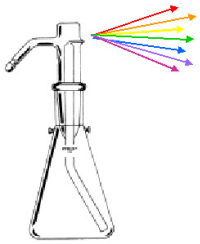
TLC Visualization Reagents



This is a brief selection of the many available TLC visualization reagents. Below each title is the type of compounds or structure which can be detected with the specific reagent. When beginning work with these reagents, acquired any MSDS (material safety data sheets) to see if there are any extra precautions needed in safely using them.

Before spraying, plates should be well dried in the hood of residual solvents and components. Amines and organic acids used in the mobile phases may adversely affect the visualization reaction being attempted. If heating to remove these components is done, consideration should be given so that loss of components or their decomposition is avoided (by lowering the temperature or using a shorter time in the oven).

Always spray any of these reagents onto plates in a well ventilated hood while wearing safety glasses. Also apply moderate amounts to the plate so it always appears dull and flat (if it looks wet, you have sprayed too much). You can always overspray to enhance the detection.

When information about the results of using the visualization reagents was available, this was put under each reagent as 'Results'. If not give, the user will have to do a few experiments to see what the results might be. Always remember to look under normal light and also short and long wavelength UV light so as not to miss any possibilities.

Many of the reagents for these visualizers can be found in the EMD Chemicals catalog or on the website (www.emdchemicals.com).

Aluminium chloride
For flavonoids
Spray plate with a 1% ethanolic solution of aluminum chloride.
Results: Yellow fluorescence in long wavelength UV light (360nm)

4-Aminoantipyrine/potassium hexacyanferrate (III) (Emerson reaction)
(Emerson reagent) for the detection of phenols and arylamines
Solution I: 1g aminoantipyrine (4-aminophenazone) in 100ml 80% ethanol
Solution II: 4g potassium hexacyanoferrate (III) in 20ml water, fill to 100ml with ethanol
Procedure:
Spray with solution I
Dry 5 minutes with warm air
Place chromatogram in a chamber with vapor from 25% ammonium solution, making sure that the layer does not contact the liquid.
Results: Red-orange to salmon pink spots

2-Aminoethyl diphenylborate, see Ethanolamine diphenylborate

Ammonium metavanadate, ammonium monovanadate, see Vanadium(V) / sulfuric acid

Ammonium molydbate
For detection of phosphoric acid derivatives
Solution I: 1M perchloric acid in water/acetone (1:1)
Solution II: ammonium molybdate soln: 5g $(NH_4)_6Mo_7O_{24}.4\ H_2O$ in 35ml semi-conc. Nitric acid and 65ml water.
Solution III: Tin (II) chloride soln: 0.5g $SnCl_2.2\ H_2O$ in 100ml 0.5M hydrochloric acid:

Dry developed chromatogram and heat to 60 C
Hydrolyse di- and triphosphates by spraying perchloric acid (solution I) onto the warm plate. After spraying 2 times, dry plate slowly at 50 C. Amidophosphates might not be decomposed.
In any case, spray the still warm plate with ammonium molybdate solution (solution II)
Then spray the still wet plate with tin (II) chloride solution (solution III)
Results: Phosphates appear as blue to blue-green spots. Polyphosphates can also be detected by dipping the plates in a solution of ammonium molybdate (1g) dissolved in water (8ml) and perchloric acid (3ml, ca. 70%), filled up to 100ml with acetone. Then phosphates appear as yellow-green spots on a blue background. Also see Molybdenum blue reaction according to Dittmer and Lester.

Aniline phthalate
For the detection of reducing sugars
Dry the developed chromatogram
Spray with 0.93g aniline and 1.66g o-phthalic acid dissolved in 100ml n-butanol saturated with water.
Briefly dry with hot air, then heat to 105 C for 10 minutes
Results: Substance spots show different colors on an almost colorless background. Some spots give fluorescence at 365nm.

p-Anisaldehyde – sulfuric acid
For detection of phenols, sugars, steroids, and terpenes
Spray with a solution of freshly prepared 0.5ml p-anisaldehyde in 50ml glacial acetic acid and 1ml 97% sulfuric acid.
and heat to 105°C until maximum visualization of spots. The background might be brightened by water vapor.
Results: Lichen constituents, phenols, terpenes, sugars, and steroids turn violet, blue, red, grey or green.
For detection of sugars
Spray with a solution of freshly prepared 1ml p-anisaldehyde, 1ml 97% sulfuric acid in 18ml ethanol and heat at 110°C.
Results: Sugar phenylhydrazones produce green-yellow spots in 3 min. Sugars will produce blue, green, violet spots in 10min. Also detects digitalis glycosides.

p-Anisidine Hydrochloride
For detection of carbohydrates / sugars
Mix a solution of 3% p-anisidine hydrochloride in n-butanol
Spray and heat at 100°C for 2-10min.
Results: Aldohexoses are seen as green-brown spots, ketohexoses as yellow spots, aldopentoses as green spots, and uronic acids as red spots.

