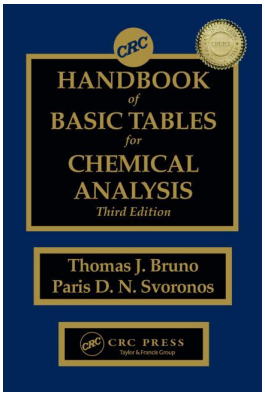

Handbook of Basic Tables for Chemical Analysis Third Edition

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Thin Layer Chromatography

Publication details
https://www.routledgehandbooks.com/doi/10.1201/b10385-4
J. Bruno Thomas, D. N. Svoronos Paris
Published online on: 13 Dec 2010

CHAPTER 3

Thin Layer Chromatography

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STRENGTH OF COMMON TLC SOLVENTS

The following table contains the common solvents used in thin layer chromatography, with a measure of their "strengths" on silica gel and alumina. The solvent strength parameter, ε°, is defined as a relative energy of adsorption per unit area of standard adsorbent [1–3]. It is defined as zero on alumina when pentane is used as the solvent. This series is what was called the eluotropic series in the older literature. For convenience, the solvent viscosity is also provided. Note that the viscosity is tabulated in cP for the convenience of most users. This is equivalent to mPa·s in the SI convention. Additional data on these solvents may be found in the tables on high performance liquid chromatography.

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Strength of Common TLC Solvents

Solvent	ε° (Al_2O_3)	Viscosity cP, 20 °	ε° (SiO_2)
Fluoroalkanes	–0.25	—	
n-Hexane	0.00	0.23	0.00
n-Pentane	0.001	0.23	0.00
2,2,4-Trimethylpentane (isooctane)	0.01	0.54	
n-Heptane	0.01	0.41	
n-Decane	0.04	0.92	
Cyclohexane	0.04	1.00	–0.05
Cyclopentane	0.05	0.47	
Carbon disulfide	0.15	0.37	0.14
Tetrachloromethane (carbon tetrachloride)	0.18	0.97	
1-Chloropentane (n-pentylchloride)	0.26	0.43	
Diisopropyl ether	0.28	0.37	
2-Chloropropane (isopropyl chloride)	0.29	0.33	
Methylbenzene (toluene)	0.29	0.59	
1-Chloropropane (n-propyl chloride)	0.30	0.35	
Chlorobenzene	0.30	0.80	
Benzene	0.32	0.65	0.25
Bromoethane (ethyl bromide)	0.37	0.41	
Diethyl ether (ether)	0.38	0.23	0.38
Trichloromethane (chloroform)	0.40	0.57	
Dichloromethane (methylene chloride)	0.42	0.44	
Tetrahydrofuran	0.45	0.55	
1,2-Dichloroethane	0.49	0.79	
Butanone (methyl ethyl ketone)	0.51	0.43	
Propanone (acetone)	0.56	0.32	0.47
1,4-Dioxane	0.56	1.54	0.49
Ethyl ethanoate (ethyl acetate)	0.58	0.45	0.38
Methyl ethanoate (methyl acetate)	0.60	0.37	
1-Pentanol (n-pentanol)	0.61	4.1	
Dimethyl sulfoxide (DMSO)	0.62	2.24	
Aminobenzene (aniline)	0.62	4.4	
Nitromethane	0.64	0.67	
Cyanomethane (acetonitrile)	0.65	0.37	0.50
Pyridine	0.71	0.94	
2-Propanol (isopropanol)	0.82	2.3	
Ethanol	0.88	1.20	
Methanol	0.95	0.60	
Ethylene glycol	1.11	19.9	
Ethanoic acid (acetic acid)	large	1.26	
Water	large	1.00	

MODIFICATION OF THE ACTIVITY OF ALUMINA BY ADDITION OF WATER

The following table describes five different activity grades of commercial alumina used in chromatography [1–3]. The activity grades are defined by the degree of adsorption of azobenzene (called azobenzene number) on the types of hydrated alumina. Those types are prepared by heating commercial alumina to redness, giving grade I, and then adding controlled amounts of water and allowing equilibration in a closed vessel. The azobenzene number decreases with the amount of water added. The R_f value is the ratio of distance traveled by the solute spot to that traveled by the solvent.

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Modification of the Activity of Alumina by Addition of Water

Water Added (wt/wt%)	Activity Grade	Azobenzene Number [maximum adsorption of azobenzene (10^{-5} mol/g)]	R_f (p-Amino-azobenzene)
0	I	26	0.00
3	II	21	0.13
6	III	18	0.25
10	IV	13	0.45
15	V	0	0.55

STATIONARY AND MOBILE PHASES

The following table provides a comprehensive guide to the selection of thin layer chromatography media and solvents for a given chemical family. Mixed mobile phases are denoted with a slash, /, between components, and where available, the proportions are given. Among the references are several excellent texts [1–3,60], review articles [4–24], and original research papers and reports [25–59,61–98]. A table of abbreviations follows this section.

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Stationary and Mobile Phases

Abbreviations/Solvent Table

Abbreviation	Solvent Name	Abbreviation	Solvent Name
Ac	acetone	Et_2O	diethylether
Ace	acetate	Foram	amylformate
AcOH	acetic acid	HCl	hydrochloric acid
n-AmOH	n-amyl alcohol	H_3BO_3	boric acid
t-AmOH	t-amyl alcohol	Hex	hexane
$AmSO_4$	ammonium sulfate	HForm	formic acid
i-BuAc	isobutylacetate	MeCl	methylene chloride
BuFor	n-butylformate	MeCN	acetonitrile
i-BuOH	isobutanol	MEK	methylethylketone
n-BuOH	n-butanol	MeOH	methanol
$i\text{-}Bu_2O$	diisobutylether	NaAc	sodium acetate
CCl_4	carbon tetrachloride	NH_3	ammonia, aqueous
C_2HCl_3	trichloroethene	Petet	petroleum ether
$CHCl_3$	chloroform	Ph	phosphate
$(CH_2)_6$	cyclohexane	PhOH	phenol
C_6H_6	benzene	PrAc	propylacetate
$n\text{-}C_6H_{14}$	n-hexane	PrFor	propylformate
$n\text{-}C_7H_{16}$	n-heptane	Progl	propylene glycol
$i\text{-}C_8H_{18}$	isooctane	i-PrOH	isopropanol
$(ClCH_2)_2$	dichloroethane	n-PrOH	n-propanol
DEAE	diethyl aminoethyl	$i\text{-}Pr_2NH$	diisopropylamine
Diox	dioxane	$i\text{-}Pr_2O$	diisopropylether
DMF	dimethylformamide	Py	pyridine
EtFor	ethylformate	THF	tetrahydrofuran
EtOAc	ethylacetate	Tol	toluene
EtOH	ethanol	w	water
Et_2NH	diethylamine	m-X	m-xylene

Stationary and Mobile Phases

Family	Stationary Phase	Mobile Phase	Ref.
Adrenaline and derivatives	alumina (two dimensional)	C_6H_6/EtOAc (60:40)	25
		$CHCl_3$/EtOH/Tol (90:6.5:3.1)	
Adrenochromes	cellulose	AcOH(2 %)/w	26,27
	Whatman #1 (descending)	AcOH(2 %)/w	26–29
Alcohols	silica gel (G-coated)	EtOAc/Hex	30
Alcohols, polyhydric	alumina or Kieselguhr (impregnated with polyamide) or silica gel	$CHCl_3$/Tol/HForm or n-BuOH/NH_3 or $CHCl_3$	31
Aldehydes	silica gel (G-coated)	EtOAc/Hex	30
Aldehydes, 2,4-dinitro-phenylhydrazones	alumina	C_6H_6 or $CHCl_3$ or Et_2O or C_6H_6/Hex	3
	alumina IB	MeCl or Tol/THF (4:1)	32
	silica gel	Hex/EtOAc (4:1 or 3:2)	3
	silica gel IB	MeCl or Tol/THF (4:1)	32
Alkaloids	alumina	i-BuOH/AcOH or i-BuOH/NH_3 or i-PrOH/AcOH	33
	alumina		3
	cellulose (impregnated with formamide)	$CHCl_3$ or EtOH or $(CH_2)_6$/$CHCl_3$ (3:7)	3
	paper (S&S #2043b)	C_6H_6/n-C_7H_{16}/$CHCl_3$/Et_2NH (6:5:1:0.02)	34
	paper electrophoresis	n-BuOH/HCl(25 %)/w (100:26:39)	35
	silica gel	i-BuOH/AcOH or i-BuOH/NH_3 or i-PrOH/HOAc	3
		C_6H_6/EtOH (9:1) or $CHCl_3$/Ac/Et_2NH (5:4:1)	
Amides	Kieselguhr (adipic acid impregnated)	i-Pr_2O/Petet/CCl_4/HForm/w	36
	silica gel	i-Pr_2O/Petet/CCl_4/HForm/w	36
Amines	alumina	Ac/n-C_7H_{16} (1:1)	3
	alumina G	i-BuAc or i-BuAc/AcOH	37
	Keiselguhr G	Ac/w (99:1)	3
	silica gel	EtOH (95 %)/NH_3 (25 %) (4:1)	3
	silica gel (aromatic only)		38
Amino acids	alumina	n-BuOH/AcOH/w (3:1:1) or Py/w	3
	cellulose	n-BuOH/AcOH/w (4:1:1)	3
	cellulose (two-dimensional)	n-BuOH/Ac/NH_3/w (10:10:5:2)	3
	silica gel	followed by i-PrOH/HForm/w (20:1:5)	3
		n-BuOH/AcOH/w (3 or 4:1:1) or PhOH/w (3:1) or n-PrOH/NH_3 (34 %) (2:1)	
Aminochromes	Whatman #1 (acid washed)	w or AcOH/w or MeOH/w or EtOH/w or n-BuOH/AcOH/w or i-PrOH/w	26,39
Barbiturates	silica gel	$CHCl_3$/n-BuOH/NH_3 (25 %) (14:8:1)	3
Benzophenones, hydroxy	alumina or cellulose or Kieselguhr (impregnated with adipic acid triethylene glycol polyester) or silica gel	HForm/m-X	40

