 (by volume).

Spray solution II: Mix 8 ml 0.8% aqueous cobalt(II) nitrate solution with 4 ml acetate buffer solution (c = 0.2 mol/L; 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.

Literature:
O. Crépy, O. Judas, B. Lachese, J. Chromatog. **16**, 340 (1964).

Chemicals:
1-(2-Pyridylazo)-2-naphthol (PAN) metal indicator, Ord. No. 1.07531
Cobalt(II) nitrate hexahydrate GR, Ord. No. 1.02536
Acetate buffer solution pH 4.66, Ord. No. 1.07827
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Dichloromethane GR ACS, ISO, Ord. No. 1.06050

255. Quercetin for cations of the hydrogen sulfide group, aluminium, magnesium, uranyl and tungstate ions.

Spray solution: 0.2% ethanolic quercetin solution.

After-treatment: Spray with 25% ammonia solution or place into a chamber with ammonia. In long-wave UV light fluorescing spots.

Literature:
A. Weiss, S. Fallab, Helv. Chim. Acta **37**, 1253 (1954).
E. Pfeil, A. Friedrich, T. Wachsmann, Z. anal. Chem. **158**, 429 (1957).

Chemicals:
Quercetin cryst. LAB, Ord. No. 1.07546
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

256. Quinalizarin for cations.

Spray solution: 0.05% solution of quinalizarin in 70% ethanol.

After-treatment: Place the chromatogram into a chamber saturated with ammonia vapours.

Literature:
O.H. Johnson, H.H. Krause, Anal. Chim. Acta **11**, 128 (1954).

Chemicals:
1,2,5,8-Tetrahydroxyanthraquinone (quinalizarin)
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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 for synthesis, Ord. No. 8.02410
1-Butanol GR ACS, ISO, Ord. No. 1.01990
Pyridine GR ACS, Ord. No. 1.09728

258. Resorcinol - zinc chloride - sulfuric acid for plasticisers (especially phthalate esters).

Spray solution I: Add to a 20% ethanolic resorcinol solution some zinc chloride.

Spray solution II: Sulfuric acid (c = 2 mol/L).

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-Peereboom, J. Chromatog. **4**, 323 (1960).
D. Braun, Chimia (Switz.) **19**, 77 (1965).

Chemicals:
Resorcinol GR, Ord. No. 1.07593
Zinc chloride GR ACS, ISO, Ord. No. 1.08816
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Potassium hydroxide pellets GR, Ord. No. 1.05033

259. Resorcyl aldehyde - sulfuric acid for 16-dehydrosteroids.

Solution a: 0.5% solution of resorcyl 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.

After-treatment: Heat at 100-110°C until maximal visualisation of the spots.

Literature:
D.B. Gower, J. Chromatog. **14**, 424 (1964).

Chemicals:
Resorcyl aldehyde
Acetic acid 96% GR, Ord. No. 1.00062
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

260. Rhodamine B

100 ml ready to use spray solution for chromatography (c = ca. 0.2% in ethanol).

Ord. No. 1.07602

261. Rhodamine B, general spray reagent.

Spray solution: 0.025-0.25% ethanolic solution of rhodamine B. Inspect in long-wave UV light.

Literature:
H.P. Kaufmann, J. Budwig, Fette u. Seifen, Anstrichmittel **53**, 390 (1951).

Chemicals:
Rhodamine B (C.I. 45170) GR, Ord. No. 1.07599
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

262. Rhodamine 6 G for lipids.

Spray solution: Dissolve 0.001 g rhodamine 6 G in 100 ml acetone. Inspect in long-wave UV light.

Literature:
R.F. Witter, G.V. Marinetti, A. Morrison, Arch. Biochem. Biophys. **68**, 15 (1957).

Chemicals:
Rhodamine 6 G
Acetone GR ACS, ISO, Ord. No. 1.00014

263. Rhodanine for carotenoid aldehydes.

Spray solution I: 1-5% ethanolic solution of rhodanine.

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. Hegedues, Chimia (Switz.) **14**, 18 (1960).

Chemicals:
Rhodanine
Ammonia solution 25% GR, Ord. No. 1.05432
Sodium hydroxide solution min. 27% (1,3) GR, Ord. No. 1.05591

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.

Literature:
T.V. Arden, F.H. Burstall, G.R. Davies, J.A. Lewis, R.P. Linstead, Nature **162**, 691 (1948).

Chemicals:
Rhodizonic acid disodium salt indicator, Ord. No. 1.06595
Ammonia solution 25% GR, Ord. No. 1.05432

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 vapours.

Literature:
F.W.H.M. Merkus, Pharm. Weekblad **98**, 955 (1963).
J.A. Lewis, J.M. Griffiths, Analyst **76**, 388 (1951).

Chemicals:
Rubeanic acid GR, Ord. No. 1.00629
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432

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.

Literature:
W.J. Burke, A.D. Potter, R.M. Parkhurst, Anal. Chem. **32**, 727 (1960).

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Acetone GR ACS, ISO, Ord. No. 1.00014

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 vapours (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% sodium thiosulfate solution. After rinsing for 2 hours under running water dry the chromatogram.

