Preparation of chromatography spray reagents

Spray reagent selection guide

Acids organic	Bromocresol Green
Aldehydes	Fuchsin
Alkaloids	Dragendorff's; Iodoplatinate
Amines	Iodoplatinate; Ehrich's; Ninhydrin
Amino acids and related compounds	Ninhydrin
Antioxidants	Phosphomolybdic acid
Barbiturates	Mercuric diphenylcarbazone
Flavanoids	Antimony trichloride
Glycopids	Bial's; Orcinol; Diphenylamine
Lipids	Antimony trichloride; Bial's; Orcinol; Bromothymol blue; Cupric acetate; 2,7-Dichlorofluorescein; Diphenylamine; Dragendorff's; Molybdenum blue; Ninhydrin; Potassium- Dichromate/H2SO4; Rhodamine B; Phodamine 6G
Phenols	Phosphormolybdic acid; Rhodamine B
Steroids	Antimony pentachloride; Antimony trichloride; Phosphormolybdic acid
Terpenes	Antimony pentachloride
Vitamins	Antimony pentachloride; Iodoplatinate
Charring Reagent	Potassium dichromate/H2SO4

Alphabetical search for TLC Visualization Spray Reagents

B C D E F G H I J K L M N O P Q R S T
U V W X Y Z

Aluminium chloride

For flavonoids
Dissolve 1 gm aluminum chloride in 100 ml 95% ethanol
Look for yellow fluorescence in UV light (360nm)

4-Aminoantipyrine/potassium hexacyanferrate (III) (Emerson reaction) for the detection of phenols and aryl amines
Solution I: 1g aminoantipyrine (4-aminophenazone) in 100ml 95% ethanol
Solution II: 4g potassium hexacyanoferrate (III) in 50 ml water and 50ml 95% ethanol
Spray with solution I
Dry 5 minutes with warm air(heat gun)
Spray with solutionn II
Dry again for 5 minutes
Place plate in an ammonia vapor chamber (25% ammonia solution) to develop.
Red-orange to pink spots develop.

2-Aminoethyl diphenylborate, see Ethanolamine diphenylborate

Ammonia
25% w/v in water.For neutralizing ninhydrin fixer for chromatograms and numerous other applications.

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

Ammonium molydbate For detection of phosphoric acid derivatives
Solution I: 10 mls perchloric acid in 50 ml water and 50 ml acetone
Solution II: ammonium molybdate soln: 5g $(NH4)6Mo_7O_{24}.4\ H2O$ in 40 ml 1:1 nitric acid and 60ml water.
Solution III: (Stannous chloride)Tin (II) chloride soln: Dissolve 0.5g $SnCl_2.2\ H2O$ in minimum conc. HCl and make up to 100 ml solution.
Dry developed chromatogram and heat to 60° C in TLC oven.
Spray twice with perchloric acid reagent.
Then spray the still warm plate with ammonium molybdate solution (solution II) followed by the Tin II chloride reagent. (solution III)
Phosphates appear as blue-green spots. Polyphosphates can be detected by using molybdenum blue spray reagent.

Aniline-Diphenylamine For detecting reducing sugars.
Dissolve 1.8gm aniline and 1.8gm diphenylamine in 80ml acetone and add 20ml conc.HCl.

Aniline phthalate For the detection of reducing sugars
Dry the 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 110°C for 10 minutes
Spots show different colors. 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 conc. sulfuric acid.
Heat to 105°C until maximum visualization of spots. Enhance background with water vapor spray.
Components give violet, blue, red, grey or green spots.

p-Anisaldehyde / ethanol For detection of sugars
Spray with a solution of freshly prepared 1ml p-anisaldehyde, 1ml conc. sulfuric acid in 20ml ethanol.
Heat at 110°C.
Sugar phenylhydrazones give green-yellow spots in less tham 5 mins. Sugars will produce blue, green, violet spots in 10min.

p-Anisidine Hydrochloride For detection of carbohydrates / sugars
Mix a solution of 3gm p-anisidine hydrochloride in 100ml n-butanol
Spray and heat at 100°C.
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.
Hexoses, green; pentoses, red-violet, methylpentoses, yellow-green; uronic acids,brown.

Antifoam
25% antifoam reagent in methylene chloride.
For preventing/dispersing foam.