(Continued)

Stationary and Mobile Phases (Continued)

Family	Stationary Phase	Mobile Phase	Ref.
Bile acids	silica gel	C_6H_6/Et_2O (4:1) or Et_2O/AcOH (99.6:0.4) or $CHCl_3$/MeOH (9:1)	41
Caffeine	chromatography paper	n-BuOH/NH_3 or n-BuOH/HForm	42
Carboxylic acids	Keiselguhr/polyethylene glycol	i-Bu_2O/HForm/w (90:7:3)	43
	Polyamide powder	i-Pr_2O/Petet/CCl_4/HForm/w (50:20:20:8:1) or MeCN/EtOAc/ HForm or BuForm/EtOAc/HForm	43
	silica gel (G-coated)	EtOH/NH_3/THF	30
	silica gel ($CaSO_4$ impregnated)	n-PrOH/NH_3 or EtOH/$CHCl_3$/NH_3	44
	silica gel/polyethylene glycol M-1000	i-Pr_2O/HForm/w (90:7:3)	45,46 43
Carboxylic acids, unsaturated	silica gel	$CHCl_3$/MeOH	47
Catecholamines	alumina		48
	boric acid gel (neutral pH)	HCl (0.025N)	48
	Kieselguhr		
	phenylboronate		49
	phosphocellulose	Dilute acids	50
		Ph buffer (pH = 6.2)/EDTA	51
Catecholamines, dansyl derivatives	alumina (two dimensional)	C_6H_6/EtOAc (60:40) or $CHCl_3$/EtOH/ Tol (90:6.5:3.5)	25
	Amberlite IRC50		52
Catecholamines, o-methyl derivatives	silica gel (sodium borate impregnated)		53
Corticosteroids	silica gel	EtOH(5 %)/MeCl or EtOH/$CHCl_3$	54,55
Coumarins	polyamide	MeOH/w (4:1 or 3:2)	3
	silica gel G	Petet/EtOAc (2:1)	3
	silica gel G (impregnated with NaAc)	Tol/EtFor/HForm (5:4:1)	3
	silicic acid (starch bound)	EtOAc/Skellysolve B	3
Dicarboxylic acids	Kieselguhr/polyethylene glycol	i-Pr_2O/HForm/w (90:7:3)	43
	polyamide powder	i-Pr_2O/Petet/CCl_4/HForm/w (50:20:20:8:1) or MeCN/EtOAc/ HForm (9:1:1) or BuFor/EtOAc/ HForm (9:1:1)	43
	polyamide	MeCN/PrFor/PrAc/HForm (45:45:10:10) or i-Pr_2O/Petet/ CCl_4/ HForm/w (50:20:20:8:1) or n-AmOH/CCl_4/HFor (3:2:1)	56
	Woelm DC powder		
	silica gel	i-Pr_2O/HForm/w (90:7:3)	43
	silica gel (G-coated)	EtOH/NH_3/THF	30
Diols (see Alcohols, polyhydric)			
Disulfides	alumina	Hex	57
Disulfides, 3,5-dinitro-benzoates	Whatman #3 (impregnated with 10 % paraffin oil in cyclohexane), $(CH_2)_6$	DMF/MeOH/w or Foram/MeOH/w	58

Stationary and Mobile Phases (Continued)

Family	Stationary Phase	Mobile Phase	Ref.
Flavinoids	paper	n-BuOH/AcOH/w or EtOAc/w or AcOH/w or C_6H_6/AcOH/w	60
	polyamide		3
	silica gel	MeOH/H_2O	59
	silica gel (impregnated with NaAc)	C_6H_6/Py/AcOH (36:9:5) Petet/EtOAc (2:1)	3
	silicic acid (starch bound)	Tol/EtForm/HFor (5:4:1)	3
		EtOAc/Skellysolve B	
Glycerides	silica gel G	$CHCl_3/C_6H_6$ (7:3)	3
	silica gel G (impregnated with silver nitrate)	$CHCl_3$/AcOH (99.5:0.5)	3
Glycolipids	silica gel G	n-PrOH/NH_3 (12 %) (4:1)	3
Glycols, polyethylene	paper	n-PrOH/EtOAc/w (7:1:2) or n-BuOH/AcOH/w (4:1:5) or t-AmOH/n-PrOH/w (8:2:3) or EtOAc/AcOH/w (9:2:2)	60
	silica gel	Ac or n-BuOH/AcOH/w	61
Hydroxamates	silica gel	i-Pr_2O or i-Pr_2O/EtOAc (1:4) or i-Pr_2O/i-C_8H_{18}	62
Hydroxamic acids	Kieselguhr G (impregnated with diethy-lene glycol or triethylene glycol adipate polyesters)	i-Pr_2O/Petet/CCl_4/HForm/w (50:20:20:8:1)	98
Indoles	acetylated (ascending)	$CHCl_3$/MeOH/w (10:10:6)	63
	cellulose (thin-layer)	w or HCl (0.005N) or n-BuOH/AcOH/w (12:3:5) or C_6H_6/AcOH/w (125:72:3)	64
α-Ketoacids	silica gel ($CaSO_4$ impregnated)	EtOH/$CHCl_3$/NH_3	44
Ketones, 2,4-dinitro-phenyl hydrazones	alumina IB	MeCl or Tol/THF (4:1)	32
	silica Gel IB	MeCl or Tol/THF (4:1)	32
Lactams	silica gel	i-Pr_2O or i-Pr_2O/EtOAc (1:4) or i-Bu_2O/i-C_8H_{18}	62
Lactones	silica gel	i-Pr_2O or i-Pr_2O/EtOAc (1:4) or i-Bu_2O/i-C_8C_{18}	62
Lipids	alumina	Petet/Et_2O (95:5)	3
	silica gel G	Petet/Et_2O/AcOH (90:10:1)	3
	silicic acid	$CHCl_3$/MeOH/w (80:25:3)	3
Mercaptans (see Thiols)			
Nitrosamines	silica gel	Hex/Et_2O/MeCl	65
	Kieselgel	MeCl/Hex/Et_2O (2:3:4) (aliphatic, aromatic); MeCl/Hex/Et_2O (5:7:10) (cyclic)	66
Nucleotides	cellulose	$AmSO_4$ (sat'd)/NaAc(1 M)/ i-PrOH (80:18:2)	3
	cellulose (on DEAE)		3
		HCl (aq)	
Oximes	silica gel G	C_6H_6/EtOAc or C_6H_6/MeOH (abs)	67
Peroxides	silicone filter paper	w/EtOH/$CHCl_3$	68
Phenols	alumina	Et_2O	3
	alumina/AcOH	C_6H_6	3

(Continued)

Stationary and Mobile Phases (Continued)

Family	Stationary Phase	Mobile Phase	Ref.
	silica gel A	$CHCl_3$/AcOH (5:1) or $CHCl_3$/Ac/AcOH (10:2:1) or C_6H_6/AcOH (5:1) or Petet (80 °)/CCl_4/AcOH (4:6:1) or $CHCl_3$/Ac/Et_2NH (4:2:0.2)	69
	silica gel G	C_6H_6/Diox/AcOH (90:25:4)	3
	silica gel/oxalic acid	C_6H_6	70
	silica gel/potassium carbonate	MeCl/EtOAc/Et_2NH (92:5:3 or 93:5:2)	70
Phosphates, esters	alumina	Hex/C_6H_6/MeOH (2:1:1) or Hex/MeOH/Et_2O	71
	Kieselgel		71
		Hex/C_6H_6/MeOH (2:1:1) or Hex/MeOH/Et_2O	
Phospholipids	silica gel G	$CHCl_3$/MeOH/w	3
Polynuclear aromatics	alumina	CCl_4	3
	alumina	C_6H_6/$(CH_2)_6$ (15:85)	72
	silica gel	Hex or CH_3CHCl_2 or C_2HCl_3 or CCl_4	73,74
Polypeptides	Sephadex G-25	w or NH_3 (0.05 M)	3
	silica gel G	$CHCl_3$/MeOH (9:1) or $CHCl_3$/Ac (9:1)	3
Pyridines	Whatman #1 (descending)	n-BuOH/w or n-BuOH/w/NH_3 or Ac or i-BuOH/w or MEK/AcOH/w	75
Pyridines, quaternary salts (descending)	Whatman #1	Ac/w or $AmSO_4$/Ph buffer (pH = 6.8)/n-PrOH(2 %) or n-PrOH	75
Purines	silica gel	Ac/$CHCl_3$/n-BuOH/NH_3 (25 %) (3:3:4:1)	3
Pyrrole, tri-carboxylic acid	silica gel	n-BuOH/EtOH/NH_3/w (10:10:1:1)	64
Skatoles, hydroxy	silica gel G	i-Pr_2O or $(ClCH_2)_2$/i-Pr_2NH (6:1)	76
Steroids	alumina	$CHCl_3$EtOH (96:4)	3
	paper	Petet/Tol/MeOH/w or Petet/C_6H_6/MeOH/w	60
	paper (impregnated with kerosene)		77
		n-PrOH/w	78,79
	silica gel G	EtOAc/$(CH_2)_6$/EtOH(abs) or EtOAc/$(CH_2)_6$ or $CHCl_3$/EtOH (abs) or C_6H_6/EtOH or n-C_6H_{14}/EtOAc or EtOAc/n-C_6H_{14}/EtOH(abs)/AcOH or EtOAc/n-C_6H_{14}/AcOH	80,81
Sugars	cellulose	n-BuOH/Py/w (6:4:3) or EtOAc/Py/w (2:1:2)	3
	Kieselguhr G (buffered with 0.02N NaAc)	EtOAc/i-PrOH/w	3
	silica gel (buffered with H_3BO_3)	C_6H_6/AcOH/MeOH (1:1:3)	3
	silica gel (impregnated with sodium bisulfite)	EtOAc/AcOH/MeOH/w (6:1:5:1) or n-PrOH/w (85:15) or i-PrOH/EtOAc/w (7:1:2) or MEK/AcOH/w (6:1:3)	3
	silica gel G	n-PrOH/conc NH_3/w (6:2:1)	3
	Whatman #1 (descending-two dimensional)	PhOH or n-BuOH/AcOH	82