Literature:
C. Petronici, G. Safina, Chem. Abstr. **47**, 11297 (1953).

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Sodium thiosulfate pentahydrate GR ACS, ISO, Ord. No. 1.06516
Acetone GR ACS, ISO, Ord. No. 1.00014
Ammonia solution 25% GR, Ord. No. 1.05432

268. Silver nitrate - ammonia for reducing substances (Tollens or Zaffaroni reagent).

Solution a: Silver nitrate solution (c = 0.1 mol/L).

Solution b: Ammonia solution (c = 5 mol/L).

Spray solution: Mix a and b in the ratio 1:5 freshly before use.
Caution! Formation of explosive silver azide by prolonged standing.

After-treatment: Heat 5-10 min at 105°C until the dark spots have become most intense.

Literature:
A.C. Bath-Smith, R.G. Westall, Biochim. et biophys. Acta **4**, 427 (1950).

Chemicals:
Silver nitrate solution 0.1 mol/l, Ord. No. 1.09081
Ammonia solution 25% GR, Ord. No. 1.05432

269. Silver nitrate - ammonia - fluorescein for halogen ions.

Spray solution I: Dissolve 1 g silver nitrate in 100 ml ammonia solution (c = 0.5 mol/L).

Spray solution II: 0.1% ethanolic fluorescein solution.

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

Literature:
H. Seiler, T. Kaffenberger, Helv. Chim. Acta **44**, 1282 (1961).

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Fluorescein (C.I. 45350)
Ammonia solution 25% GR, Ord. No. 1.05432
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

270. Silver nitrate - ammonia - sodium chloride for thioacids.

Spray solution I: Mix freshly before use 50 ml silver nitrate solution (c = 0.1 mol/L) with 50 ml 10% ammonia solution.
Longer standing may lead to **formation of explosive silver azide**!

Spray solution II: 10% 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:
Silver nitrate solution 0.1 mol/l, Ord. No. 1.09081

Ammonia solution 25% GR, Ord. No. 1.05432
Sodium chloride GR ACS, ISO, Ord. No. 1.06404

271. Silver nitrate - ammonia - sodium methoxide for sugars.

Solution a: 0.3% 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.

After-treatment: Heat 10 min at 110°C.

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Sodium rods, Ord. No. 1.06260
Methanol GR ACS, ISO, Ord. No. 1.06009
Ammonia solution 25% GR, Ord. No. 1.05432

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% 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.

Literature:
H. Michl, F. Harberler, Mh. Chem. **85**, 779 (1954).

Chemicals:
Bromophenol blue indicator pH 3.0-4.6 ACS, Ord. No. 1.08122
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Acetone GR ACS, ISO, Ord. No. 1.00014

273. Silver nitrate - fluorescein for alkyl- and arylsulfonic acids.

Solution a: 10% aqueous silver nitrate solution.

Solution b: 0.2% ethanolic fluorescein sodium solution.

Spray solution: Mix freshly before use 10 ml a and 50 ml b. Yellow spots on salmon-pink background.

Literature:
F.H. Pollard, G. Nicklas, K.W.C. Burton, J. Chromatog. **8**, 507 (1962).
C.M. Coyne, G.A. Maw, J. Chromatog. **14**, 552 (1964).

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Fluorescein sodium (C.I. 45350) extra pure, Ord. No. 1.03992
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

274. Silver nitrate - formaldehyde for chlorinated insecticides (e.g. dieldrin, aldrin and lindane).

Spray solution I: Silver nitrate solution (c = 0.05 mol/L).

Spray solution II: 35% formaldehyde solution.

Spray solution III: Methanolic potassium hydroxide solution (c = 2 mol/L).

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 dark for 12 hours, and expose to daylight. Dark green spots on light grey background.

Literature:
L.C. Mitchell, J. Assoc. Off. Agr. Chemists **35**, 920 (1952).

Chemicals:
Silver nitrate solution 0.1 mol/l, Ord. No. 1.09081
Formaldehyde solution min. 37% GR, Ord. No. 1.04003
Potassium hydroxide pellets GR, Ord. No. 1.05033
Methanol GR ACS, ISO, Ord. No. 1.06009
Hydrogen peroxide 30% H_2O_2 (Perhydrol®) GR ISO, Ord. No. 1.07209
Nitric acid 65% GR ISO, Ord. No. 1.00456

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.

After-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.

Literature:
M.F. Kovacs, J. Assoc. Off. Agr. Chemists **46**, 884 (1963).

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512

Acetone GR ACS, ISO, Ord. No. 1.00014
Hydrogen peroxide 30% H_2O_2 (Perhydrol®) GR ISO, Ord. No. 1.07209
Ethylene glycol monophenyl ether for synthesis, Ord. No. 8.07291

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 II and re-dry again in the air. Then spray with III.

Literature:
H. Weidmann, Dissertation Berlin 1961.