Anisidine phthalate
For detection of carbohydrates and reducing sugars
Spray with a solution of 1.23 g p-anisidine and 1.66g phthalic acid in 100ml 95% ethanol.
Results: Hexoses, green; pentoses, red-violet – sensitivity 0.5ug; methylpentoses, yellow-green; uronic acids, brown – sensitivity 0.1-0.2ug.

Antimony (III) chloride
For detection of flavonoids
Spray with a 10% solution of antimony (III) chloride in chloroform
Results: Fluorescing spots in long wavelength light (360nm).

Antimony (III) chloride
For detection of vitamins A & D, carotenoids, steroids, sapogenins, steroid glycosides, terpenes
Spray with a solution of 25g antimony (III) chloride in 75ml chloroform (generally a saturated solution of antiomony (III) chloride in chloroform or carbon tetrachloride is used).
Heat 10min at 100C, view under long wavelength light (360nm).

Bromine / Carbon tetrachloride
For detection of organothiophosphorous pesticides
Place chromatogram in a chamber with a 10% bromine and tetrachloride without contact with the liquid.

Bromocresol green
For detection of organic acids
Dip chromatogram in a solution of 0.1g bromocresol green in 500ml ethanol and 5ml 0.1M NaOH
Results: Acids yield yellow spots on a blue background.

Bromthymol blue
For detection of lipids and phospholipids
Reagent: 0.1% bromthymol blue in 10% aqueous ethanol made just alkaline with NH_4OH
Spray dried plate.
Results: Compounds above produce blue-green colors; sensitivity 0.1-1µg.

Chloranil reagent
For detection of phenols
Spray with a solution of 1% tetrachloro-p-benzoquinone in toluene

Chlorine / o-tolidine
For detection of of compounds forming chloroamines, e.g., urea derivatives, carbamated, antibiotics
Solution I: 160mg o-tolidine in 30ml glacial acetic acid, filled to 500ml with distilled water, plus 1g KI solution
Solution II: saturated solution of o-tolidine in 2% acetic acid/0.85% KI solution (1:1, v/v)
Procedure A
Place chromatogram 15-20min in a chlorine atmosphere (e.g., Potassium permanganate +10% Hydrochloric acid)
Leave 5 minutes at ambient temperature until the chlorine is evaporated completely (spray corner of plate to insure no blue color is seen, showing complete absence of chlorine).
Spray with solution I
Procedure B
Spray with 2% potassium hypochlorite solution in water
Leave 1-1.5hr at ambient temperature
Spray with solution II

Copper sulfate / phosphoric acid
Used as a charring reagent for polymer bound TLC plates (the newer hard layer plates)
Spray with a solution of 10% copper (II) sulfate in 10% phosphoric acid
Heat 5-30min at 110°C
Results: View frequently (every 5-10min) to see if colored or fluorescent spots (at 254 and 360nm) can be seen. Charring can be continued until spots are brown, grey or black.

Chromosulfuric acid
See under **Potassium dichromate / sulfuric acid**

DDQ Reagent (Dichlorodicyanobenzoquinone)
For detection of phenols
Spray with a solution of 2% 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in toluene

Dichlorofluorescein
For the detection of sweeteners saccharine & cyclamate
Spray with a 0.2% solution of dichlorofluorescein in 96% ethanol
Dry with warm air; if necessary, spray with water
View under 360nm UV light

Dichlorofluorescein / fluorescein sodium salt
For detection of N-substituted barbiturates
Spray with a 0.1% ethanolic solution of dichlorofluorescein
Then spray with a 0.1% ethanolic solution of fluorescein sodium salt

2,6-Dichloroquinone -4- chloroimide

For detection of antioxidants, phenols, primary and secondary aliphatic amines, secondary and tertiary aromatic amines, aromatic hydrocarbons, pharmaceuticals, phenoxyacetic acid herbicides, etc
Spray with a freshly prepared 0.5-2% solution of 2,6-dichloroquinone-4-chloroimide in ethanol (reagent stable for 3 weeks if refrigerated).
Heat 10min at 110 C; treat with ammonia vapor

p-Dimethylaminobenzaldehyde
For detection of sulfonamides
Spray with a solution of 1% p-dimethylaminobenzaldehyde in 5% hydrochloric acid; add 5% ethanol
Detects sulfonamides

p-Dimethylaminobenzaldehyde / hydrochloric acid reagent (Ehrlich's reagent)
For detection of amines, indole derivatives
Spray with a solution of 1% p-dimethylaminobenzaldehyde in conc. hydrochloric acid/methanol (2:2)
Heat plates for 20min at 50 C

2,4-Dinitrophenylhydrazine
For detection of aldehydes and ketones
Spray plate with solution of 0.4 g 2,4-DNPH in 100ml 2N hydrochloric acid, add 1ml ethanol
Results: Yellow-red spots will be seen.