Stationary and Mobile Phases (Continued)

Family	Stationary Phase	Mobile Phase	Ref.
Sugars, aldoses	paper	EtOAc/Py/w (2:1:2) or n-BuOH/AcOH/w (4:1:5) or n-BuOH/EtOH/H_2O (5:1:4) or EtOAc/AcOH/w (9:2:2) or EtOAc/AcOH/HForm/w or EtOAc/Py/NaAc (sat'd)	60
	Whatman #1	PhOH or n-BuOH/AcOH	82
Sugars, carbamates	silica gel	n-BuOH/H_3BO_3 (0.03 M) (9:1)	
Sugars, deoxy	Whatman #1	PhOH or n-BuOH/AcOH	82
Sugars, ketoses	paper	EtOAc/Py/w (2:1:2) or n-BuOH/AcOH/w (4:1:5) or n-BuOH/EtOH/H_2O (5:1:4) or EtOAc/AcOH/w (9:2:2) or w/PhOH (pH = 5.5)	60
	Whatman #1	PhOH or n-BuOH/AcOH	82
Sulfides	alumina	Hex	75
	alumina	$CHCl_3$/MeOH	96
	silica gel	CCl_4 or C_6H_6	83
	silica gel DF-5	Ac/C_6H_6 or Tol/EtOAc	95
Sulfilimines, p-nitrosobenzene sulfonyl	Whatman #4 (impregnated with formamide)	C_6H_6 or C_6H_6/$(CH_2)_6$	84
Sulfonamides	Kieselguhr	$CHCl_3$/MeOH (9:1) or $CHCl_3$/MeOH/NH_3	85
	silica gel		86
	silica gel (neutral)	Et_2O or $CHCl_3$/MeOH (10:1)	87
	silica gel (G)	n-BuOH/MeOH/Ac/Et_2NH (9:1:1:1)	3
		$CHCl_3$/EtOH/n-C_7H_{16}	
Sulfones	alumina	Et_2O or Hex/Ac (1:1)	57
	silica gel DF-5	Ac/C_6H_6 or Tol/EtOAc	95
Sulfones, esters	alumina	Et_2O or Hex/Ac (1:1)	57
Sulfones, hydroxy-ethyl	alumina	Hex/w (1:3)	57
Sulfoxides	alumina	C_6H_6/Py (20:1) and Diox	88
	alumina	Ac/CCl_4 (1:4)	97
	silica gel	Ac or EtOAc or $CHCl_3$/Et_2O	83
	silica gel DF-5	Ac/C_6H_6 or Tol/EtOAc	95
	Whatman #1	PhOH/w (8:3) or n-BuOH/AcOH/w (9:1:2.5)	89
Sulfoxides, hydroxy-ethyl	alumina	Et_2O or Hex/Ac (1:1) or Hex/Et_2O (1:3)	57
Terpenes	alumina	C_6H_6 or C_6H_6/Petet or C_6H_6/EtOH	3
	silica gel G	i-Pr_2O or i-Pr_2O/Ac	3
	silica gel/gypsum	$CHCl_3$/C_6H_6 (1:1)	90
	silicic acid (starch bond)	n-C_6H_{14}/EtOAc (85:15)	3
Thiobarbiturates	paper	n-AmOH/n-BuOH/25 % NH_3 (2:2:1)	91
Thiolactones	silica gel	i-Pr_2O or i-Pr_2O/EtOAc (1:4) or i-Bu_2O/i-C_8H_{18}	62

(Continued)

Stationary and Mobile Phases (Continued)

Family	Stationary Phase	Mobile Phase	Ref.
Thiols	alumina	Hex	57
	alumina (activated)	AcOH/MeCN (3:1)	96
	alumina (5 % cetane impregnated)	AcOH/MeCN (3:1)	96
		EtOAc or $CHCl_3$	83
	silica gel		
Thiophenes	alumina G	Petet (40–60 °C)	92
	silica gel	MeOH or $C_6H_6/CHCl_3$ (9:1)	92
Thiophosphate, esters		Petet or $C_6H_6/CHCl_3$ or Ac or EtOH or EtOAc or MeOH	93
Ureas	acetylated plates	CCl_4/EtOAc/EtOH (100:5:2)	94
	silica gel	CCl_4/MeCl/EtOAc/HOAc (70:50:15:10)	94
Urethanes (See ureas)			

TYPICAL STATIONARY AND MOBILE PHASE SYSTEMS USED IN THE SEPARATION OF VARIOUS INORGANIC IONS

The following table lists a series of stationary and mobile systems that are used in the separation of various inorganic ions [1–8]. The list is far from detailed and the reader is advised to consult the given references for details.

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Typical Stationary and Mobile Phase Systems Used in the Separation of Various Inorganic Ions

Stationary Phase	Mobile Phase	Solvent Ratio	Separated Ions
Silica Gel G	butanol/1.5N HCl/2,5-hehanedione	100:20:0.5	hydrogen sulfide group
Silica gel G	acetone/conc. HCl/2,5-hexanedione	100:1:0.5	ammonium sulfide group
Silica gel G	water sat'd ethyl acetate/ tributyl phosphate	100:4	U, Ga, Al
Silica gel G	ethanol/acetic acid	100:1	alkali metals
Silica gel G	acetone/1-butanol/conc. NH_4OH/ water	65:25:10:5	halogens
Silica gel G	methanol/conc. NH_4OH/10 % trichloroacetic acid/water	50:15:5:30	phosphates
Dowex 1-cellulose (1:1)	1 M aqueous sodium nitrate		halogens
Cellulose	HCl (or HBr)/alcohol mixtures	variable	Groups IA, IIA, IIIB, IVB, VB, VIB, transition metals
Cellulose	1-butanol/water/HCl	8:1:1	Fe, Al, Ga, Ti, In
Cellulose	acetic acid/pyridine/conc. HCl	80:6:20	ammonium sulfide group
DEAE cellulose	sodium azide/HCl	variable	Cd, Cu, Hg
Amberlite CG 400 and CG 120	HCl/HNO_3	variable	Pb, Bi, Sn, Sb, Cu, Cr, Hg

Sat'd = Saturated.
Conc. = Concentrated.

SPRAY REAGENTS IN THIN LAYER CHROMATOGRAPHY

The following table lists the most popular spray reagents needed to identify organic compounds on chromatographic plates. These reagents have been thoroughly covered in several books [1–3], and reviews [4–23]. Due to the aerosol nature of the spray and the chemical hazards associated with several of these chemicals, the use of a fume hood is highly recommended. The original references of the spray reagents are given in order to provide information about their results with individual compounds [24–138]. A list and description of some complicated protocols follows this section of the chapter.

Note: $1\gamma = 1\ \mu g/cm^2$ on a TLC plate.