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Potassium dichromate GR ACS, ISO, Ord. No. 1.04864
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Acetone GR ACS, ISO, Ord. No. 1.00014
Methanol GR ACS, ISO, Ord. No. 1.06009

277. Silver nitrate - potassium permanganate for reducing compounds.

Solution a: Mix freshly before use 1 part silver nitrate solution (c = 0.1 mol/L), 1 part ammonia solution (c = 2 mol/L) and 2 parts sodium hydroxide solution (c = 2 mol/L).

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.

Literature:
J. Kellen, Chem. listy **51**, 973 (1957).

Chemicals:
Potassium permanganate GR ACS, Ord. No. 1.05082
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391
Silver nitrate solution 0.1 mol/l, Ord. No. 1.09081
Sodium hydroxide solution 2 mol/l, Ord. No. 1.09136
Ammonia solution 25% GR, Ord. No. 1.05432

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: Nitric acid (c = 0.5 mol/L).

Procedure: Dip into I, dry the chromatogram in the air 10 min and dip into II. Dip the red-dyed chromatogram into III, thus discolouring the background, leaving the purines as red spots.

Literature:
R.M. Reguera, I. Asimov, J. Am. Chem. Soc. **73**, 5781 (1950).

Chemicals:
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Sodium dichromate dihydrate GR, Ord. No. 1.06336
Nitric acid 65% GR ISO, Ord. No. 1.00456

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 precepitate.

Spray solution II: Sodium hydroxide solution (c = 0.5 mol/L) 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 GR ACS, ISO, Ord. No. 1.01512
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Acetone GR ACS, ISO, Ord. No. 1.00014
Methanol GR. Ord. No. 1.06009

280. Sodium dithionite for antimony, arsenic, mercury, silver and bismuth ions.

Spray solution: 0.1% aqueous sodium dithionite solution.

Literature:
F.H. Pollard, J.F.H. McOmie, Chromatographic Methods of Inorganic Analysis, Butterworths Scientific Publications, London, 1953, p. 47.

Chemicals:
Sodium dithionite LAB, Ord. No. 1.06507

281. Sodium hydroxide for Δ^4-3-ketosteroids.

Spray solution: 10% sodium hydroxide solution in 60% methanol.

After-treatment: Heat 10 min at 80°C. Δ^4-3-ketosteroids show yellow fluorescence in long-wave UV light.

Literature:
I.E. Bush, Biochem. J. **50**, 370 (1951).

Chemicals:
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Methanol GR ACS, ISO, Ord. No. 1.06009

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.
Caution: Benzidine is carcinogenic!

Literature:
J.A. Cifonelli, F. Smith, Anal. Chem. **26**, 1132 (1954).

Chemicals:
Sodium metaperiodate GR ACS, Ord. No. 1.06597
Benzidine
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Acetone GR ACS, ISO, Ord. No. 1.00014

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 hydrochloric acid (c = 1 mol/L) 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 vapours. Additionally you can spray with III, the white spots turn dark.
Caution: Benzidine is carcinogenic!

Literature:
D. Waldi, J. Chromatog. **18**, 417 (1965).

Chemicals:
Sodium metaperiodate GR ACS, Ord. No. 1.06597
Benzidine
Silver nitrate GR ACS, ISO, Ord. No. 1.01512
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Acetone GR ACS, ISO, Ord. No. 1.00014
Ammonia solution 25% GR, Ord. No. 1.05432

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 dissolution 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.

Literature:
R. Consden, A.H. Gordon, A.J.P. Martin, Biochim. J. **40**, 33 (1946).

Chemicals:
Sodium metaperiodate GR ACS, Ord. No. 1.06597
Mercury(II) iodide red extra pure, Ord. No. 1.04420
Potassium iodide GR ISO, Ord. No. 1.05043
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498

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% 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 glycals show yellow spots which fluoresce strongly in long-wave UV light. The colour changes to green by spraying with 5% methanolic sodium hydroxide solution.

Literature:
J.T. Edward, D.M. Waldron, J. Chem. Soc. **1952**, 3631.

Chemicals:
Sodium metaperiodate GR ACS, Ord. No. 1.06597
4-Nitroaniline, Ord. No. 1.06760
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid fuming 37% GR ISO,Ord. No. 1.00317
Sodium hydroxide pellets, Ord. No. 1.06498
Methanol GR ACS, ISO, Ord. No. 1.06009

286. Sodium nitrite - hydrochloric acid for indoles and thiazoles.

Spray solution: Freshly prepared solution of 1 g sodium nitrite in 100 ml hydrochloric acid (c = 1 mol/L). Heat at 100°C.

Note: Indoles turn red and thiazole derivatives light green.

Alternative:

Spray solution: 0.5% aqueous sodium nitrite solution.

After-treatment: Place the chromatogram into a chamber with hydrogen chloride vapours.

Literature:
D. v. Denffer, M. Behrens, A. Fischer, Naturwissenschaften **39**, 258 (1952).

Chemicals:
Sodium nitrite GR ACS, Ord. No. 1.06549
Hydrochloric acid 1 mol/l Titrisol®, Ord. No. 1.09970
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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 hydrochloric acid (c = 2 mol/L). 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: Respraying 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!**

Literature:
G. Tonnies, J.J. Kolb, Anal. Chem. **23**, 823 (1951).