Antimony (III) chloride For detection of vitamins A & D, carotenoids, steroids, sapogenins, steroid glycosides, terpenes
Spray with a saturated solution of 25g antimony (III) chloride in chloroform.
Heat 10min at 100°C, then view at 360nm.

B

Bial reagent See Orcinol
Boute reaction for detection of phenols
Reaction of constituents with nitrogen dioxide and ammonia vapor
Dry and heat the developed chromatogram
Place hot plate for 3 – 10 minutes in a chamber with NO2 vapour (from conc. nitric acid)
Then treat with NH3 vapour (from conc. ammonia)
Bromine / Carbon tetrachloride For detection of organothiophosphorous pesticides
Place chromatogram in a chamber with a 10ml bromine in 90ml tetrachloride. No contact with the liquid.
Bromocresol Green
Dissolve 0.04gm bromocresol green in 100ml ethanol
For detecting organic acids and bases
Bromocresol green For tank development of organic acids
Dip chromatogram in a solution of 0.1g bromocresol green in 500ml ethanol and few drops dilute NaOH
Acids yield yellow spots on a blue background.
Bromthymol blue For detection of lipids and phospholipids
Dissolve 0.1gm bromthymol blue in 10% aqueous ethanol made just alkaline with NH4OH
Spray dried plate.
Compounds produce blue-green colors.

C

Chloranil reagent (tetrachloro-p-benzoquinone) For detection of phenols
Spray with a solution of 1gm tetrachloro-p-benzoquinone in 100ml toluene
Chlorine / o-tolidine For detection of chloroamine forming compounds, e.g., urea derivatives, carbamated, antibiotics
Solution I: Dissolve 160mg o-tolidine in 30ml glacial acetic acid, add 500ml distilled water, and add 1gm KI
Solution II: saturated solution of o-tolidine in dilute acetic acid with 0.85gm KI added.
Chloramine-T
Dissolve 5gm of reagent and 0.5gm sodium hydroxide in 100ml water.
For detection of phenolic compounds.
Cupric Acetate
Dissolve 3gm cupric acetate and 15mls phosphoric acid in 85mls water..
For detecting prostaglandins.
Copper sulfate / phosphoric acid Used as a charring reagent for *polymer bound* TLC plates.
Spray with a solution of 10gm copper (II) sulfate 10mls phosphoric acid in 90mls water.
Heat 5-30min at 110°C
View every few minutes to see if colored or fluorescent spots at 254 and 360nm appear.
Spots become brown, grey or black.
Chromosulfuric acid See under Potassium dichromate / sulfuric acid
a-Cyclodextrin
Dissolve 1gm of the reagent in 100ml ethanol.
Distinguishes saturated from unsaturated lipids.

D

DDQ Reagent (Dichlorodicyanobenzoquinone) For detection of phenols
Spray with a solution of 2gm 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in 100ml toluene
2 Dichlorofluorescein