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Spray Reagents in Thin Layer Chromatography

Family/Functional Group	Test	Result	Ref.
Adrenaline (and derivatives)	2,6-dichloroquinonechloroimide (0.5 % in absolute ethanol)	Variety of colors	1
	potassium ferricyanide (0.6 % in 0.5 % sodium hydroxide	Red spots	25
Adrenochromes	4-N,N-diemthylaminocinnamaldehyde	Blue-green to grey-green spots	26
	Ehrlich reagent	Blue-violet to red-violet spots	26
	zinc acetate (20 %)	Blue or yellow fluorescent spots	26
Alcohols	ceric ammonium sulfate (or nitrate)	Yellow/green spots on red background	1,3
	2,2-Diphenylpicrylhydrazyl (0.06 % in chloroform)	Yellow spots on purple background after heating (110 °C, 5 min)	3
	vanillin (1 % in conc. sulfuric acid)	Variety of spots after heating (120 °C); good only for higher alcohols	27
Aldehydes	o-dianisidine (saturated solution in acetic acid)	Variety of spots	28
	2,4-dinitrophenylhydrazine	Blue colors (saturated ketones); olive green colors (saturated aldehydes); slow developing colors (unsaturated carbonyl compounds)	1
	2,4-diphenylpicrylhydrazyl (0.06 % in chloroform)	Yellow spots on a purple background after heating (110 °C, 5 min)	3
	hydrazine sulfate (1 % in 1N hydrochloric acid)	Spots under UV (especially after heating)	3
Aldehydes, carotenoids	Tollens reagent	Dark spots	1
	Rhodamine (1–5 % in ethanol)	Variety of spots after treatment with strong alkali (sensitivity 0.03 μg)	1
Alkaloids	Bromcresol green (0.05 % in ethanol)	Green spots, especially after exposure to ammonia	3
	Chloramine-T (10 % aqueous)	Rose spots after exposure to hydrochloric acid and heat	4
	cobalt (II) thiocyanate	Blue spots on a light pink background	29
	p-N,N-dimethylaminobenzaldehyde (4 % in 1:3 hydrochloric acid/methanol)	Characteristic spots for individual alkaloids	4
	iodine/potassium iodide (in 2N acetic acid)	Variety of spots	3
	Kalignost test	Orange/red spots fluorescing under long-wave UV	30
	Sonnenschein test	Variety of spots	1
Alkaloids (ergot or fungal)	p-N,N-dimethylaminobenzaldehyde/ sulfuric acid	Blue spots	31
Amides	chlorine/pyrazolinone/cyanide	Red spots turning blue (detection limit 0.5 μg)	32
	hydroxylamine/ferric chloride	Variety of spots	33

Amines (all types unless specified)	alizarin (0.1 % in ethanol)	Violet spots on yellow background	3
	chlorine/pyrazolinon/cyanide	Red spots turning blue (aromatic only)	32
	cobalt (II) thiocyanate	Blue spots on white/pink background	29
	diazotization and α-naphthol coupling	Variety of spots (1 ° aromatic amines only)	1
	Ehrlich reagent	Yellow spots for aromatic amines	34
	Fast Blue B Salt	Variety of spots (only for amines that can couple)	1
	Glucose/phosphoric acid (4 %)	Variety of spots (aromatic amines only) especially after heating	35
	malonic acid (0.2 %)/salicylaldehyde (0.1 %) (in ethanol)	Yellow spots after heating (120 °C, 15 min)	3,4
Amines (all types unless specified; cont.)	1,2-naphthoquinone-4-sulfonic acid, sodium salt (0.5 % in 1N acetic acid)	Variety of colors after 30 min (aromatic amines only)	36
	ninhydrin	Red colors when exposed to ammonium hydroxide	37
	p-nitroaniline, diazotized	Variety of colored spots	38
	nitroprusside (2.5 %)/acetaldehyde (5 %)/sodium carbonate (1 %)	Variety of spots (2 ° aliphatic only)	39
	picric acid (3 % in ethanol)/sodium hydroxide (10 %) (5:1)	Orange spots	4
	potassium iodate (1 %)	Variety of spots for phenylethylamines (after heating)	3
	vanillin-potassium hydroxide	Variety of colors	40
Amino acids	dehydroascorbic acid (0.1 % in 95 % n-butanol)	Variety of colored spots	3
	2,4-dinitrofluorobenzene	Variety of spots	1
	Isatin-zinc acetate	Variety of colors	1
	Folin reagent	Variety of colors	1
	ninhydrin	Red colors when exposed to ammonium hydroxide	36
	vanillin/potassium hydroxide	Variety of colors	40
Amino alcohols	alizarin (0.1 % in ethanol)	Violet on yellow background	3
Aminochromes	p-N,N-dimethylaminocinnamaldehyde	Variety of colors	41
	Ehrlich reagent	Violet spots	26,41
	ferric chloride (3 %)	Gray-brown spots	41
	p-nitroaniline, diazotized	Red/brown spots	26,41
	sodium bisulfite, aqueous	Yellow fluorescence under UV	41,42
Aminosugars	ninhydrin	Red colors when exposed to ammonium hydroxide	37
Ammonium salts, quaternary	cobalt (II) thiocyanate	Variety of spots	29

(Continued)

Spray Reagents in Thin Layer Chromatography (Continued)

Family/Functional Group	Test	Result	Ref.
Anhydrides	hydroxylamine/ferric chloride	Variety of spots	33
Arginine	Sakaguchi reagent	Orange/red spots	1
Azulenes	EP reagent	Blue spots (room temperature) that fade to green/yellow shades and can be regenerated with steam	1
Barbiturates	cobalt (II) nitrate (2 %)/lithium hydroxide (0.5 %)	Variety of colors	1
	cupric sulfate/quinine/pyridine	Variety of colors (white, yellow, violet)	1
	s-diphenylcarbazone (0.1 % in ethanol)	Purple spots	3
	ferrocyanide/hydrogen peroxide	Yellow/red colors	1
	fluorescein (0.005 % in 0.5 M ammonia)	Variety of spots under long or short-wave UV	43
	mercurous nitrate (1 %)	Variety of spots	1
	Zwikker reagent	Variety of spots	1
Bile acids	anisaldehyde/sulfuric acid	Variety of spots	4
	antimony trichloride (in chloroform)	Variety of spots	44
	perchloric acid (60 %)	Fluorescent spots (long wave UV) after heating (150 °C, 10 min)	44
	sulfuric acid	Variety of spots	44,45
Bromides	fluorescein/hydrogen peroxide	Nonfluorescent spots	1
Caffeine	chloramine-T	Pink–red spots	1
	silver nitrate (2 % in 10 % sulfuric acid)	Carmine-red spots (limit 2γ)	46
Carboxylic acids	Bromcresol blue (0.5 % in 0.2 % citric acid)	Yellow spots on blue background	3
	Bromothymol blue (0.2 % in ethanol, pH = 7)	Yellow spots upon exposure to ammonia	47
	2,6-dichlorophenol/indophenol (0.1 % in ethanol)	Red spots on blue background after heating	48
	hydrogen peroxide (0.3 %)	Blue fluorescence under long-wave UV	49
	Schweppe reagent	Dark brown spots	1
Carboxylic acids, ammonium salts			
Catechins	p-toluenesulfonic acid (20 % in chloroform)	Fluorescent spots under long-wave UV	50
Catecholamines	ethylenediamine (50 %)	Spots under short/long wave UV after heating (50 °C, 20 min)	1

Chlorides, alkyl	2,6-dichlorophenol indophenol (0.2 %)/silver nitrate (3 %) in ethanol	Variety of spots	1
	silver nitrate (0.5 % in ethanol)	Dark spots upon UV irradiation	51
	silver nitrate/formaldehyde	Dark grey spots	1
	silver nitrate/hydrogen peroxide	Dark spots	1
Chlorinated insecticides and pesticides	diphenylamine (0.5 %)/zinc chloride (0.5 %) in acetone	Variety of colors upon heating (200 °C)	1
	2-phenoxyethanol (5 %) in 0.05 % silver nitrate	Variety of spots	4
	silver nitrate/formaldehyde	Dark grey spots	1
	o-toluidine (0.5 %) in ethanol	Green spots under UV (sensitivity 0.5 μg)	4
Choline derivatives	dipicrylamine (0.2 % in 50 % aqueous acetone)	Red spots on yellow background	1
Corticosteroids	Blue Tetrazolium (0.05 %)/sodium hydroxide (2.5 M)	Violet spots (limit 1γ/cm^2)	1,52
	2,3,5-Triphenyl-H-tetrazolium chloride (2 % in 0.5 NaOH)	Red spots after heating (100 °C, 5 min)	1
Coumarins	Benedict reagent	Fluorescent spots under long-wave UV	1
	potassium hydroxide (5 % in methanol)	Variety of spots under long-wave UV	1
Dextrins	iodine/potassium iodide	Blue–black spots (α-dextrins); brown–yellow spots (β- or γ-dextrins)	53
Dicarboxylic acids	bromocresol purple (0.04 % in basic 50 % ethanol, pH = 10)	Yellow spots on blue background	4,54
Diols (1,2-)	lead tetraacetate (1 % in benzene)	White spots after heating (110 °C, 5 min; limit 2 μg)	55
Disulfides	iodine (1.3 % in ethanol)/sodium azide (3.3 % in ethanol)	White spots on brown iodine background	3
	nitroprusside (sodium)	Red spots	56
Diterpenes	antimony (III) chloride/acetic acid	Reddish yellow to blue-violet	57
Esters	hydroxylamine/ferric chloride	Variety of spots	33
Flavonoids	aluminum chloride	Yellow fluorescence on long-wave UV	58
	antimony (III) chloride (10 % in chloroform)	Fluorescence on long-wave UV	59
	Benedict's reagent	Fluorescence on long-wave UV (only for o-dihydroxy compounds)	59
	lead acetate (basic, 25 %)	Fluorescent spots	4,50
	p-toluenesulfonic acid (20 % in chloroform)	Fluorescent spots under long-wave UV after heating (100 °C, 10 min)	
Fluorescamines	perchloric acid (70 %)	Blue fluorescent spots	60
Glycols, polyethylene	quercetin/sodium tetraphenylborate	Orange–red spots	61
Glycolipids	diphenylamine (5 % in ethanol) dissolved in 1:1 hydrochloric acid/acetic acid	Blue–grey spots	1

(Continued)

Spray Reagents in Thin Layer Chromatography (Continued)