Variation for thiolactones:

Spray solution I: Sodium hydroxide solution (c = 1 mol/L).

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.

Literature:
F. Korte, J. Vogel, J. Chromatog. **9**, 381 (1962).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391
Hydrochloric acid 25% GR, Ord. No. 1.00316
Ammonia solution 25% GR, Ord. No. 1.05432
Methanol GR ACS, ISO, Ord. No. 1.06009
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sodium cyanide pure, Ord. No. 1.06437
Sodium hydroxide solution 1 mol/l Titrisol®, Ord. No. 1.09956

288. Sodium nitroprusside - acetaldehyde for secondary aliphatic and alicyclic amines.

Spray solution: Dissolve 5 g sodium nitroprusside in 100 ml 10% aqueous acetaldehyde solution. Before use mix 1 part of this solution with 1 part 2% aqueous sodium carbonate solution.

Literature:
F. Feigl, Spot Test in Organic Analysis, Elsevier Pub. Co., 7th Ed., 1966, p. 251.
K. Macek, J. Hacaperková, B. Kakai-, Pharmazie **11**, 533 (1956).
E. Stein, V. Kamienski, Planta Med. **50**, 291 (1957).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391
Acetaldehyde for synthesis, Ord. No. 8.00004

289. Sodium nitroprusside - ammonia for hemlock alkaloids.

Spray solution I: 1% aqueous sodium nitroprusside solution.

Spray solution II: 10% ammonia solution.

Procedure: Spray with I and then with II.

Note: γ-Coniceine turns red.

Literature:
F. Mall, Arch. Pharm. **296**, 205 (1963).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Ammonia solution 25% GR, Ord. No. 1.05432

290. 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.

Literature:
E. Hofmann, A. Wuensch, Naturwissenschaften **45**, 338 (1958).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium hydroxide solution min. 10% (1.11) GR, Ord. No. 1.05588
Hydrogen peroxide 30% H_2O_2 (Perhydrol®), Ord. No. 1.07209

291. Sodium nitroprusside - hydroxylamine for thiourea derivatives (Grote reagent).

Spray solution: Dissolve 0.5 g sodium nitroprusside in 10 ml water, add 0.5 g hydroxylamine hydrochloride and 1 g sodium hydrogen carbonate. After gas generation is complete, add 2 drops bromine and fill up to 25 ml with water. The reagent is stable for about 2 weeks.

Literature:
I.W. Grote, J. Biol. Chem. **93**, 25 (1931).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium hydrogen carbonate GR ISO, Ord. No. 1.06329
Bromine GR ISO, Ord. No. 1.01948
Hydroxylammonium chloride GR ACS, ISO, Ord. No. 1.04616

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 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.

Literature:
J. Roche et al., Biochim. et biophys. Acta **14**, 71 (1954).
L. Fishbein, M.A. Cavanaugh, J. Chromatog. **20**, 283 (1965).
L. Fishbein, Rec. trav. chim. **84**, 465 (1965).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Potassium hexacyanoferrate(III) GR ACS, ISO, Ord. No. 1.04973
Sodium hydroxide solution min. 10% (1.11) GR, Ord. No. 1.05588
Acetone GR ACS, ISO, Ord. No. 1.00014

293. Sodium nitroprusside - potassium permanganate for sulfonamides (Roux reagent).

Spray solution: Dissolve 10 g sodium nitroprusside in 100 ml water, add 2 ml 33% aqueous sodium hydroxide and 5 ml potassium permanganate solution (c = 0.02 mol/L) and filter after mixing.

Procedure: Spray and inspect in UV light.

Literature:
E. Vitolo, Bull. Chim. Farm. **89**, 351 (1950).
G. Wagner, Pharmazie **9**, 979 (1954).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Potassium permanganate solution 0.02 mol/l Titrisol®, Ord. No. 1.09935

294. Sodium nitroprusside - sodium hydroxide for methyl ketones and activated methylene groups.

Spray solution: Dissolve 1 g sodium nitroprusside in 100 ml of a mixture of sodium hydroxide (c = 2 mol/L) and ethanol (1+ 1). Red to violet spots.

Literature:
F. Feigl, Spot Tests in Organic Analysis, Elsevier Publ. Co., 1966, 7th Ed., p. 208.

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sodium hydroxide solution 2 mol/l, Ord. No. 1.09136

295. Sodium nitroprusside - sodium meta-periodate for deoxy-sugars.

Spray solution I: 2.5% aqueous sodium meta-periodate solution.

Spray solution II: Mixture of 1 part 7% aqueous sodium nitroprusside solution, 3 parts water and 20 parts of a saturated solution of piperazine in ethanol.

Procedure: Spray with I, dry 10 min at room temperature, then spray with II.

Literature:
J.T. Edward, D.M. Waldron, J. Chem. Soc. **1952**, 3631.