Dissolve 0.2gm of the reagent in 100ml ethanol.
For detecting saturated and unsaturated lipids.
Dichlorofluorescein For the detection of sweeteners saccharine and cyclamate
Dissolve 0.2gm of dichlorofluorescein in 100ml ethanol
Spray and dry with warm air.
View under 360nm UV light
Dichlorofluorescein / fluorescein sodium salt For detection of N-substituted barbiturates
Spray with a solution of 0.1gm dichlorofluorescein in 100ml ethanol
Then spray with a solution of 0.1gm fluorescein sodium salt in 100ml ethanol
2,6-Dichloroquinone -4- chloroimide For detection of antioxidants, phenols, amines, aromatic hydrocarbons, pharmaceuticals, phenoxyacetic acid herbicides, etc
Spray with a freshly prepared solution of 1.0gm 2,6-dichloroquinone-4-chloroimide in 100ml ethanol.
Heat 10min at 110°C and treat with NH3 vapor
p-Dimethylaminobenzaldehyde(Ehrlich reagent). For detection of sulfonamides, amines, indoles, and ergot alkaloids
Dissolve 1gm p-dimethylaminobenzaldehyde in 50ml hydrochloric acid and add 50ml methanol
Heat plates for 20min at 50°C
4- Dimethylaminocinnamaldehyde For detecting indoles.
Dissolve 0.2gm of the reagent in 80ml ethanol and 20ml conc. HCl.
N,N-Dimethyl-p-phenylenediamine dihydrochloride For detecting organic peroxides.
Dissolve 1gm of the reagent in 80ml methanol and 20ml conc. HCl.
Diphenylhexatriene For detecting lipids.
Dissolve 0.03gm of the reagent in 100ml chloroform.
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
Observe yellow-red spots.
Diphenylamine For detection of glycosides, glycolipids
Dissolve 5gm diphenylamine in 50ml ethanol, add 40ml conc. HCl and 10ml glacial acetic acid
Spray and cover plate with another glass plate, heat 30-40min at 110°C until spots appear
Glycolipids produce blue spots.
s-Diphenylcarbazone For detection of barbiturates
Spary with a solution of 0.1gm s-diphenylcarbazone in ethanol
Barbiturates give purple spots
Diphenylcarbozone/Mercuric sulphate For detection of barbiturates.
DPC is 0.01% in chloroform. Mercuric sulphate is 0.01% in dilute sulphuric acid.
2,2-Diphenylpicrylhydrazyl For detection of aldehydes and ketones
Dissolve 15mg of 2,2-DPPH in 25ml chloroform
Spray, heat 5-10min at 110°C
Yellow spots on a purple background.
Dithizone For detection of heavy metal ions
Dissolve 20mg dithizone in 100ml acetone, store in a brown bottle in a refrigerator
Spray with dithizone solution
Spray with 25% ammonia solution
Dittmer and Lester See Molybdenum blue
Dragendorffs reagent. For detecting alkaloids and quaternary nitrogen compounds
Dissolve 0.11gm potassium iodide and 0.18gm bismuth subnitrate ($OBiNO_3$) in 20mls acetic acid and make up to 100ml.

E

Erhlich reagent See p-Dimethylaminobenzaldehyde
Ethanolamine diphenylborate (flavone reagent according to Neu) For detection of flavonoids
1. Dissolve 1gm of ethanolamine diphenylborate in 100ml methanol
2. Dissolve 5gm polyyethylene glycol in 100ml ethanol
Spray with solution 1, then solution 2. Observe at 365nm UV light
Erhlich reagent See p-Dimethylaminobenzaldehyde

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

F

Fast Blue B reagent For detection of cannabinoids, phenols, tanning agents.
Spray with a freshly prepared soution of 0.5g Fast Blue B (tetraazotized di-o-anisidine) in 90ml acetone and 10ml water.
Then overspray with 0.1M sodium hydroxide solution
Ferric chloride For detecting phenols and phenolic acids.
2.7gm of salt dissolved in 100ml 2M hydrochloric acid.
Ferric Chloride / sulfuric acid Charring reagent for *polymer bound TLC plates*.
Spray with a solution of 2g $FeCl_3$ in 83ml n-butanol and 15ml conc. sulfuric acid.
Heat at 110°C
View at 5 min intervals to see if spots appear at 254 and 360nm.
Heat until spots are brown, grey or black.
Ferric chloride-potassium ferricyanide
Dissolve 3gm of each solid in 100ml 2M hydrochloric acid
For detecting aromatic amines and phenolic compounds.
Flavone reagent according to Neu See ethanolamine diphenyl borate
Fluorescamine
0.005gm of the reagent in 100ml acetone, prepared fresh.
For detecting primary amino acids and amines.
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 100ml acetone prepared fresh daily
For stabilization of fluorescence at 360nm spray with 10g triethylamine, made up 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 off. They are activated at 254nm or 360nm
When activated the fluorescent indicator will turn a green or white and the compounds appear as dark spots. The compounds might also have some fluorescence of their own, so various colors may also appear.
Formaldehyde / sulfuric acid For detection of alkaloids, aromatic hydrocarbons, e.g., antihypertensive drugs
Spray with a solution of 37% formaldehyde (commercial) in conc. sulfuric acid (1:10) as soon as the plate is removed from the developing chamber.
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 conc. phosphoric acid with stirring at room temperature.
The reagent is stable for several weeks.
Fuchsin reagent For detection of aldehydes
Dissolve 0.05 gm Fuchsin (4-rosaline hydrochloride) in 50 ml water. Add 2 ml saturated sodium bisulphite solution, leave on bench for 1 hour. Add 1 ml conc. HCl and allow to stand overnight to give a colorless liquid.
Gives violet to purple spots.
Furfural / sulfuric acid For detection of carbamate esters
Solution I: Dissolve 1gm of furfural in 100ml acetone
Solution II: Dissolve 10ml conc. sulfuric acid in 90ml acetone
Spray with I, then II.