Family/Functional Group	Test	Result	Ref.
Glycosides, triterpene	Liebermann–Burchard reagent	Fluorescence under long-wave UV	1
Hydroxamates	ferric chloride (10 % in acetic acid)	Brown spots	62
Hydroxamic acids	ferric chloride (1–5 % in 0.5N hydrochloric acid)	Red spots	1
Imidazoles	p-anisidine/amyl nitrite	Red/brown spots	3
Indoles	chlorine/pyrazolinone/cyanide	Red spots turning blue after a few minutes (limit 0.5 μg)	32
	cinnamaldehyde/hydrochloric acid	Red spots	1
	p-N,N-dimethylaminocinnamaldehyde	Variety of colored spots	63
	Ehrlich reagent	Purple for indoles; blue for hydroxyindoles	9,34,64
	ferric chloride (0.001 M) in 5 % perchloric acid	Red spots	3
	naphthoquinone/perchloric acid	Orange spots	65
	perchloric acid (5 %)/ferric chloride (0.001 M)	Variety of colored spots	66
	Prochazka reagent	Fluorescent (yellow/orange/green) spots under long wave UV	1
	Salkowski reagent	Variety of colored spots	67
	van Urk (or Stahl) reagent	Variety of colored spots	1
	xyanthydrol (0.1 % in acidified ethanol)	Variety of colored spots after heating (100 °C)	68
Iodides	Sonnenschein test	Variety of spots	1
α-Ketoacids	2,6-dichlorophenol/indophenol (0.1 % in ethanol)	Pink spots upon heating	4,48
	o-phenylenediamine (0.05 % in 10 % trichloroacetic acid or 0.2 % in 0.1N H_2SO_4/ethanol)	Green fluorescence under long wave UV after heating (100 °C, 2 min)	1
Ketones	o-dianisidine (saturated solution in acetic acid)	Characteristic spots	28
	2,4-dinitrophenylhydrazine	Yellow–red spots	3
Lactones	hydroxylamine/ferric chloride	Variety of colors	33
Lipids	α-cyclodextrin	Variety of spots (for straight chain lipids)	53
	2′,7′-dichlorofluorescein (0.2 %) in ethanol	Spots under long-wave UV	1,69
	fluorescein	Spots after treatment with steam	1
	Rhodamine 6G (1 % in acetone)	Spots under long-wave UV	70
	tungstophosphoric acid (20 % in ethanol)	Variety of colored spots after heating	71

Mercaptans (see Thiols)			
Nitrocompounds	p-N,N-dimethylaminobenzaldehyde/ stannous chloride/ hydrochloric acid	Yellow spots	3
Nitrosamines	diphenylamine/palladium chloride	Violet spots after exposure to short-wave UV (limit 0.5γ)	1,72
	sulfanilic acid (0.5 %)/α-naphthylamine (0.05 %) in 30 % acetic acid	Spraying is preceded by short-wave UV irradiation (3 min); aliphatic nitrosamines yield red/violet spots, while aromatic ones green/blue spots (limit 0.2–0.5γ)	1,72,73
Oximes	cupric chloride (0.5 %)	Immediate green spots (β-oximes); green-brown spots after 10 min (α-oximes)	74
Peroxides	ammonium thiocyanate (1.2 %)/ferrous sulfate (4 %)	Brown-red spots	74
	N,N-dimethyl-p-phenylene diammonium dichloride	Purple spots	76
	ferrous thiocyanate	Red-brown spots	1,75
	iodide (potassium)/starch	Blue spots	1
Persulfates	benzidine (0.05 % in 1N acetic acid)	Blue spots	77
Phenols	anisaldehyde/sulfuric acid	Variety of colors	1,78
	p-anisidine/ammonium vanadate	Variety of spots on pink background	3
	benzidine, diazotized	Variety of colors	79
	ceric ammonium nitrate (46 % in 2 M nitric acid)	Variety of spots	80
	α,α′-dipyridyl (0.5 %)/ferric chloride (0.5 %) in ethanol	Variety of spots	4,81
	emerson	Red-orange to pink spots	1
	fast Blue B salt	Variety of spots	1
	ferric chloride (1–5 % in 0.5N HCl)	Blue-greenish spots	1
	Folin–Denis reagent	Variety of spots	82
	Gibbs reagent	Variety of colors	1
	Millon reagent	Variety of colors after heating	1
	naphthoquinone/perchloric acid	Yellow spots (phenol, catechol); dark blue spots (resorcinol)	65
	p-nitroaniline, diazotized	Variety of colored spots	38
	p-nitrobenzenediazonium fluoroborate	Variety of spots	84
	silver nitrate (saturated in acetone)	Pink to deep green colors	84
	stannic chloride (5 %) in equal volumes of chloroform/ acetic acid	Variety of spots after heating (100 °C, 5 min)	1
	tetracyanoethylene (10 % in benzene)	Variety of colors	85
	Tollen's (or Zaffaroni) reagent	Dark spots	86
	vanillin (1 % in sulfuric acid)	Variety of colors after heating	27

(Continued)

Spray Reagents in Thin Layer Chromatography (Continued)

Family/Functional Group	Test	Result	Ref.
Phenols, chlorinated	Folin–Denis reagent	Variety of spots	82
Phenothiazines	ferric chloride (5 %)/perchloric acid (20 %)/nitric acid (50 %; 1:9:10)	Variety of colors	4,87
	formaldehyde (0.03 % in phosphoric acid)	Variety of spots	88,89
	palladium (II) chloride (0.5 % pH < 7)	Variety of spots	1
Phosphates, esters	cobalt (II) chloride (1 % in acetone or acetic acid)	Blue spots upon warming the plate at 40 °C	90
Polynuclear aromatics	formaldehyde (2 %) in conc. sulfuric acid	Variety of colors	91
	tetracyanoethylene (10 % in benzene)	Variety of colors	85
Purines	fluorescein (0.005 % in 0.5 M ammonia)	Variety of spots under long- or short-wave UV	43
Pyrazolones	ferric chloride (5 %)/acetic acid (2N; 1:11)	Variety of colors	4
Pyridines	König reagent	Variety of spots (for free α-position pyridines)	92,93
Pyridines, quaternary	König reagent	Blue-white fluorescence under UV	93
Pyrimidines	Fluorescein (0.005 % in 0.5 M ammonia)	Variety of spots under long- or short-wave UV	43
Pyrones (α- and γ-)	Neu reagent	Fluorescent spots under long-wave UV	1
Quinine derivatives	formic acid vapors	Fluorescent blue spots	3
Sapogenins	Komarowsky reagent	Yellow/pink spots	94
	paraformaldehyde (0.03 % in 85 % phosphoric acid)	Variety of spots	88
	zinc chloride (30 % in methanol)	Fluorescent spots after heating (105 °C, 1 h) in a moisture-free atmosphere	94
Steroids	anisaldehyde/sulfuric acid	Variety of colors	95,96,97
	antimony (III) chloride (in acetic acid)	Variety of colors	57,96
	Carr-Price reagent	Variety of colors	1
	chlorosulfonic acid/acetic acid	Fluorescence under long-wave UV	78,95
	Dragendorff reagent	Variety of spots	88,89
	formaldehyde (0.03 % in phosphoric acid)	Variety of spots	96
	Hanes and Isherwood reagent	Variety of spots (only for 3-hydroxy-Δ^5-steroids)	1
	Liebermann–Burchard reagent	Fluorescence under long-wave UV	1,44
	perchloric acid (20 %)	Fluorescent spots (long-wave UV) after heating (150 °C, 10 min)	96
	phosphomolybdic acid	Blue color	95,96,98
	phosphoric acid (50 %)	Fluorescent spots after heating (120 °C) (limit 0.005γ)	99

	phosphotungstic acid (10 % in ethanol)	Variety of spots	1, 100
	stannic chloride (5 %) in equal volumes of chloroform/acetic acid (1:1)	Variety of spots after heating (100 °C, 5 min)	1
	sulfuric acid	Variety of spots	50
	p-toluenesulfonic acid (20 % in chloroform)	Fluorescent spots under long-wave UV	96
	trichloroacetic acid (50 % aqueous)	Variety of colors	95,96
	Zimmerman reagent	Variety of colors	
Sterols	antimony (III) chloride (50 % in acetic acid)	Variety of spots	99
	bismuth (III) chloride	Fluorescence under long-wave UV	1
	chlorosulfonic acid/acetic acid	Fluorescence under long-wave UV	1
	Liebermann–Burchard reagent	Fluorescence under long-wave UV	1
	1,2-naphthoquinone-4-sulfonic acid/perchloric acid	Pink spots that change to blue upon prolonged heating (cholesterol limit 0.03γ)	65,101
	phosphoric acid (50 %)	Fluorescent spots after heating (120 °C, 15 min)	98,102
	phosphotungstic acid (10 % in ethanol)	Variety of spots	99
	stannic chloride (5 %) in equal volumes of chloroform/acetic acid	Variety of spots after heating (100 °C, 5 min)	1
	sulfuric acid	Variety of spots	102
Sugars	o-aminodiphenyl (0.3 %)/orthophosphoric acid (5 %)	Brown spots after heating	103
	aniline/phosphoric acid	Variety of colors	104
	anisaldehyde/sulfuric acid	Variety of colors	1,78
	Anthrone test	Yellow spots	105
	benzidine/trichloroacetic acid	Red-brown/dark spots	106
	carbazole/sulfuric acid	Violet spots on blue background	103
	Lewis-Smith reagent	Brown spots	107
	naphthoquinone/perchloric acid	Pink–brown spots (glucose, mannose, lactose, sucrose)	65
	naphthoresorcinol (0.2 % in ethanol)/phosphoric acid (10:1)	Variety of spots after heating (100 °C, 5–10 min)	1
	naphthoresorcinol (0.1 %)/sulfuric acid (10 %)	Variety of spots after heating (100 °C, 5–10 min)	1
	orcinol reagent	Variety of spots	1
	permanganate, potassium (0.5 % in 1N sodium hydroxide)	Variety of spots after heating (100 °C)	108
	phenol (3 %)/sulfuric acid (5 % in ethanol)	Brown spots after heating (100 °C, 10 min)	103