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium metaperiodate GR ACS, Ord. No. 1.06597
Piperazine hexahydrate Ph Eur, BP, Ord. No. 1.07327
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

296. Sodium pentacyanoamineferrate(II) for urea, thiourea and guanidines (Fearon reagent).

Sodium pentacyanoamineferrate(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 sodium carbonate solution (c = 0.5 mol/L) 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 pentacyanoaminoferrate(II) solution and 1 drop Perhydrol®. Stable for about 24 hours.

Literature:
P.H. List, Hoppe-Seylers Z. physiol. Chem. **305**, 27 (1956).

Chemicals:
Sodium nitroprusside dihydrate GR ACS, Ord. No. 1.06541
Sodium carbonate anhydrous GR ISO, Ord. No. 1.06392
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Ammonia solution 25% GR, Ord. No. 1.05432
Hydrogen peroxide 30% H_2O_2 (Perhydrol®), Ord. No. 1.07209
Creatinine, Ord. No. 1.05208
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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**, 957 (1963).

Chemicals:
Sodium sulfide hydrate GR, Ord. No. 1.06638

298. Sodium tetraphenylboron (Kalignost®) for alkaloids.

Spray solution I: 1% sodium tetraphenylboron (sodium tetraphenyl borate) 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. Chromatog. **11**, 364 (1963).

Chemicals:
Sodium tetraphenyl borate (Kalignost®) GR ACS, Ord. No. 1.06669
Quercetin cryst. LAB, Ord. No. 1.07546
Fisetin
Methanol GR ACS, ISO, Ord. No. 1.06009
Ethyl methyl ketone GR ACS, Ord. No. 1.09708

299. Sodium tetraphenylboron(Kalignost®) - rhodamine B for potassium ions.

Spray solution I: Sodium hydroxide solution (c = 0.1 mol/L).

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:
Sodium tetraphenyl borate (Kalignost®) GR ACS, Ord. No. 1.06669
Sodium hydroxide solution 0.1 mol/l Titrisol®, Ord. No. 1.09959
Rhodamine B (C.I. 45170) GR, Ord. No. 1.07599
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

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.

Literature:
G.P. Heisig, F.H. Pollard, Anal. Chim. Acta **16**, 234 (1957).

Chemicals:
Copper(II) acetate GR, Ord. No. 1.02711
Sodium thiosulfate pentahydrate GR ACS, ISO, Ord. No. 1.06516
Acetic acid 96% GR, Ord. No. 1.00062

301. Starch for amylases.

Spray solution I: 2% aqueous starch solution.

Spray solution II: Iodine solution (c = 0.005 mol I_2/L).

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).

Chemicals:
Starch soluble GR ISO, Ord. No. 1.01252
Iodine solution 0.05 mol I_2/l, Ord. No. 1.09910

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.

Literature:
I.M. Hais, K. Macek, Handbuch der Papierchromatographie I, G. Fischer, Jena, 1958, p. 743.

Chemicals:
Sulfanilamide extra pure, Ord. No. 1.08035
Sodium nitrite GR ACS, Ord. No. 1.06549
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
1-Butanol GR ACS, ISO, Ord. No. 1.01990

303. Sulfanilic acid diazotised for phenols, coupling amines and heterocycles (Pauly reagent).

Spray solution: Dissolve 4.5 g sulfanilic acid in 45 ml hydrochloric acid (c = 12 mol/L) 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.

Literature:
H. Jatzkewitz, Hoppe-Seylers Z. physiol. Chem. **292**, 99 (1953).
N.R. Grimmett, E.L. Richards, J. Chromatog. **20**, 171 (1965).

Chemicals:
Sulfanilic acid GR ACS, Ord. No. 1.00686
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
Sodium nitrite GR ACS, Ord. No. 1.06549
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391

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.

Literature:
R. Preussmann, D. Daiber, H. Hengy, Nature **201**, 502 (1964).
R. Preussmann, G. Neurath, G. Wulf-Lorentzen, D. Daiber, H. Hengy, Z. anal. Chem. **202**, 187 (1964).

Chemicals:
Sulfanilic acid GR ACS, Ord. No. 1.00686
1-Naphthylamine GR, Ord. No. 1.06245
Acetic acid 96% GR, Ord. No. 1.00062

305. Sulfuric acid as general visualisation reagent (in particular for sterols, steroids, bile acids and gibberellins).

*Spray solution*s:

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 during subsequent heating: cholesterol and esters first turn red, then red-violet and brown while vitamin A and esters first 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 complete oxidation to CO_2.

Literature:
D.F. Jones, J. McMillan, M. Radley, Phytochemistry **2**, 307 (1964) (gibberellins).
W.L. Anthony, W.T. Beher, J. Chromatog. **13**, 570 (1964).
H. Jatzkewitz, E. Mehl, Hoppe-Seylers Z. physiol. Chem. **320**, 251 (1960)
H. Metz, Naturwissenschaften **48**, 569 (1961).

Chemicals:
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1-Butanol GR ACS, ISO, Ord. No. 1.01990
Acetic anhydride GR ACS, ISO, Ord. No. 1.00042
Acetic acid 96% GR, Ord. No. 1.00062

306. Sulfuric acid - hypochlorite for digitalis glycosides.

Spray solution: Mix 10 ml sulfuric acid (c = 1 mol/L) and 3 ml sodium hypochlorite solution.