Diphenylamine
For detection of glycosides, glycolipids
Reagent: 10ml 10% diphenylamine in ethanol, 100ml HCl and 80ml glacial acetic acid
Spray lightly, cover plate with another glass plate, heat 30-40min at 110°C until positive areas appear
Results: Glycolipids produce blue spots.

s-Diphenylcarbazone
For detection of barbiturates
Spary with a solution of 0.1% s-diphenylcarbazone in 95% ethanol
Results: Barbiturates will produce purple spots

2,2'-Diphenylpicrylhydrazyl
For detection of aldehydes and ketones
Reagent: dissolve 15mg of 2,2'-DPPH in 25ml chloroform
Spray, heat 5-10min at 110°C;
Results: Yellow spots on a purple background will be seen.

Dithizone
For detection of heavy metal ions
Dissolve 20mg dithizone in 100ml acetone, store in a brown bottle in a refrigerator
Procedure:
Spray with dithizone solution
Spray with 25% ammonia solution

Dittmer and Lester
See Molybdenum blue

Dragendorff reagent
For detection of nitrogen compounds, alkaloids, antiarrhythmic drugs, surfactants
Solution 1) 1.7g basic bismuth nitrate and 20g tartaric acid in 80ml water
Solution 2) 16 g potassium iodide in 40ml water
Stock solution (stable for several weeks in a refrigerator):
Mix equal volumes of solutions 1 and 2
Procedure:
Spray with a solution of 10g tartaric acid, 50ml water and 5ml stock solution

Ethanolamine diphenylborate (flavone reagent according to Neu)
For detection of flavonoids
Spray with a 1% solution of ethanolamine diphenylborate in methanol
Spray with a 5% ethanolic solution of polyyethylene glycol for fluorescence stabilization
Irradiate 2 minutes with intense 365nm UV light
View under 365nm UV light

Erhlich's reagent
See p-Dimethylaminobenzaldehyde

Emerson reagent
See 4-aminoantipyrine/potassium hexa-cyanoferrate (III)

Fast Blue B reagent
For detection of cannabinoids, phenols, tanning agents, amines with can be coupled
Spray with a solution of 0.5g Fast Blue B (tetraazotized di-o-anisidine) in acetone/water (9:1, v/v), always prepared fresh
Then overspray with 0.1M sodium hydroxide solution
Results: Cannabinoids turn dark red/purple in color

Ferric Chloride / sulfuric acid
Used as a charring reagent for polymer bound TLC plates (the newer hard layer plates)
Spray with a solution of 2g $FeCl_3$ in 83ml n-butanol and 15ml conc. sulfuric acid.
Heat 5-30min at 110°C
Results: View frequently (every 5-10min) to see if colored or fluorescent spots (at 254 and 360nm) can be seen. Charring can be continued until spots are brown, grey or black.

Flavone reagent according to Neu
See ethanolamine diphenyl borate

Fluorescamine
For detection of primary and secondary amines, peptides, sulfonamides, e.g., nitrosoamines after photolysis
Spray plate with a solution of 0.1mg/ml 4-phenyl-spiro[furan-2(3H),1-phthalan]-3,3-dione in acetone prepared fresh daily
For stabilization of fluorescence at 366nm spray with 10g triethylamine, brought to 100ml with dichloromethane.

Fluorescent Indicator
For detection of compounds which absorb UV light
Some TLC plates when manufactured have an inorganic fluorescent indicator added to the slurry poured to make the final plates. This type of indicator will not dissolve or elute off. They are activated at 254nm or 360nm (see recommendations of the manufacturer for that type of plate).
Results: When activated the fluorescent indicator will turn a green or white (depending on the indicator added) and the compounds appear as dark spots or shadows against this background. If viewing at other than the activation wavelength, the compounds might also have some fluorescence of their own, so various colors against a dark background would be seen.

Formaldehyde / sulfuric acid
For detection of alkaloids, aromatic hydrocarbons, e.g., antihypertensive drugs
Spray with a solution of 37% formaldehyde in conc. sulfuric acid (1:10) immediately after taking the plate from the developing chamber. Heating is not necessary.
Results: various colored spots.

Formaldehyde / phosphoric acid
For detection of steroid alkaloids, steroid sapogenins and phenothiazine derivatives

Spray with a solution of 0.03g formaldehyde in 100ml of 85% phosphoric acid with stirring at room temperature. The reagent is stable for several weeks.

Furfural / sulfuric acid
For detection of carbamate esters
Spray solution I: 1% solution of furfural in acetone
Spray solution II: 10% solution of sulfuric acid in acetone
Spray plate with I, then II.