(Continued)

Spray Reagents in Thin Layer Chromatography (Continued)

Family/Functional Group	Test	Result	Ref.
	silver nitrate (0.2 % in methanol)/ammonia (saturated)/ sodium methoxide (2 % in methanol)	Variety of spots after heating (110 °C, 10 min)	1
	silver nitrate/sodium hydroxide	Variety of spots	1
	sulfuric acid	Variety of spots	108
	thymol (0.5 %) in sulfuric acid (5 %)	Pink spots after heating (120 °C, 20 min)	103
Sugars, deoxy	metaperiodate/p-nitroaniline	Fluorescent (long-wave UV) yellow spots	109
Sugars, ketoses	Anthrone test	Bright purple (pentoses); orange-yellow (heptoses); blue fluorescence (aldoses)	110
	dimedone (0.3 %)/phosphoric acid (10 % in ethanol)	Dark-grey spots (white light); dark-pink fluorescing spots (UV) after heating (110 °C, 15 min)	1,111
Sugars, reducing	4-aminohippuric acid	Fluorescence under long-wave UV	112
	aniline/diphenylamine/phosphoric acid	Variety of colors	113,114,115
	aniline hydrogen phthalate	Variety of colors (limit 1 μg)	116
	p-anisidine phthalate	Variety of colors	1
	3,5-dinitrosalicylic acid (0.5 % in 4 % sodium hydroxide)	Brown spots (sensitivity 1 μg)	3
Sulfides	ceric ammonium nitrate (in 2 M HNO_3)	Colorless spots (limit < 100 μg/spot)	117
	chloranil (1 %) in benzene	Yellow–brown spots	119
	2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2 %) in benzene	Purple–blue spots changing to orange upon ammonia exposure	119
	Gibbs reagent	Yellow–brown spots changing to blue-orange upon exposure to ammonia	119
	iodine vapors	Brown spots	118
	tetracyanoethylene (2 %) in benzene	Orange spots	119
	N,2,6-trichloro p-benzoquinoneimine (2 %) in ethanol	Brown spots	119
Sulfilimines	potassium permanganate	Colorless spots	120
Sulfilimines, p-nitro-benzene-sulfonyl	tin chloride/4-N,N-dimethylaminobenz-aldehyde	Yellow spots	121
Sulfites	malachite green oxalate	White spots on blue background	4
Sulfonamides	chlorine/pyrazolinone/cyanide	Red spots changing to blue	32
	diazotization and coupling	Variety of spots (limit 0.25γ)	122,123
	Ehrlich	Variety of colors	124

	chloranil (1 %) in benzene	Pink turning to violet or green after heating	119
	2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2 %) in benzene	Lilac-violet turning to yellow-green upon ammonia exposure	119
	Gibbs reagent	Violet turning to tan upon exposure to ammonia and heat	119
Sulfones	iodine vapors	Brown spots	118
	tetracyanoethylene (2 %) in benzene	Pink to yellow upon exposure to ammonia and heat	119
Sulfonic acids	Pinacryptol yellow (0.1 %)	Yellow–orange spots under long-wave UV	124
	silver nitrate/fluorescein	Yellow spots under long-wave UV	125,126
Sulfoxides	acetyl bromide	Yellow–orange spots	127
	ceric ammonium nitrate (40 %) in 2 M nitric acid	Brown spots after heating (especially good for α-polychlorosulfoxides); limit 80 μg/spot	117
	chloranil (1 %) in benzene	Yellow–blue spots	119
	2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2 %) in benzene	Orange–crimson spots	119
	Dragendorff reagent	Orange–brown–red spots (limit 30–150γ)	128
	Gibbs reagent	Yellow turning to brown upon ammonia exposure	168
	Iodide (sodium)/starch	Brown spots (limits 0.01 μmol/20 μl solution)	129
	iodine vapors	Brown spots	118,130
	tetracyanoethylene (2 %) in benzene	Yellow or crimson turning to white or tan upon exposure to ammonia	119
	N,2,6-trichloro-p-benzoquinoneimine (2 %) in ethanol	Yellow spots	119
Terpenes	anisaldehyde/sulfuric acid	Variety of colors	1
	antimony (V) chloride	Variety of colors	1
	Carr-Price reagent	Variety of colors	1
	diphenylpicrylhydrazyl in chloroform	Yellow spots on purple background after heating (110 °C) (limit 1γ/0.5 cm diameter)	131
	phenol (50 % in carbon tetrachloride)	Variety of spots upon exposure to bromine vapors	3
	vanillin (1 % in 50 % H_3PO_4)	Variety of spots after heating (120 °C, 20 min)	4
Tetracyclines	ammonium hydroxide	Yellow fluorescence under long-wave UV	132
Thioacids	silver nitrate/ammonium hydroxide/sodium chloride	Yellow-brown spots	1
Thiobarbiturates	cupric sulfate (0.5 %)/diethylamine (3 % in methanol)	Green spots (limit 15γ)	3,133
Thiolactones	nitroprusside (sodium), basic	Red spots	62

(Continued)

Spray Reagents in Thin Layer Chromatography (Continued)

Family/Functional Group	Test	Result	Ref.
Thiols (Mercaptans)	ceric ammonium nitrate (in 2 M nitric acid)	Colorless spots on yellow background (limit < 100 μg/spot)	117
	iodine (1.3 % in ethanol)/ethanol	White spots in brown iodine background	3
	nitroprusside (sodium; 3 %)	Red spots	134
Thiophenes	Isatin (0.4 % in conc. sulfuric acid)	Variety of colors	135
Thiophosphates, esters	ferric chloride/sulfosalicylic acid	White spots on violet background	136
	palladium (II) chloride (0.5 % in acidified water)	Variety of spots	1,136
	periodic acid (10 % in 70 % perchloric acid)	Variety of spots	3
Unsaturated compounds	fluorescein (0.1 % in ethanol)/bromine	Yellow spots on a pink background upon exposure to bromine vapors	1
	osmium tetroxide vapors	Brown/black spots	3,95
Ureas	p-N,N-dimethylaminobenzaldehyde (1 % in ethanol)	Characteristic spots after exposure to hydrochloric acid	4
Vitamin A	antimony (V) chloride	Variety of colors	1
	Carr-Price reagent	Variety of colors	1
	sulfuric (50 % in methanol) followed by heating	Blue spots that turn brown	1
Vitamin B1	dipicyrlamine	Characteristic spots	3
	Thiochrome	Variety of spots under long-wave UV	137
Vitamin B6	N,2,6-trichloro-p-benzoquinoneimine (0.1 % in ethanol)	Blue spots after exposure to ammonia	3
Vitamin B6, acetal	2,6-dibromo-p-benzoquinone-4-chlorimine (0.4 % in methanol)	Characteristic spots	138
Vitamin C	cacotheline (2 % aqueous)	Purple spot after heating (100 °C)	3
	iodine (0.005 %) in starch (0.4 %)	White spot on blue background	3
	methoxynitroaniline/sodium nitrite	Blue spots on orange background	3
Vitamin D	antimony (V) chloride	Variety of colors	139
	Carr-Price reagent	Variety of colors	1
	trichloroacetic (1 % in chloroform)	Variety of spots after heating (120 °C, 5 min)	1
Vitamin E	2′,7′-dichlorofluorescein (0.01 % in ethanol)	Spots under long-wave UV light	1
	α,α′-dipyridyl (0.5 %)/ferric chloride (0.5 % in ethanol)	Variety of colors	1

PROTOCOL FOR REAGENT PREPARATION

The following section gives a summary for the preparation of the major spray reagents listed in the previous section (Spray Reagents in Thin Layer Chromatography). Reference to the original literature is recommended for any reagents not listed here [1–4].

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2. Bobbitt, J. B. *Thin Layer Chromatography*. New York: Reinhold, 1963.
3. Touchstone, J. C., and M. F. Dobbins. *Practice of Thin Layer Chromatography*. New York: John Wiley and Sons, 1983.
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acetic anhydride-sulfuric acid

See Liebermann–Burchard reagent.

alizarin

A saturated solution of alizarin in ethanol is sprayed on the moist plate, which is then placed in a chamber containing 25 % ammonium hydroxide solution to yield a variety of colors.

aluminum chloride

A 1 % aluminum chloride solution in ethanol is sprayed on the plate, which is then observed under long-wave UV light.

4-aminoantipyrine-potassium ferricyanide

See Emerson reagent.

4-aminobiphenyl-phosphoric acid

See Lewis-Smith reagent.