G

Gentian Violet / Bromine For detection of lipids
Spray 0.1gm gentian violet (crystal violet) in 100ml methanol onto plate and place in a tank containing bromine vapor.
Look for blue spots on a yellow background.
Gibbs reagent For detection of phenols.
Dissolve 3gm of 2,6-dibromo-N-chloro-p-benzoquinone imine in 100ml methanol.
HPT Reagent For detecting bile acids.
Dissolve 0.025gm of 8-hydroxy-1,3,6-pyrenetrisulphonic acid trisodium salt in 100ml methanol.

H

Hydroxylamine / iron (III) chloride For detection of amides, lactones, carboxylic acid esters and anhydrides
Solution I: Make up a solution of 7g hydroxylammonium chloride in 100ml methanol and a solution of 7.2 g potassium hydroxide in 100ml methanol. Mix both solutions together and filter.
Solution II: Dissolve 2gm of iron (III) chloride and 1ml conc. HCl in 100ml water.
Dry plate in air
Spray with solution I, then with solution II

I

Iodoplatinate For detecting alkaloids, amines, and organic nitrogen compounds.
Dissolve 0.15gm potassium chloroplatinate and 3gm potassium iodide in 100ml dilute hydrochloric acid.
Iodoplatinic acid For detection of alkaloids, cocaine metabolites, amines, analgesics and biotin.
Dissolve 3ml. of chloroplatinic acid (hydrogen hexachloroplatinate) in 100ml. water, then mix with a solution of 6gm of potassium iodide in 100ml water.
Iron (III) chloride / potassium hexacyanoferrate / sodium arsenate (according to Patterson & Clements)
For detection of iodine compounds, e..g., thyroid gland hormones
Solution I: Dissolve 2.7gm iron (III) chloride hexahydrate in 100ml of 2N hydrochloric acid
Solution II: Dissolve 3.5gm potassium hexacyanoferrate in 100ml 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
Isatin For detecting proline, hydroxyproline and other amino acids and peptides.
0.2gm isatin in 100ml methanol.

J K L

Lead tetraacetate/2,7-dichlorofluorescein for detection of vicinal diols, glycosides and phenols, e.g. sugar acids
Solutions:
1. 2gm lead tetraacetate in 100ml glacial acetic acid
2. 1gm 2,7-dichlorofluorescein in 100ml ethanol
Mix 5 ml each of solution 1 and 2 and make up up to 200 ml with dry toluene. This reagent solution is stable for only about 2 hours.

M

Mandelin reagent See Vanadium(V) / sulfuric acid
Manganese / salicylaldehyde For detection of organothiophosphorus pesticides
Solution I: Dissolve 100mg manganese chloride ($MnCl_2.4H_2O$) in 100ml ethanol.
Solution II: Dissolve 1.3g 2-hydrozine quinoline in a minimum 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 C2-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.
Mercury (II) chloride / diphenylcarbazone For detection of barbiturates
Solution I: Dissolve 2gm mercury (II) chloride in ethanol and make up to 100ml with water.
Solution II: Dissolve 0.2gm diphenlycarbazone in ethanol and make up to 100ml with water.
Mix equal parts of both solutions together just before use.
Mercury (II) chloride / dithizone For detection of barbiturates
Solution I: Dissolve 1.5gm mercury (II) chloride in 100ml ethanol.
Solution II: Dissolve 1.5gm dithizone in 100ml ethanol.
Spray with a mixture of equal volumes of solution I and solution II.
View under 360nm UV light
4-Methoxybenzaldehyde / sulfuric acid / ethanol For detection erythromycin and metabolites
Make up reagent by adding 10ml of 4-methocybenzaldehyde and 10ml sulphuric acid to 90ml ethanol.
Heat 1 minute at 105°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 make up to 100ml with ethanol.
Dry at ambient temperature
Irradiate 5 min with UV light.
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 stand at room temperature overnight until solution turns light blue.
Solution II: Boil 1.78 g molybdenum powder and 500ml of solution I for 15 min, cool and decant from the remaining residue.
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.
Molybdenum blue For detecting phospholipids and related compounds.
Dissolve 1.3gm molybdenun oxide and 23.5ml conc. sulphuric acid in 100ml water.