Gentian Violet – Bromine
For detection of lipids
Spray 0.1% gentian violet (crystal violet) in methanol onto plate and place in a tank containing bromine vapor.
Results: lipids produce blue spots on a yellow background.

Gibb's reagent
For detection of phenols. For further applications see 2,6-dichloroquinone-4-chloroimide
Spray with a solution of 3% 2,6-dibromo-N-chloro-p-benzoquinone imine in toluene or methanol.

Hydroxylamine / iron (III) chloride
For detection of amides, lactones, carboxylic acid esters and anhydrides
Solution 1) Mix 1 vol part of 7g hydroxylammonium chloride in 100ml methanol w 1 vol part of a solution of 7.2 g potassium hydroxide in 100ml methanol. Filter from precipitated potassium chloride.
Solution 2) 2% solution of iron (III) chloride in 1% aqueous hydrochloride acid
Spray air dried plate first with solution 1, then with solution 2

Iodine containing compounds
Detection by decomposition under UV
Dry plates at 100 C
After cooling spray with a small amount of 50% acetic acid
Irradiate some minutes with unfiltered UV light.
Results: Iodine compounds show weakly violet to brown spots. The color can be enhanced by spraying with 10% acetic acid and irradiation with UV light (sudden appearance of blue spots).

Iodine vapor
Relatively unspecific universal reagent for many organic compounds
Charge chamber with some crystals of iodine
Place developed, dried chromatogram in iodine vapor
Results: spots turn tan-brown in color

Iodoplatinate
For detection of organic nitrogen compounds, alkaloids, e.g., cocaine metabolites
Spray with a freshly prepared mixture of 3ml hexachloroplatinic (IV) acid solution (10%) in 97ml/min water and 100ml aqueous potassium iodide solution.
Note - use 5% ethanol or methanol in water to prepare these solutions for the new polymer bound TLC plates.

Iron (III) chloride / potassium hexacyanoferrate / sodium arsenate (according to Patterson & Clements)
For detection of iodine compounds, e..g., thyroid gland hormones
Solution I: 2.7% iron (III) chloride hexahydrate in 2N hydrochloric acid
Solution II: 3.5% potassium hexacyanoferrate in water
Solution III: dissolve 3.8g arsenic trioxide in 25ml 2N sodium hydroxide solution heating slightly, cool to 5 C, and add 50ml 2N sulfuric acid, fill to 200ml with water
Immediately before use mix 5ml solution I, 5ml solution II and 1ml solution III.
Spray onto the dry layer and dry carefully (temp below 50 C)
Cover with glass plate and leave 15min in the dark
Results: Iodine containing compound show light blue spots on a yellowish background.

Lead tetraacetate / 2,7-dichlorofluorescein
For detection of vicinal diols, glycosides and phenols, e.g., sugar acids
Solution I: 2% (w/v) lead tetraacetate in glacial acetic acid
Solution II: 1% (w/v) 2,7-dichlorofluorescein in ethanol
Mix 5ml of each solution 1 and 2, fill to 200ml with dry toluene. This reagent solution is stable for only about 2 hours.

Manganese / salicylaldehyde
For detection of organothiophosphorus pesticides
Solution I: dissolve 100mg manganese chloride ($MnCl_2.4H_2O$) in 100ml 80% alcohol
Solution II: dissolve 1.3g 2-hydrozine quinoline in the lowest possible volume of hot ethanol. Dissolve 1 g salicyaldehyde in 5ml ethanol and add 1-2 drops glacial acetic acid. Combine both solutions and reflux 30 minutes. The crystals of salicyl-2-aldehyde –2-quinolinehydrazone precipitated during the cooling are recrystallized from ethanol. For solution dissolve 50mg of the salicylate derivative in 100ml ethanol
Spray with a mixture of equal volumes of solutions 1 and 2.

Mandelin's reagent
See Vanadium(V) / sulfuric acid

Mercury (II) chloride / diphenylcarbazone
For detection of barbiturates
Solution I: 2% ethanolic mercury (II) chloride
Solution II: 0.2% ethanolic diphenlycarbazone
Mix freshly before use in equal parts
Results: Pink spots on a violet background

Mercury (II) chloride / dithizone
For detection of barbiturates
Spray with a freshly prepared 1:1 mixture of 1-2% mercury (II) chloride in ethanol and 0.1-0.2% dithizone in ethanol.
View under 360nm UV light

4-Methoxybenzaldehyde / sulfuric acid / ethanol
For detection erythromycin and metabolites
Spray with 4-methocybenzaldehyde/sulfuric acid/ethanol (1:1:9)
Heat 1 minute at 100 C

Methyl yellow
For detection of chlorinated insecticides and antimicrobial compounds
Spray dried plate with a solution of 0.1g methyl yellow (N,N-dimethyl-4-phenylazoaniline) in 70ml ethanol, add 25ml water and fill to 100ml with ethanol.
Dry at ambient temperature
Irradiate 5 min with UV light without a filter
Results: Red spots on a yellow background