4-aminohippuric acid

A 0.3 % 4-aminohippuric acid solution in ethanol is sprayed on the plate, which is then heated at 140 °C (8 min) and observed under long-wave UV light.

ammonium hydroxide

The chromatogram is placed in a chamber containing 25 % ammonium hydroxide, dried, and then observed under long-wave UV light.

aniline-diphenylamine-phosphoric acid

An aniline (1 g)/diphenylamine (1 g)/phosphoric acid (5 mL) solution in acetone (50 mL) is sprayed on the plate, which is then heated at 85 °C (10 min) yielding a variety of colors.

aniline-phosphoric acid

A 20 % aniline solution in n-butanol, saturated with an aqueous (2N) orthophosphoric acid solution is sprayed on the plate, which is then heated at 105 °C (10 min) yielding a variety of colors.

aniline phthalate

An aniline (1 g)/o-phthalic acid (1.5 g) solution in n-butanol (100 mL; saturated with water) is sprayed on the plate, which is then heated at 105 °C (10 min) yielding a variety of colors.

anisaldehyde-sulfuric acid

A 1 % anisaldehyde solution in acetic acid (acidified by conc. sulfuric acid) is sprayed on the plate, which is then heated at 105 °C to yield a variety of colors.

p-anisidine phthalate

A 0.1 M solution of p-anisidine and phthalic acid in ethanol is sprayed on the plate, which is then heated at 100 °C (10 min) to yield a variety of colors.

anthrone

A 1 % anthrone solution in 60 % aqueous ethanol solution acidified with 10 mL 60 percent phosphoric acid is sprayed on the plate, which is then heated at 110 °C (5 min) to yield yellow spots.

antimony (III) chloride

See Carr–Price reagent.

antimony (III) chloride-acetic acid

A 20 % antimony (III) chloride solution in 75 % chloroform-acetic acid solution is sprayed on the plate, which upon heating at 100 °C (5 min) yields a variety of colors.

antimony (V) chloride

A 20 % antimony (V) chloride solution in chloroform or carbon tetrachloride is sprayed on the plate yielding a variety of colors upon heating.

Benedict's reagent

A solution that is 0.1 M in cupric sulfate, 1.0 M in sodium citrate and 1.0 M in sodium carbonate is sprayed on the plate, which is then observed under long-wave UV light.

benzidine diazotized

A 0.5 % benzidine solution in 0.005 % hydrochloric acid is mixed with an equal volume of 10 % sodium nitrite solution in water; the mixture is sprayed on the plate to yield a variety of colors.

benzidine-trichloroacetic acid

A 0.5 % benzidine in (1:1:8) acetic acid/trichloroacetic acid/ethanol is sprayed on the plate to yield red-brown spots upon heating (110 °C) or exposure to unfiltered UV light (15 min).

bismuth (III) chloride

A 33 % ethanol solution of bismuth (III) chloride is sprayed on the plate, which upon heating (110 °C) yields fluorescent spots under long-wave UV light.

carbazole-sulfuric acid

A 0.5 % carbazole in ethanol/sulfuric acid (95:5) is sprayed on the plate, which yields violet spots (on blue background) after heating at 120 °C (10 min).

Carr–Price reagent

A 25 % antimony (III) chloride solution in chloroform or carbon tetrachloride is sprayed on the plate, which is heated at 100 °C (10 min) to yield a variety of colors.

ceric ammonium sulfate

A 1 % solution of ceric ammonium sulfate in strong acids (phosphoric, nitric) is sprayed on the plate to yield yellow/green spots on a red background, after heating at 105 °C (10 min).

chloramine-T

A 10 % chloramine-T solution is sprayed on the plate, followed by 1 N hydrochloric acid. The chromatogram is dried and exposed to 25 % ammonium hydroxide and warmed.

chlorine-pyrazolinone-cyanide

An equal volume mixture of 0.2 M 1-phenyl-3-methyl-2-pyrazolin-5-one solution (in pyridine) and 1 M aqueous potassium cyanide solution is sprayed on the plate that has been previously exposed to chlorine vapors. The resulting red spots turn blue after a few minutes.

chlorosulfonic acid-acetic acid

A 35 % chlorosulfonic acid solution in acetic acid is sprayed on the plate, which is then heated at 130 °C (5 min) to produce fluorescence under long-wave UV.

cinnamaldehyde-hydrochloric acid

A 5 % cinnamaldehyde solution in ethanol (acidified with hydrochloric acid) is sprayed on the plate, which is then placed in a hydrochloric acid chamber to yield red spots.

cobalt (II) thiocyanate

An ammonium thiocyanate (15 %)/cobalt (II) chloride (5 %) solution in water is sprayed on the plate yielding blue spots.

cupric sulfate-quinine-pyridine

A solution that is 0.4 % in cupric sulfate, 0.04 % in quinine hydrochloride and 4 % in pyridine in water is sprayed on the plate followed by a 0.5 % aqueous potassium permanganate solution. A variety of colors (white, yellow, violet) is detected on the chromatogram.

α-cyclodextrin

A 30 % α-cyclodextrin solution in ethanol is sprayed on the plate, which is further developed in an iodine chamber.

diazonium

See Fast Blue B salt.

diazotization and coupling reagent

A 1 % sodium nitrite solution (in l M hydrochloric acid) is sprayed on the plate, followed by a 0.2 % α-naphthol solution in l M potassium hydroxide and drying.

4-N,N-dimethylaminobenzaldehyde-sulfuric acid

A 0.125 % solution of 4-N,N-dimethylaminobenzaldehyde in 65 % sulfuric acid mixed with 5 % ferric chloride (0.05 mL per 100 mL solution) is sprayed on the plate giving a variety of spots.

4-N,N-dimethylaminocinnamaldehyde

A 0.2 % solution of 4-N,N-dimethylaminocinnamaldehyde in 6N HCl/ethanol (1:4) is sprayed on the plate, which is then heated at 105 °C (5 min) revealing a variety of colored spots. Vapors of aqua regia tend to intensify the spots.

2,4-dinitrofluorobenzene

A 1 % sodium bicarbonate solution in 0.025 M sodium hydroxide is sprayed on the plate followed by a 2,4-dinitrofluorobenzene (10 %) solution in methanol. Heating the plate in the dark (40 °C, one hour) and further spraying it with diethyl ether yields a variety of spots.

2,4-dinitrophenylhydrazine

A 0.4 % solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid is sprayed on the plate followed by a 0.2 % solution of potassium ferricyanide in 2N hydrochloric acid yielding orange/yellow spots.

Dragendorff reagent

A 1.7 % aqueous solution of basic bismuth nitrate in weak acids (tartaric, acetic) mixed with an aqueous potassium iodide or barium chloride solution is sprayed on the plate to yield a variety of spots.

Ehrlich reagent

A 1 % 4-N,N-dimethylaminobenzaldehyde solution in ethanol is sprayed on the plate, which is dried and then placed in a hydrochloric acid chamber to yield various spots.

Emerson reagent

A 2 % 4-aminoantipyrine solution in ethanol is sprayed on the plate, followed by an 8 % aqueous potassium ferricyanide solution. The chromatogram is then placed in a chamber containing 25 % ammonium hydroxide.

EP

A 0.3 % solution of 4-N,N-dimethylaminobenzaldelyde in acetic acid/phosphoric acid/water (10:1:4) is sprayed on the plate to yield a variety of spots.

Fast Blue B Salt (diazonium)

A 0.5 % aqueous solution of Fast Blue B Salt is sprayed on the plate followed by a 0.1 M sodium hydroxide.

ferric chloride–perchloric acid

A solution made out of 5 mL 5 % aqueous ferric chloride, 45 mL 20 % perchloric acid and 50 mL 50 % nitric acid is sprayed on the plate to yield a variety of spots.

ferric chloride–sulfosalicylic acids

The plate is first exposed to a bromine atmosphere then sprayed with a 0.1 % ethanolic solution of ferric chloride. After air drying (15 min) the chromatogram is sprayed with a 1 % ethanolic solution of sulfosalicylic acid to yield a variety of spots.

ferrocyanide–hydrogen peroxide

0.5 g ammonium chloride is added to a 0.1 % potassium ferrocyanide solution in 0.2 % hydrochloric acid and the resulting solution is sprayed on the plate, which is then dried (100 °C). The chromatogram is further sprayed with 30 % hydrogen peroxide, heated (150 °C, 30 min) and sprayed with 10 % potassium carbonate to yield yellow/red spots.

ferrous thiocyanate

A 2:3 mixture of a 4 % aqueous ferrous sulfate and 1.3 % acetone solution of ammonium thiocyanate is sprayed on the plate yielding red-brown spots.

fluorescein-hydrogen peroxide

A 0.1 % fluorescein solution in 50 % aqueous ethanol is sprayed on the plate followed by a 15 % hydrogen peroxide in glacial acetic acid and heated (90 °C, 20 min) yielding nonfluorescent spots.

Folin reagent

A 0.02 % sodium 1,2-naphthoquinone-4-sulfonate in 5 % sodium carbonate is sprayed on the plate, which is then dried to yield a variety of colors.

Folin–Denis reagent

A tungstomolybdophosphoric acid solution is sprayed on the plate, which is then exposed to ammonia vapors.