After-treatment: Heat 10-15 min at 125°C.

Note: Digitalis glycosides of series A - E show fluorescence of various colours in long-wave UV light.

Literature:
L. Fauconnet, R. Fazan, Bull. Soc. vaud. sci. nat. **66**, 307 (1956).
L. Fauconnet, M. Waldesbuehl, Pharm. Acta Helv. **38**, 423 (1963).

Chemicals:
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Sodium hypochlorite solution (about 13% activated chlorine), Ord. No. 1.05614

307. Tetracyanoethylene for aromatic hydrocarbons, phenols and herocyclic 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.

Literature:
P.V. Peurifoy, S.C. Slaymaker, M. Nager, Anal. Chem. **31**,1740 (1959).
J. Janák, J. Chromatog. **15**, 15 (1964).
N. Kucharczyk, F. Fohl, J. Vymétal, J. Chromatog. **11**, 55 (1963).

Chemicals:
Benzene GR ACS, ISO, Ord. No. 1.01783
Tetracyanoethylene for synthesis, Ord. No. 8.08240

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.

Literature:
J. Binkert, E. Angliker, A. v. Wartburg, Helv. Chim. Acta **45**, 2122 (1962).

Chemicals:
Potassium hydroxide pellets GR, Ord. No. 1.05033
Benzene GR ACS, ISO, Ord. No. 1.01783
2,2',4,4'-Tetranitrodiphenyl
Methanol GR ACS, ISO, Ord. No. 1.06009

309. Tetraphenyldiboroxide for flavones. PC.

Prepare tetraphenyldiboroxide according to the directions by R. Neu from 3 g sodium tetraphenylboron (Kalignost®), 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.

Literature:
R. Neu, Z. anal. Chem. **143**, 30 (1954).
R. Neu, Z. anal. Chem. **151**, 321 (1956).

Chemicals:
Sodium tetraphenyl borate (Kalignost®) GR ACS, Ord. No. 1.06669
Hydrochloric acid 25% GR, Ord. No. 1.00316
Petroleum benzine GR boiling range 40-60°C, Ord. No. 1.01775

310. Tetrazolium blue for corticosterids and other reducing compounds.

Spray solution: Mix freshly before use equal parts of 0.5% methanolic tetrazolium blue solution and sodium hydroxide solution (c = 6 mol/L) in water or water-methanol mixture. Violet spots at room temperature or after short warming.

Literature:
O. Adamec, Steroids **1**, 495 (1963).
T. Feher, Mikrochim. Acta **1965**, 105.
U. Freimuth, B. Zawta, M. Buechner, Acta Biol. et Med. Ger. **13**, 624 (1964).
O. Nishikaze, R. Abraham, H. Staudinger, J. Biochem. (Tokyo) **54**, 427 (1963).
I.E. Bush, M. Willoughby, Biochem. J. **67**, 689 (1957).

Chemicals:
Tetrazolium blue, Ord. No. 1.08103
Sodium hydroxide pellets GR ISO, Ord. No. 1.06498
Methanol GR ACS, ISO, Ord. No. 1.06009

311. Thiobarbituric acid for sorbic acid.

Spray solution: Saturated aqueous solution of thiobarbituric acid. Sorbic acid shows red spots.

Literature:
J.W. Copius-Peereboom, H.W. Beekes, J. Chromatog. **14**, 417 (1964).

Chemicals:
2-Thiobarbituric acid, Ord. No. 1.08180

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 caution.

After-treatment: Heat 15-20 min at 120°C. Sugars show pink spots.

Literature:
S. Adachi, J. Chromatog. **17**, 295 (1965).

Chemicals:
Thymol cryst. extra pure Ph Eur, Ord. No. 1.08167
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731

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 sulfuric acid (c = 0.005 mol/L). Yellow spots on red background.

Literature:
V.M. Ingram, J. Biol. Chem. **202**, 193 (1953).

Chemicals:
Thymol blue indicator ACS, Ord. No. 1.08176
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
1-Butanol GR, Ord. No. 1.09990
Sulfuric acid 0.005 mol/l Titrisol®, Ord. No. 1.09982

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.

Literature:
M. Jurecek, J. Churácek, V. Cervinka, Mikrochim. Acta **1960**, 102.

Chemicals:
Tin(II) chloride dihydrate GR ACS, Ord. No. 1.07815
4-Dimethylaminobenzaldehyde GR ACS, Ord. No. 1.03058
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317
1-Butanol GR ACS, ISO, Ord. No. 1.01990

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.

Literature:
F.H. Burstall, G.R. Davies, R.P. Linstead, R.A. Wells, J. Chem. Soc. **1950**, 516.

Chemicals:
Tin(II) chloride dihydrate GR ACS, Ord. No. 1.07815
Potassium iodide GR ISO, Ord. No. 1.05043
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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.