Molybdatophosphoric acid
See under **Phosphomolybdic acid**

Molybdenum blue reaction according to Dittmer and Lester
For detection of phospholipids and phosphoric acid derivatives
Solution I: Boil 40.11g MoO_3 in 1 liter 25N sulfuric acid for 3-4 hours until the molybdenum oxide is completely dissolved. Let the light yellow solution slowly cool to ambient temperature overnight. The solution will turn light blue.
Solution II: Boil 1.78 g molybdenum powder and 500ml of solution I for 15min, cool and decant from the remaining residue.

For preparation of the spray reagent, add equal volumes of solutions I and II to 4.5 volume parts water. A dark green solution is formed.
Solutions I and II are stable for several months when stored in the dark. The spray reagent has to be prepared weekly.

Ninhydrin
For detection of amino acids, amines, amino sugars.
Spray with a solution of 0.2g ninhydrin in 100ml ethanol and heat to 110 C until spots appear.
Results: reddish spots appear

Ninhydrin / cadmium acetate
For detection of amino acids and heterocyclic amines
Dissolve 1g ninhydrin and 2.5g cadmium acetate in 10ml glacial acetic acid and fill to 500ml with ethanol.
Spray and heat 20min at 120 C
Results: Red, pink, or purple spots are seen.

Ninhydrin / pyridine / glacial acetic acid
For detection of peptides
Spray with a 1% ninhydrin in pyridine/glacial acetic acid (5:1, v/v)
Heat 5 min at 100 C

Nitric acid / ethanol
For detection of amines and alkaloids
Spray with a solution of 50 drops 65% nitric acid in 100ml ethanol (higher acid concentrations are also possible).
If necessary, heat to 120 C for some time.

Orcinol (Bials reagent)
For detection of glycosides, glycolipids
Reagent: dissolve 0.1g orcinol in 40.7ml conc. HCl, add 1ml 1% ferric (111) chloride, and dilute to 10ml
Spray and heat at 80°C for 90 minutes.
Results: Glycolipids produce violet spots.

Patterson and Clements
See under Iron (III) chloride/potassium hexacyanoferrate/sodium arsenate

Paraffin oil
For enhancement of fluorescence spots – more stable and greater intensity
1% paraffin oil in hexane
Spray evenly over the TLC plate
Results: spots should be more stable (no fading with time) for scanning and are of greater intensity

m-Phenylenediamine
For detection of reducing sugars
Spray with a solution of 3.6g m-phenylenediamine dihydrochloride in 100ml 70% ethanol and heat briefly at 105°C
Results: Intensely fluorescence colors in UV light (wavelength not specified, so check 254 and 366nm).

o-Phenylenediamine – trichloroacetic acid
For detection of alpha-keto acids
Spray with a solution of 0.05g 1,2-phenylenediamine in 100ml 10% aqueous trichloroactic acid and heat plate at 100C for no more than 2 minutes.
Results: Green fluorescence spots in long wavelength UV light.

p-Phenylenediamine – phthalic acid
For detection of conjugated 3-ketosteroids

Spray with a solution of 0.9 p-phenylenediamine and 1.6g phthalic acid in 100ml 1-butanol saturated with water and heat plate at 100-110°C
Results: Yellow to orange spots

Phenylhydrazine sulfonate
For detection of some antimicrobial compounds
Solution I: dissolve 3.5g phenylhydrazine 4-sulfonic acid hemihydrate in 10ml water and 20ml 1N NaOH solution
Solution II: mix 30ml 1N sodium hydroxide solution with 40ml acetone
The spray reagents have to be prepared fresh each time.
Procedure:
Wet chromatogram evenly with spray solution 1
After air drying the plate, shake spray solution 2 and spray plate.

Phosphoric acid
For detection of sterols, steroids, and bile acids
Spray heavily until the layer appears transparent with a solution of 85% phosphoric acid with water (1:1, v/v)
Then heat 10-15minutes at 120°C
Results: Sterols, steroids and bile acids and bile acids produce various colors under visible and UV light.

Phosphoric acid – bromine
For detection of digitalis glycosides
Spray solution I: 10% aqueous phosphoric acid solution
Spray solution II: Mix 2ml saturated aqueous potassium bromide, 2ml saturated solution aqueous potassium bromate and 2ml 25% hydrochloric acid.
Procedure: Spray plate with I and heat 12 min at 120°C.
Results: Digitalis glycosides of the series B, D, and E show blue fluorescence in long wavelength UV light
Procedure continued: Heat the plate again at 120°C and spray lightly with II
Results: Glycosides of the series A show orange, of the series C show grey-green to grey-blue fluorescence in UV light.