N

Ninhydrin For detection of amino acids, amines, amino sugars(amphetamines).
Dissolve 0.2gm ninhydrin in 100ml ethanol, or in 94ml water and 6ml acetone.
Spray and heat to 110°C until reddish spots appear.
Ninhydrin Fixer reagent
Acidified ethanol containing 1% saturated cupric nitrate.
Fixative for ninhydrin chromatograms.
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
Red, pink, or purple spots are seen.
Ninhydrin / pyridine / glacial acetic acid For detection of peptides
Spray with a solution of 1gm ninhydrin in 95mls pyridine and 5 mls glacial acetic acid.
Heat 5 min at 100 C
Nitric acid / ethanol For detection of amines and alkaloids
Spray with a solution of 50 drops conc. nitric acid in 100ml ethanol. If necessary, heat to 120 C for some time.
N-4-Nitrobenzyl-N-n-propylamine hydrochloride (NBPA)
0.2 millimole reagent
For detection of isocyanates.

O

1. Orcinol Ferric Chloride (Bials reagent)
For detecting sugars,glycosides, and sulfolipids.
Dissolve 0.9gm ferric chloride and 0.55gm orcinol in 80mls ethanol and 20 mls HCl.
2. Orcinol (Bials reagent)
For detection of glycosides, glycolipids
Dissolve 0.1g orcinol in 40.7ml conc. HCl, add 1ml 1% ferric(111) chloride, and dilute to 100ml
Spray and heat at 80°C for 90 minutes.
Glycolipids produce violet spots.
Oxime (8-hydroxyquinoline)
Dissolve 0.25g in 8o ml etanol and 20 ml water
For detection of cations, AL, Cr, Fe, Co, Ni, Mn, Zn.

P

Patterson and Clements See under Iron (III) chloride/potassium hexacyanoferrate/sodium arsenate
Paraffin oil For enhancement of fluorescence spots.
1% paraffin oil in hexane
Spray evenly over the TLC plate
Spots remain stable.
m-Phenylenediamine For detection of reducing sugars
Spray with a solution of 3.6g m-phenylenediamine dihydrochloride in 100ml ethanol and heat briefly at 105°C.
Intense fluorescence in UV light.
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 100°C for 2 minutes only.
Green fluorescence spots in long wavelength UV light.
p-Phenylenediamine - phthalic acid For detection of conjugated 3-ketosteroids
Dissolve 0.9 p-phenylenediamine and 1.6g phthalic acid in 100ml 1-butanol saturated with waterand heat plate at 100-110 °C
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 2N sodium hydroxide solution with 40ml acetone
The spray reagents must be prepared fresh.
Wet chromatogram evenly with spray solution 1 and then air dry the plate.
Shake spray solution II and spray plate.
1. Phosphomolydbic acid For detection of reducing substances, e.g, alcohols, bile acids, lipids, fatty acids, steroids
Spray with a solution of 0.25gm 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 1 week even in the dark. Also used as a charring reagent for polymer bound TLC plates.
When used as a charring reagent, view every 5-10min to observe spots at 254 and 360nm. Spots are brown, grey or black.
2. Phosphomolybdic acid
For detection of Fatty acid methyl esters, keto acids, lipids, steroids, and sulphated bile acids.
Dissolve 5gm reagent in 100 ml propan-2-ol.
Phosphoric acid For detection of sterols, steroids, and bile acids
Spray heavily until the layer appears transparent with a solution of 50 mls conc phosphoric acid 50 mls water.