Gibbs reagent

A 0.4 % methanolic solution of 2,6-dibromoquinonechloroimide is sprayed on the plate followed by a 10 % aqueous sodium carbonate yielding a variety of spots.

glucose–phosphoric acid

A 2 % glucose solution in phosphoric acid/water/ethanol/n-butanol (1:4:3:3) is sprayed on the plate followed by heating (115 °C, 10 min) to yield a variety of spots.

hydroxylamine-ferric chloride

A 1:2 mixture of a 10 % hydroxylammonium chloride/10 % potassium hydroxide in aqueous ethanol is sprayed on the plate followed by drying. The chromatogram is then sprayed with an ether solution of ferric chloride in hydrochloric acid to yield a variety of spots.

iodide (potassium) starch

A 1 % potassium iodide solution in 80 % aqueous acetic acid is sprayed on the plate followed by a 1 % aqueous starch solution. A pinch of zinc dust is recommended as an addition to the potassium iodide solution.

iodide (sodium) starch

A solution made by mixing a 5 % starch/0.5 % sodium iodide solution with an equal volume of concentrated hydrochloric acid is sprayed on the plate, which is then exposed to dry sodium hydroxide (desiccator) and evacuated (30–60 min) to yield brown spots.

isatin-zinc acetate

An isatin (1 %)/zinc acetate (1.5 %) solution in isopropanol acidified with acetic acid is sprayed on the plate, which is then heated to yield a variety of spots.

Kalignost reagent

A 1 % solution of sodium tetraphenylborate in aqueous butanone is sprayed on the plate, followed by a 0.015 % methanolic solution of fischtin or quercetin to yield orange-red spots that fluoresce under long-wave UV.

Komarowski reagent

A 2 % methanolic solution of p-hydroxybenzaldehyde that is 5 % in sulfuric acid is sprayed on the plate, which is then heated (105 °C, 3 min) to yield yellow or pink spots.

König reagent

A 2 % p-aminobenzoic acid in ethanolic hydrochloric acid (0.6 M) is sprayed on the plate that has been exposed (1 hr) to vapors of cyanogen bromide.

Lewis–Smith reagent

o-Aminobiphenyl (0.3 g dissolved in 100 ml of a 19:1 ethanol/phosphoric acid mixture) is sprayed on the plate, which is then heated at 110 °C (15 min).

Liebermann-Burchard reagent

A freshly prepared mixture of 5 mL acetic anhydride/5 mL conc. sulfuric acid in 50 mL cold absolute ethanol is sprayed on the plate, which is heated at 100 °C (10 min) and observed under long-wave UV light.

malachite green oxalate

A 1 % ethanolic potassium hydroxide solution is sprayed, the plate heated (150 °C, 5 min), and further sprayed with a buffered (pH = 7) water/acetone solution of malachite green oxalate to yield white spots on blue background.

metaperiodate (sodium)-p-nitroaniline

A 35 % saturated solution of sodium metaperiodate is sprayed on the plate, which is left to dry (10 min). The chromatogram is then sprayed with a 0.2 % p-nitroaniline solution in ethanol/hydrochloric acid (4:1) to yield fluorescing (long-wave UV) yellow spots.

methoxynitroaniline - sodium nitrite

A 0.02 M 4-methoxy-2-nitroaniline solution in 50 % aqueous acetic acid/5N sulfuric acid is sprayed on the plate, which is dried and re-sprayed with 0.2 % sodium nitrite to yield blue spots on an orange background.

Millon reagent

A solution of mercury (5 g) in fuming nitric acid (10 mL) diluted with water (10 mL) is sprayed on the plate to yield yellow/orange spots that are intensified by heat (100 °C).

1,2-naphthoquinone-4-sulfonic acid/perchloric acid

A 0.1 % 1,2-naphthoquinone-4-sulfonic acid solution in ethanol/perchloric acid/40 % formaldehyde/water (20:10:1:9) is sprayed on the plate, which is then heated (70 °C) to yield pink spots that turn to blue on prolonged heating.

Neu reagent

A 1 % methanolic solution of the β-aminoethylester of diphenylboric acid is sprayed on the plate to yield fluorescent spots under long wave UV light.

ninhydrin

A ninhydrin solution (0.3 % in acidified n-butanol or 0.2 % in ethanol) is sprayed on the plate, which is then heated (110 °C). The resulting spots are stabilized by spraying with a solution made of 1 mL saturated aqueous cupric nitrate, 0.2 mL 10 % nitric acid and 100 mL 95 % ethanol, to yield red spots when exposed to ammonium hydroxide (25 %).

p-nitroaniline, diazotized

A solution made by mixing 0.1 % aqueous p-nitroaniline/0.2 % aqueous sodium nitrite/10 % aqueous potassium carbonate (1:1:2) is sprayed on the plate to yield colored spots.

p-nitroaniline, diazotized (buffered)

A solution of 0.5 % p-nitroaniline (in 2N hydrochloric acid), 5 % aqueous sodium nitrite and 20 % aqueous sodium acetate (10:1:30) is sprayed on the plate to yield a variety of colored spots.

nitroprusside (sodium)

A solution made by mixing sodium nitroprusside (1.5 g), 2N hydrochloric acid (5 mL), methanol (95 mL), and 25 % ammonium hydroxide (10 mL) is sprayed on the plate to yield a variety of colors.

nitroprusside (sodium), basic

A 2 % sodium nitroprusside solution in 75 % ethanol is sprayed on the plate, which has already been treated with lN sodium hydroxide to yield red spots.

orcinol

A mixture consisting of 0.6 % ethanolic orcinol and 1 % ferric chloride in dilute sulfuric acid is sprayed on the plate, which is further heated (100 °C, 10 min) to yield characteristic spots.

Prochazka reagent

A 10 % formaldehyde solution in 5 % hydrochloric acid solution in ethanol is sprayed on the plate, which is then heated to yield fluorescent spots (yellow/orange/green) under long-wave UV.

quercetin-sodium tetraphenylborate

A mixture of quercetin (0.015 % in methanol) and sodium tetraphenyl-borate (1 % in n-butanol saturated with water) is sprayed on the plate to yield orange/red spots.

quinaldine

A 1–1.5 % solution of 3,5-diaminobenzoic acid dihydrochloride in 30 % phosphoric acid is sprayed on the plate, which is then heated (100 °C, 15 min) to yield fluorescent (green/yellow) spots under long-wave UV or (in case of high concentrations) brown spots in daylight.

Sakaguchi reagent

A 0.1 % acetone solution of 8-hydroxyquinoline is sprayed on the plate followed by a 0.2 % 0.5N sodium hydroxide solution to yield orange/red spots.

Salkowski reagent

A 0.01 M aqueous ferric chloride/35 % perchloric acid solution is sprayed on the plate, which is then heated (60 °C, 5 min) to yield a variety of colors intensified when exposed to aqua regia.

Schweppe reagent

A mixture of 2 % aqueous glucose/2 % ethanolic aniline in n-butanol is sprayed on the plate, which is heated (125 °C, 5 min) to yield a variety of spots.

silver nitrate-ammonium hydroxide-sodium chloride

A mixture of silver nitrate (0.05 M)/ammonium hydroxide (5 %) is sprayed on the plate, followed by drying and further spraying with 10 % aqueous sodium chloride to yield yellow/brown spots.

silver nitrate-fluorescein

A mixture of silver nitrate (2 %)/sodium-fluorescein (0.2 %) in 80 % ethanol is sprayed on the plate to yield yellow spots on pink background.

silver nitrate-formaldehyde

The plate is consecutively sprayed with 0.05 M ethanolic silver nitrate, 35 % aqueous formaldehyde, 2 M potassium hydroxide and, finally, a solution made of equal volumes of hydrogen peroxide (30 %) and nitric acid (65 %). Each spraying is preceded by a 30 min. drying and at the end the plate is kept in the dark for 12 hr before exposing to sunlight to yield dark grey spots.

silver nitrate-hydrogen peroxide

A 0.05 % silver nitrate solution in water/cellosolve/acetone (1:10:190; to which a drop of 30 % hydrogen peroxide has been added) is spayed on the plate, which is then treated under unfiltered UV to yield dark spots.

silver nitrate-sodium hydroxide

A saturated silver nitrate solution is sprayed on the plate followed by a 0.5 M aqueous/methanol solution. Subsequent drying (100 °C, 2 min) yields a variety of spots.

Sonnenschein reagent

A 2 % ceric sulfate solution in 20 % aqueous trichloroacetic acid (that has been acidified with sulfuric acid) is sprayed on the plate. A variety of colors appears upon heating (110 °C, 5 min).

Stahl

See van Urk reagent.

sulfanilic acid-1-naphthylamine

A mixture of 2 % sulfanilic acid/1-naphthylamine in 30 % acetic acid is sprayed on the plate to yield a variety (violet/green/blue) of colors.

thiochrome

A 0.3 M aqueous potassium ferricyanide solution that is 15 % in sodium hydroxide is sprayed on the plate yielding a variety of spots under long-wave UV.

Tollen's reagent

See Zaffaroni reagent.

vanillin-potassium hydroxide

A 2 % solution of vanillin in n-propanol is sprayed on the plate, which is heated (100 °C, 10 min) and sprayed again with 1 % ethanolic potassium hydroxide. Reheating yields a variety of colors observed under daylight.

van Urk (Stahl) reagent

A 0.5 % solution of 4-N,N-dimethylaminobenzaldehyde in concentrated hydrochloric acid/ethanol (1:1) is sprayed on the preheated plate, which is then subjected to aqua regia vapors to yield a variety of colors.

Zaffaroni (Tollen's) reagent

A mixture of silver nitrate (0.02 M)/ammonium hydroxide (5 M) is sprayed on the plate, which is then heated (105 °C, 10 min) to yield black spots.

Zwikker reagent

A 1 % cobaltous nitrate in absolute ethanol is sprayed on the plate, which is dried (at room temperature) and exposed to a wet chamber containing 25 % ammonium hydroxide.