After-treatment: After spraying heat the chromatogram 5-10 min at 100°C and inspect subsequently in daylight and in long-wave UV light.

Literature:
J.J. Scheidegger, E. Cherbuliez, Helv. Chim. Acta **38**, 547 (1955).

Chemicals:
Tin(IV) chloride extra pure, Ord. No. 1.07810
Chloroform GR ISO, Ord. No. 1.02445
Acetic acid 96% GR, Ord. No. 1.00062

317. Titan yellow for cadmium ions.

Spray solution: 0.1% aqueous titan yellow solution.

After-treatment: Spray either with 25% ammonia solution or place the chromatogram sprayed with titan yellow solution into a chamber saturated with ammonia vapours.

Literature:
I.I.M. Elbeih, M.A. Abou-Elnaga, Anal. Chim. Acta **17**, 397 (1957).

Chemicals:
Titan yellow (C.I. 19540) GR, Ord. No. 1.01307
Ammonia solution 25% GR, Ord. No. 1.05432

318. *p*-Toluenesulfonic acid for steroids, flavonoids and catechins.

Spray solution: 20% solution of *p*-toluenesulfonic acid in chloroform.

After-treatment: After spraying heat a few minutes at 100°C. Inspect the spots in long-wave UV light.

Literature:
D.G. Roux, Nature **180**, 973 (1957).
H.J. Zeitler, J. Chromatog. **18**, 180 (1963).
H. Silbermann, R.H. Thorp, J. Pharm. Pharmacol. **6**, 546 (1954).

Chemicals:
4-Toluenesulfonic acid monohydrate GR, Ord. No. 1.09613
Chloroform GR ISO, Ord. No. 1.02445

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.

Literature:
D. Hamerman, Science **122**, 924 (1955).

Chemicals:
Formaldehyde solution min. 37% GR, Ord. No. 1.04003
Toluidine blue 0
Acetone GR ACS, ISO, Ord. No. 1.00014
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Acetic acid 96% GR, Ord. No. 1.00062

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.

After-treatment: Heat 5-10 min at 120°C. Inspect the spots in daylight and in long-wave UV light.

Literature:
B.J. Aldrich, M.L. Frith, S.E. Wright, J. Pharm. Pharmacol. **8**, 1042 (1956).
H.J. Zeitler, J. Chromatog. **18**, 180 (1963).

Chemicals:
Trichloroacetic acid GR ACS, Ord. No. 1.00807
Chloroform GR ISO, Ord. No. 1.02445
Hydrogen peroxide 30% H_2O_2 (Perhydrol®) GR ISO, Ord. No. 1.07209

321. Trifluoroacetic acid for steroids.

Spray solution: 1% trifluoroacetic acid in chloroform.

After-treatment: Heat 5 min at 120°C.

Chemicals:
Trifluoroacetic acid for synthesis , Ord. No. 8.08260
Chloroform GR ISO, Ord. No. 1.02445

322. 2,4,6-Trinitrobenzoic acid for cardiac glycosides.

Spray solution I: 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 dihydrogen phosphate solution.

Procedure: 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.

Literature:
T. Momose, T. Matsukuma, Y. Ohkura, J. Pharm. Soc. Japan **84**, 783 (1964).

Chemicals:
N,N-Dimethylformamide GR ISO, Ord. No. 1.03053
Sodium carbonate 10-hydrate GR ISO, Ord. No. 1.06391
Sodium dihydrogen phosphate GR, Ord. No. 1.06346
2,4,6-Trinitrobenzoic acid

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 sodium hydroxide solution (c = 1 mol/L).

After-treatment: Heat 5-10 min at 100°C. Reducing compounds show red spots.

Note: Tetrazolium blue is more sensitive.

Literature:
F.G. Fischer, H. Doerfel, Hoppe-Seylers Z. physiol Chem. **297**, 164 (1954).

Chemicals:
2,3,5-Triphenyltetrazolium chloride, Ord. No. 1.08380
Methanol GR ACS, ISO, Ord. No. 1.06009
Sodium hydroxide solution 1 mol/l Titrisol®, Ord. No. 1.09956

324. Tungstophosphoric acid for reducing compounds, lipids, sterols and steroids.

Spray solution: 20% ethanolic solution of tungstophosphoric acid.

After-treatment: Heat at 120°C until maximal visualisation of the spots.

Literature:
H.P. Martin, Biochim. et biophys. Acta **25**, 408 (1957).

Chemicals:
Tungstophosphoric acid hydrate GR, Ord. No. 1.00583
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

325. Urea - hydrochloric acid for sugars.

Spray solution: Dissolve 5 g urea in 20 ml hydrochloric acid (c = 2 mol/L) and add 100 ml ethanol.

After-treatment: Heating at 100°C promotes reaction. Ketoses and oligosaccharides containing ketoses turn blue.

Literature:
R. Dedonder, Bull. soc. chim. biol. **34**, 44 (1952).

Chemicals:
Urea GR ACS, Ord. No. 1.08487
Hydrochloric acid 25% GR, Ord. No. 1.00316
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

326. Vanillin - hydrochloric acid for catechins.

Spray solution: Dissolve 0.5 g vanillin in 50 ml 37% hydrochloric acid.