Then heat 10-15minutes at 120°C
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.
1. Spray plate with solution I and heat 12 min at 120°C.
Digitalis glycosides of the series B, D, and E show blue fluorescence in long wavelength UV light
2. Heat the plate again at 120°C and spray lightly with solution II
Glycosides of the series A show orange, series C show grey-green to grey-blue fluorescence in UV light.
Phosphoric acid / cupric acetate
For detection of Phospholipids.
Dissolve 3gm cupric acetate 10 mls phosphoric acid and 90 mls water.
Phosphotungstic acid For detection of cholesterol and its esters, reducing compounds, lipids, sterols, and steroids
Dissolve 20gm phosphotungstic acid in 100ml ethanol, heat at 110°C for 5-15min or until spots appear.
Cholesterol, esters will give red spots.
Pinacryptol yellow For detection of sweetners, surfactants, alkyl- and arylsulfonic acids
Dissolve 0.10gm pinacryptol yellow in 100ml hot water or ethanol.
Spray and view under UV light.
Yellow to orange fluorescence spots under 366nm. UV lightt.
Potassium dichromate / sulfuric acid (chromosulfuric acid)
Universal visualization reagent for organic compounds (e.g., alcohols, bile acids, lipids)
Dissolve slowly and with stirring 5g potassium dichromate into 100ml conc. sulfuric acid. (Use an ice bath)
Note: do not use this reagent on polymer bound TLC plates since it will char the binder; use only with gypsum binder plates.
If necessary heat plate to 150°C
Brown, grey, or black spots show up.
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 gypsum binder plates.
Dissolve slowly and with stirring 1.6gm potassium permanganate into 100 ml conc. sulfuric acid. (Use an ice bath).
Heat plate for 15-20min at 180°C
Pyrocatechol violet reagent for detection of organotin compounds
Completely decompose metalorganic compounds by irradiation of the developed plates with UV light
Dip plates in a solution of 1gm pyrocatechol violet in 1000 ml ethanol (99.5%)

Q R

Rhodamine B
For detecting lipids, and
For detecting metals (Au, Bi, Cd, Fe, Hg, Mo, Tl, V, and W.)
Dissolve 0.25gm of regent in 100 ml ethanol or acetone.
Placing the sprayed chromatograms into an ammonia atmosphere, increases sensitivity.
Rhodamine 6G
For detecting lipids.
Dissolve 0.01 gm Rhodamine 6G in 100 ml methylene chloride.
1. Rubeanic acid (sodium dithioxide)
For detection of cations gb111A and 111B
Dissolve 1gm reagent in 100 ml ethanol.
2. Rubeanic acid for detection of heavy metal ions
Spray with a solution of 0.5gm rubeanic acid in 100 ml ethanol
Dry briefly
Spray with 25% ammonia solution or place in a chamber with ammonia vapour

S

Silver nitrate / hydrogen peroxide For detection of halogenated hydrocarbons
Make up 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)
Spray plate and irradiate with UV light. For alumina plates, about 50 minutes, for silica gel plates about 15 minutes
Look for dark spots.
Sodium azide For detection of antibiotics (penicillins and cephlosporins)
Solution I: Dissolve 0.5gm soluble starch 100 ml boiling water and cool down to room temperature.
Solution II: Dissolve 3.5gm sodium azide in 100 ml of 0.1N iodine solution
Spray with solution 1, dry, then spray with solution II
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 50 ml ethanol and 50 ml water.
Spray with I, then II.
Thiazide drugs appear as orange spots within 15min; basic drugs with primary amino groups also react. However, barbiturates do not react.
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, and heat at 100°C.
Indoles turn red and thiazole derivatives turn light green.
Sodium nitroprusside / hydrogen peroxide For detection of guanidine, urea, thiourea and derivatives, creatine and creatinine.
Mix 2ml of 5% aqueous sodium nitroprusside, 1ml of 10% aqueous sodium hydroxide and 5ml of 3% aqueous hydrogen peroxide. Dilute to 15 mls. with water.
Sodium nitroprussate / potassium hexacyanoferrate (III) For the detection of aliphatic nitrogen compounds, cyanamide, guanidine, urea, thiourea and derivatives, creatine, and creatinine.
1. Make up a solution with 15 mls 10% aqueous sodium hydroxide, 15 mls of 10% sodium nitroprussate, 15 mls of 10% potassium hexacyanoferrate (III), and 45 mls of water. Let the solution stand at room temperature for about half hour before use.
2. Mix the reagent solution with an equal part of acetone and spray.
Stannic Chloride See under Tin (IV) chloride
Sulphuric acid spray reagent
Charring reagent for organic compounds. For detection of bile acids
5% w/v of the acid in ethanol.