Phosphomolydbic acid
For detection of reducing substances, e.g, alcohols, bile acids, lipids, fatty acids, steroids
Also used as a charring reagent for polymer bound TLC plates (the newer hard layer plates)
Spray with a solution of 250mg molybdatophosphoric acid in 50ml ethanol
Heat to 120 C until spots appear (oven or heat gun)
If necessary, treat with ammonia vapors to remove some background coloration.
The reagent solution is stable for only 10 days even in the dark.
Results when using as a charring reagent: View frequently (every 5-10min) to see if colored or fluorescent spots (at 254 and 360nm) can be seen. Charring can be continued until spots are brown, grey or black.

Phosphotungstic acid
For detection of cholesterol and its esters, reducing compounds, lipids, sterols, and steroids
Spray with 20% phosphotungstic acid in ethanol, heat at 110°C for 5-15min or until maximum visualization of the spots occurs
Results: Cholesterol, esters will produce red spots.

Pinacryptol yellow
For detection of sweetners, surfactants, alkyl- and arylsulfonic acids
Dissolve 100mg pinacryptol yellow in 100ml hot water or ethanol (or some combination for polymer bound plates)
Spray with reagent solution
Results: Yellow to orange fluorescence spots under long wavelength UV light (366nm)

Potassium dichromate / sulfuric acid (chromosulfuric acid) - see below

Potassium permanganate / sulfuric acid – see below

Rhodamine B
For detection of a wide variety of compounds
Dry the developed chromatogram and spray with 0.025-0.25% ethanolic rhodamine B reagent.
Results: In most cases red-violet zones with an intense fluorescence at 365nm develop on a pink background. RP phases are less suited, because in this case the environment of the spots also forms an intense color. By placing the sprayed chromatograms into an ammonia atmosphere, the detection sensitivity can be improved.

Rhodamine 6 G
For detection of lipids
Spray plate with a solution of 1mg rhodamine 6 G in 100ml acetone
Inspect at long wavelength UV.

Silver nitrate / hydrogen peroxide
For detection of halogenated hydrocarbons
Spray plate with a solution of 0.1g silver nitrate in 1ml water, add 10ml 2-phenoxyethanol, fill to 200ml with acetone and add 1 drop hydrogen peroxide (30% solution)
Irradiate with unfiltered UV light until optimal contrast is obtained. For alumina plates, about 50 minutes, for silica gel plates about 15 minutes
Results: dark spots are formed.
Luckow [Fensenius Z. Anal. Chem., 294, 288, (1979)] uses this combination without the addition of hydrogen peroxide for the detection of pesticide ioxynil (3,5-diiodo-4-hydroxybenzonitrile).

Sodium azide
For detection of antibiotics (penicillins and cephlosporins)
Solution I: 0.5% solution of soluble starch
Solution II: 3.5% sodium azide in 0.1N iodine solution
Procedure: spray with 1), dry, spray with 2)
Results: Detects penicillin, sensitivity 0.2µg. Penicillin and penicilloic acids are also detected by starch-iodine-iodine reagents, as are cephalosporins.

Sodium 1,2-napthaquinone-4-sulfonate (NZS reagent)
For detection of thiazide drugs, basic drugs with primary amino groups
Solution I: 0.1N NaOH
Solution II: saturated solution of reagent in 1:1 ethanol: water
Spray with I, then II.
Results: Thiazide drugs appear as orange spots within 15min; basic drugs with primary amino groups also react but barbiturates do not.

Sodium nitrite / hydrochloric acid
For detection of indoles and thiazoles
Spray plate with a freshly made solution of 1g sodium nitrite in 100ml hydrochloric acid, 1mol/L and heat at 100°C.
Results: Indoles turn red and thiazole derivatives turn light green.

Sodium nitroprusside / hydrogen peroxide
For detection of guanidine, urea, thiourea and derivatives, creatine and creatinine.
Spray with a solution made up of 2ml 5% aqueous sodium nitroprusside, 1ml 10% aqueous sodium hydroxide and 5ml 3% aqueous hydrogen peroxide and dilute with 15ml water. This solution can be stored several days in the refrigerator.

Sodium nitroprussate / potassium hexacyanoferrate (III)
For the detection of aliphatic nitrogen compounds, cyanamide, guanidine, urea, thiourea and derivatives, creatine, and creatinine.

Spray with a solution of 1 volume part each of 10% aqueous sodium hydroxide, 10% sodium nitroprussate, and 10% potassium hexacyanoferrate (III) with 3 vol. parts water. Let the solution stand at least 20min at ambient temperature before use. Stored in the refrigerator, it is stable for several weeks.
Procedure: For use, mix the reagent solution with an equal part of acetone and spray.