After-treatment: Dry the chromatogram at room temperature. Catechols show red spots.

Literature:
E.A.H. Roberts, R.A. Cartwright, D.J. Wood, J. Sci. Food Agr. **7**, 637 (1957).

Chemicals:
Vanillin, Ord. No. 1.08510
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

327. Vanillin - phosphoric acid for steroids.

Spray solution: Dissolve 1 g vanillin in 100 ml 50% aqueous phosphoric acid.

After-treatment: Heat 10-20 min at 120°C.

Literature:
H. Metz, Naturwissenschaften **48**, 569 (1961).

Chemicals:
Vanillin, Ord. No. 1.08510
ortho-Phosphoric acid 85% GR ISO, Ord. No. 1.00573

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, pipecolic acid and sarcosine turn red after several hours. Glycine turns brown-green, the other amino acids faintly brown,

Literature:
G. Curzon, J. Giltrow, Nature **172**, 356 (1953).

Chemicals:
Vanillin, Ord. No. 1.08510
Potassium hydroxide pellets GR, Ord. No. 1.05033
Ethanol abs. GR, Ord. No. 1.09722
2-Propanol GR ACS, ISO, Ord. No. 1.09634

329. Vanillin - sulfuric acid for higher alcohols, phenols, steroids and essential oils.

A. Spray reagent: Dissolve 1 g vanillin in 100 ml 97% sulfuric acid.

After-treatment: Heat the chromatogram at 120°C until the spots attain maximum colour intensity.

Literature:
E. Tyihák, D. Vágujfalvi, P.L. Hágony, J. Chromatog. **11**, 45 (1963).
A.L. le Rosen, R.T. Moravek, J.K. Carlton, Anal. Chem. **24**, 1335 (1952).

B. Spray reagent: Dissolve 0.5 g vanillin in 100 ml of a mixture of 97% sulfuric acid and ethanol (40+10).
After-treatment: Heat the chromatogram at 120°C until the spots attain maximum colour intensity.

Literature:
J.S. Matthews, Biochim. et biophys. Acta **69**, 163 (1963).

Chemicals:
Vanillin, Ord. No. 1.08510
Sulfuric acid 95-97% GR ISO, Ord. No. 1.00731
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983

330. Violuric acid for alkali and alkaline earth metal ions.

Spray solution: 1.5% aqueous violuric acid solution. Violuric acid must not be heated above 60°C.

After-treatment: Heat 20 min at 120°C.

Literature:
H. Erlenmeyer, H. v. Hahn, E. Sorkin, Helv. Chim. Acta **34**, 1419 (1951)

Chemicals:
Violuric acid

331. Xanthydrol for tryptophan and other indole derivatives.

Spray solution: Dissolve 0.1 g xanthydrol in 90 ml ethanol and add 10 ml 37% hydrochloric acid freshly before use.

After-treatment: Heat at 110°C until maximal visualisation of the spots.

Literature:
S.R. Dickmann, A.L. Crockett, J. Biol. Chem. **220**, 957 (1956).

Chemicals:
Xanthydrol Reag. Ph Eur, Ord. No. 1.08696
Ethanol absolute GR ACS, ISO, Ord. No. 1.00983
Hydrochloric acid fuming 37% GR ISO, Ord. No. 1.00317

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 matter.

After-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.

Literature:
P.J. Stevens, J. Chromatog. **14**, 269 (1964).

Chemicals:
Zinc chloride GR ACS, ISO, Ord. No. 1.08816
Methanol GR ACS, ISO, Ord. No. 1.06009

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.

Literature:
H.H. Barber, I.M. Kolthoff, J. Am. Chem. Soc. **50**, 1625 (1928).

Chemicals:
Uranyl acetate dihydrate GR, Ord. No. 1.08473
Zinc acetate dihydrate GR, Ord. No. 1.08802
Acetic acid 96% GR, Ord. No. 1.00062

334. Zirconyl chloride - alizarin - hydrochloric acid for fluorine ions.

Spray solution: Dissolve 0.05 g zirconyl chloride and 0.05 g alizarinsulfonic acid sodium salt (alizarin red S) in 100 ml hydrochloric acid (c = 2 mol/L).

Literature:
H. Seiler, T. Kaffenberger, Helv. Chim. Acta **44**, 1282 (1961).

Chemicals:
Zirconium(IV) oxide chloride octahydrate GR, Ord. No. 1.08917
Alizarin red S (C.I. 58005) GR and indicator, Ord. No. 1.06279
Hydrochloric acid 25% GR, Ord. No. 1.00316

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.

Literature:
L. Hoerhammer, K.H. Mueller, Arch. Pharm. **287**, 310 (1954).

Chemicals:
Zirconium(IV) oxide chloride octahydrate GR, Ord. No. 1.08917
Citric acid monohydrate GR ACS, ISO, Ord. No. 1.00244
Hydrochloric acid 25% GR, Ord. No. 1.00316
Methanol GR ACS, ISO, Ord. No. 1.06009