T

Tetracyanoethylene - TCNE reagent For detection of aromatic hydrocarbons and heterocycles, aromatic amines, and phenols
Dissolve 0.5 - 1.0g tetracyanoethylene in 100ml dichloromethane or toluene.
Heat at 100°C for a short time.
Tetranitrodiphenyl For detection of cardiac glycosides
Solution I: Saturated solution of 2,3',4,4'-tetranitrodiphenyl in toluene
Solution II: Dissolve 10gm potassium hydroxide 50 ml water and 50 ml methanol
Spray with solution I, dry at room temperature, then spray with solution II.
Blue spots are observed.
Tetrazolium blue For detection of corticosteroids and other reducing compounds.
Solution 1. Dissolve 0.5gm tetrazolium blue 50ml water and 50 ml methanol.
Solution II. Make up 100 mls 6M NaOH.
Spray with an equal mixture of both solutions.
Violet spots 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 conc. sulfuric acid with caution.
Heat 15-20min at 120°C
Sugars show pink spots.
Tin (IV) chloride For detection of triterpenes, sterols, steroids, phenols, and polyphenols
Spray with 10ml tin (IV) chloride in 80 mls of chloroform and 80 mls of glacial acetic acid.
Heat the layer for 5-10min at 100°C and inspect in visible and long wavelength UV light.
TNF reagent (trinitrofluorenone) for detection of phenols
Spray with a solution of 2gm 2,4,7-trinitrofluorenone in 100 ml toluene
o-Tolidine, diazotized For detection of phenols
Tolidine solution - Make up 5g o-tolidine and 14ml conc. hydrochloric acid in 100ml water
Nitrate solution - Dissolve 10gm sodium nitrate in 100 ml water, prepared fresh
Mix 20ml tolidine solution and 20ml nitrate solution at 0°C stirring constantly.
The spray solution is stable for about 2-3 hours.
Note: After spraying it can take several hours until colored spots are formed.
p-Toluenesulfonic acid For detection of steroids, flavonoids and catechins
Dissolve 20gm of 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: Dissolve 25gm of trichloroacetic acid in 100 ml chloroform.
Spray solution II - for vitamin D - Dissolve 1gm of trichloroacetic acid in 100 ml chloroform.
Spray solution III - for digitalis glycosides - Dissolve 3.3g trichloroacetic acid in 100ml chloroform and add 1-2 drops hydrogen peroxide.
Spray with the appropriate solution, heat 5-10min at 120°C and observe in normal light and long UV light.
Inspect the spots in daylight and in long wavelength UV light.
Trifluoroacetic acid For detection of steroids
Spray with a solution of 1gm trifluoroacetic acid in 100 ml chloroform and heat 5min at 120°C
Triethanolamine For enhancement of fluorescence species
Dissolve 20gm of reagent in 100 ml isopropanol
Tungstophosphoric acid See under Phosphotungstic Acid

U

Ultraviolet Light - fluorescence Examine the dried chromatogram under both 254 and 360nm UV light.
Ultraviolet Light - quenching For detection of various compounds which absorb 254nm UV light
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.
Ketoses and oligosaccharides containing ketoses turn blue.

V

Vanadium (V) / sulfuric acid For detection of carbohydrates, glycols, reducing carboxylic acids, steroids, antioxidants, vitamins, phenols, aromatic amines, antihistamines
1. Spray with a solution of 1.2g ammonium monovanadate in 95ml water and 5ml conc. sulfuric acid.
2. For beta blockers: spray with saturated solution of ammonium monovanadate in conc. sulfuric acid.
Vanadium pentoxide / sulfuric acid 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 a solution of 1ml 1M potassium hydroxide added to 100ml ethanol. Dry 10 min at 110°C
View at 365nm UV
Vanillin / phosphoric acid Used as a charring reagent for *polymer bound* TLC plates.

Spray plate with a solution of 1g vanillin in a mixture of 50ml water and 50ml H_3PO_4
Heat 5-30min at 110°C.
View frequently for colored or fluorescent spots (at 254 and 360nm) to appear.
Charring can be continued until spots are brown, grey or black.
Vanillin / sulfuric acid For detection of steroids.
This charring visualization reagent can only be used with glass TLC plates in which a *G (gypsum) binder* has been incorporated.
Dissolve igm of vanillin in 100ml conc. sulfuric acid and spray plates. Dry at 120°C until maximum color development.
Another formulation of this reagent: 0.5g vanillin in 80ml sulfuric acid and 20ml ethanol.

W X Y Z

None available

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