Stannic Chloride
See under Tin (IV) chloride

Tetracyanoethylene - TCNE reagent
For detection of aromatic hydrocarbons and heterocycles, aromatic amines, and phenols
Spray with a solution of 0.5-1.0g tetracyanoethylene in dichloromethane or toluene.
Results: Aromatic hydrocarbons show various colors, some of them for a brief time. Also try heating at 100°C for a short time.

Tetranitrodiphenyl
For detection of cardiac glycosides
Spray solution I: Saturated solution of 2,3',4,4'-tetranitrodiphenyl in toluene
Spray solution II: 10% potassium hydroxide solution in 50% aqueous methanol
Spray with I, dry at room temperature, then spray with II.
Results: Blue spots are observed.

Tetrazolium blue
For detection of corticosteroids and other reducing compounds.
Spray with a freshly prepared 1:1 mix of a) 0.5% methanolic tetrazolium blue solution and 6M NaOH in water or methanol/water (1:1).
Results: violet spots are observed at room temperature or with slight warming.

Thymol / sulfuric acid
For detection of sugars
Spray with a solution of 0.5g thymol in 95ml ethanol, and add 5ml 97% sulfuric acid with caution.
Heat 15-20min at 120°C
Results: Sugars show pink spots.

Tin (IV) chloride
For detection of triterpenes, sterols, steroids, phenols, and polyphenols
Spray with a solution of 10ml tin (IV) chloride in 160ml equal volumes of chloroform and glacial acetic acid.
Heat the layer for 5-10min at 100°C and inspect in visible and long wavelength UV light.

o-Tolidine, diazotized
For detection of phenols
Tolidine solution – fill up 5g o-tolidine and 14ml conc. hydrochloric acid to 100ml water
Nitrate solution – 10% aqueous sodium nitrate solution prepared fresh
Spray solution – mix 20ml tolidine solution and 20ml nitrate solution at 0°C stirring constantly.
The spray solution is stable for about 2-3 hours.
After spraying it can take several hours until colored spots are formed.

p-Toluenesulfonic acid
For detection of steroids, flavonoids and catechins
Spray with a solution of 20% p-toluenesulfonic acid in chloroform and heat a few minutes at 100°C.
Inspect under long wavelength UV light.

Trichloroacetic acid
For detection of steroids, digitalis glycosides, veratrum alkaloids and vitamin D
Spray solution I: 25% solution of trichloroacetic acid in chloroform.
Spray solution II – for vitamin D – 1% trichloroacetic acid in chloroform.

Spray solution III – for digitalis glycosides – dissolve 3.3g trichloroacetic acid in 10ml chloroform and add 1-2 drops hydrogen peroxide.
After applying the appropriate solution, heat 5-10min at 120°C.
Inspect the spots in daylight and in long wavelength UV light.

Trifluoroacetic acid
For detection of steroids
Spray with a solution of 1% trifluoroacetic acid in chloroform and heat 5min at 120°C

Triethanolamine
For enhancement of fluorescence species
20% solution of reagent in isopropanol

Tungstophosphoric acid
See under **Phosphotungstic Acid**

Ultraviolet Light - fluorescence
For detection of various compounds which have native fluorescence.
With a plate containing a fluorescent indicator (F_{254}), examine the dried chromatogram under 360nm UVlight.
With a TLC plate containing no fluorescent indicator, examine the dried chromatogram under both 254 and 360nm UV light.

Ultraviolet Light - quenching
For detection of various compounds which absorb 254nm UV light; quenching of TLC plate fluorescence.
Examine a fluorescent indicator (F_{254}) containing plate after dried, under 254nm UV light.
Compounds absorbing this wavelength light will appear as dark spots against a green or white fluorescence caused by the UV activation of the fluorescent indicator in the plate.

Urea / hydrochloric acid
For detection of sugars
Spray with a solution of 5g urea in 20ml 2M hydrochloric acid, with 100ml ethanol added and heat to 100°C.
Results: Ketoses and oligosaccharides containing ketoses turn blue.

Vanadium (V) / sulfuric acid
For detection of carbohydrates, glycols, reducing carboxylic acids, steroids, antioxidants, vitamins, phenols, aromatic amines, antihistamines
a) Ammonium monovanadate (ammonium metavanadate) /sulfuric acid reagent
   Spray with a solution of 1.2g ammonium monovanadate in 95ml water and 5ml conc. sulfuric acid or for beta blockers: spray with saturated solution of ammonium monovanadate in conc. sulfuric acid.
b) Vanadium pentoxide / sulfuric acid reagent
   Spray with a solution of 1.82g vanadium pentoxide in 30ml 1M sodium carbonate, sonicate to achieve complete dissolution, after cooling add 46ml 2.5M sulfuric acid and fill to 100ml with acetonitrile.

Vanillin / potassium hydroxide
For detection of amines and amino acids
Spray with a solution of 1g vanillin in 50ml 2-propanol and dry 10min at 110 C
Then spray with 1ml 1M potassium hydroxide solution filled up to 100ml with ethanol and dry 10 min at 110 C
View at 365nm UV
Results: After spraying with a) ornithine will be seen as a bright green-yellow fluorescence, lysine as a green-yellow fluorescence. After spraying with b) ornithine will show a salmon color that will fade; proline, hydroxyproline, pipecolinic (pipecolic) acid and sarcosine will turn red after a few hours. Glycine will produce a green brown spot, and other amino acids will turn faint brown.

Vanillin / phosphoric acid
Used as a charring reagent for polymer bound TLC plates (the newer hard layer plates)
Spray plate with 1g vanillin in 100ml 50% aqueous H_3PO_4

Heat 5-30min at 110°C.
Results: View frequently (every 5-10min) to see if colored or fluorescent spots (at 254 and 360nm) can be seen. Charring can be continued until spots are brown, grey or black.

Vanillin / sulfuric acid - see immediately below

The charring visualization reagents given below can only be used with glass TLC plates in which a G (gypsum) binder has been used in the formulation to hold the silica gel on the plate:

Potassium dichromate / sulfuric acid (chromosulfuric acid)
Universal visualization reagent for organic compounds (e.g., alcohols, also for bile acids, lipids) – note: do not use this reagent on polymer bound TLC plates since it will char the binder; use only with G (gypsum) binder plates.
Spray with a solution of 5g potassium dichromate in 100ml conc. Sulfuric acid.
If necessary heat plate to 150 C
Results: Brown, grey, or black spots result

Potassium permanganate / sulfuric acid
Universal reagent for organic compounds, e.g., fatty acid derivatives
Note: Do not use this reagent on polymer bound TLC plates since it will char the binder; use only with G (gypsum) binder plates.
Spray with a solution of 1.6% potassium permanganate in conc. sulfuric acid (make this solution very carefully, and slowly to prevent any accidents).
Heat plate for 15-20min at 180 C

Vanillin / sulfuric acid
For detection of steroids, Note, this reagent can only be used with G (gypsum) binder plates since it will char the polymer binders in the harder layer plates.
Spray with a 1% solution of vanillin in conc. sulfuric acid and at 120 C until maximum color formation.
Another formulation of this reagent: 0.5g vanillin in 100ml sulfuric acid/ethanol (40:10).

TLC Texts with Visualization Reagent Information:

Thin Layer Chromatography, 2nd edition, E. Stahl, ed, Springer-Verlag, NY, 1969
TLC Reagents & Detection Methods – Physical & Chemical Detection Methods: Fundamentals, Reagents,I, Vol 1a, H. Jork, W. Funk, W. Fishcer, & H. Wimmer, Wiley, NY, 1990
Practice of Thin Layer Chromatography, 3rd edition, J.C. Touchstone, Wiley-Interscience, NY, 1992
TLC Reagents & Detection Methods – Physical & Chemical Detection Methods: Activation Reactions, Reagent Sequences, Reagents, II, Vol 1b, H. Jork, W. Funk, W. Fishcer, & H. Wimmer, Wiley, NY, 1994

Important Notes – Please read carefully :

1) When using the older texts, if they suggest putting the components into benzene, always substitute toluene since it is much less toxic. Likewise, as mentioned above, if the components are added to pure water, these solutions cannot penetrate well into the polymer bound layers (hard layers) now sold. Substitute 5% methanol in water or 5% ethanol in water as the makeup solvent in these instances.
2) In past years various visualization reagents were made up with **benzidine**. It had been used for the detection of terpene aldehydes, flavoinoids, carbohydrates and phenols. This reagent is no longer recommended for use since it is now classified as a carcinogen. Other suitable visualizers for the detection of the above compounds can be found in this and the newer references listed above.

3) A few words about the supports the layers might be coated onto and the use of these visualization reagents (and subsequent heating). Most of these reagents can be used on silica gel, bonded silica gel, or cellulose plates with glass, plastic, or aluminum supports. The exceptions are strong acid containing visualizers which cannot be used with aluminum supports (which obviously would react with the aluminum to dissolve it). Also when heating, plastic supports are limited to about 110°C only. Plastic supported plates when heated at any temperature should be placed on a metal or glass plate in an oven so they heat evenly. Some ovens have a metal mesh or grid, which would heat the plastic support unevenly leading to warping and lifting of the layer. If you have questions, please consult the manufacturer about the suitability of any procedure you intend on using.

ChromNotes related to TLC plate visualization (click on topic to link to it):

1) Charring for Detection on Glass, Aluminum, and Plastic Backed TLC/HPTLC Plates
2) Improving Detection Limits in TLC with Spot Compression and Fluorescence Enhancement
3) TLC Plate Visualization by Spraying and Dipping.