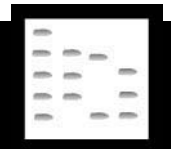
17. THIN LAYER (PLANAR) CHROMATOGRAPHY: DETECTION



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The following tables list detection methods and reagents suitable for detecting and identifying substances separated by thin-layer (planar) chromatography.

Table 1.1 Methods of detection on TLC plates (aluminium oxide) by heating (Types 150/T or 60/E)

Substances	*Temperature/time*	*Remarks*
Pesticides, e.g. aminocarb, captan, difolatan, landrin, rotenone	200°C, 45 min	Induction of fluorescence in weakly fluorescent or nonfluorescent pesticides and amplification of natural fluorescence. There are some differences between basic and acidic aluminium oxide layers
Δ^4-3-Ketosteroids, e.g. testosterone and *epi*-testosterone in urine	180°C, 20 min	Pale blue induced fluorescence ($\lambda_{fl} = 440$ nm) for Δ^4-3-ketosteroids, detection limit: 5 ng
Δ^4-3-Ketosteroids, e.g. trimethylsilyl-testosterone	180°C, 20 min or 150°C, 20 min	Conversion of Δ^4-3-ketosteroids or their trimethylsilyl or acetyl derivatives in fluorescent components, whereby the detection limits were improved by 65% for the acetates. Δ^5-3-keto- and Δ^5-3-OH-steroids also react with the same sensitivity
Testosterone	180°C, 20 min	Induced fluorescence ($\lambda_{fl} > 430$ nm, cut off filter) by thermal treatment of the chromatogram, the fluorescence increased by a factor of 2.5 by dipping in a solution of Triton X-100 − chloroform (1 + 4). Working range: 2–50 ng substance per chromatogram zone. Prewashing the layers with methanol-ammonia solution (25%) (50 + 50) increased the precision
Testosterone	180°C, 20 min	Induced fluorescence and fluorescence amplification by a factor of 25 by dipping the chromatogram in a solution of Triton X-100 − chloroform (1 + 4)
Δ^4-3-Ketosteroids, e.g. progesterone in plasma	150°C, 20 min	Conversion of Δ^4-3-ketosteroids into fluorescent derivatives ($\lambda_{fl} = 440$ nm). Relatively selective for progesterone at 150°C detection limit: 2–5 ng

Table 1.2 Methods of fluorimetric detection on TLC plates (silica gel) by heating

Substances	*Temperature/time*	*Remarks*
Essential oil components	800–900°C	Induction of fluorescence in a special apparatus
Steroids, e.g. cholesterol, triolein, androsterone; sugars, e.g. fructose, glucose, ribose; amino acids, pyrimidines, purines, alkaloids	110–150°C, 2–12 h	Conversion to fluorescent derivatives by heating
Alkaloids, e.g. raubasine and its metabolites in plasma, urine and bile	120°C, 1 h	Amplification of the natural fluorescence of raubasine ($\lambda_{fl} = 482$ nm), detection limit 20 ng
Alkaloids, e.g. reserpine, rescinnamine	105°C, 2 h	Induced fluorescence ($\lambda_{fl} > 500$ nm, cut off filter). Possibly formation of 3-dehydro derivatives
Alkaloids, e.g. reserpine, ajmaline, rescinnamine	105°C, 2 h or 105°C, 15 h	Induction of stable fluorescence ($\lambda_{fl} > 480$ nm, cut off filter), detection limits 5–20 ng
Alkaloids, e.g. cocaine, ecgonine, benzoylecgonine, ecgonine methyl ester	280°C, 8 min or 260°C, 10–30 min	Pale blue induced fluorescence ($\lambda_{fl} > 390$ nm, cut off filter), fluorescence amplification by a factor of 2 on dipping in liquid paraffin solution; detection limits: <10 ng
Alkaloids, e.g. lupanine, angustifoline, sparteine, lupinine, hydroxylupanine	130°C, 17–35 h	Induced blue fluorescence ($\lambda_{fl} = 400$ nm), detection limits: 10 ng
Pesticides, e.g. dursban, azinphos-methyl, menazon, imidan, phosalone, zinophos	200–225°C, 20–120 min	Induced fluorescence or amplification of natural fluorescence; detection limits: 10–300 ng
Organophosphorus pesticides, e.g. coumaphos, menazon, maretin, dursban	200°C, 45 min	Induced fluorescence or amplification of natural fluorescence, detection limits: 1–80 ng
Pesticides, e.g. fuberidazol	200°C, 45 min	Amplification of the natural fluorescence of some pesticides and bathochromic shift of the excitation and emission maxima; detection limits: 5–100 ng
Pesticides, e.g. coumatetralyl, methabenzthiazuron, propylisom, naptalam, thioquinox, warfarin etc.	200°C, 45 min	Induced fluorescence ($\lambda_{fl} > 430$ nm, cut off filter); detection limits: 6–600 ng
Coumaphos	200°C, 20 min	Residue analysis; induced fluorescence on heating ($\lambda_{fl} > 400$ nm); detection limit: 1 ng
Potasan, coumaphos, coroxon	200°C, 20 min	Induced blue fluorescence ($\lambda_{fl} = 430$ nm or 450 nm), idenfication of the fluorescent derivatives as chlorferon or 4-methylumbelliferone
Coumaphos	200°C, 20 min	Residue determination in honey, induced fluorescence ($\lambda_{fl} > 400$ nm, cut off filter); detection limit: 0.5 ng
Rubratoxin B	200°C, 10 min	Induced fluorescence that can be intensified by gassing the previously heated chromatogram plates with ammonia vapours (10 min). This also alters the colour of the emitted light to pale blue
Glucose or methyglucosides	135°C, 3 min or 140°C, 10 min	Induced yellow fluorescence
Sugar derivatives	'Mild heating over a Bunsen burner'	No details of whether fluorescence was produced or if a carbonization reaction occurred
Sugars, e.g. glucose, fructose, galactose, mannose etc.	160°C, 10 min	Production of fluorescence by heating the chromatogram after covering it with a glass plate. Sugar alcohols and C_1–C_1 bonded oligosaccharides do not react; detection limit: 10 ng

Table 1.2 *Continued*

Substances	*Temperature/time*	*Remarks*
Sugars, e.g. glucose, glucosamine, fucose, raffinose, cellobiose, methylated sugars	80 → 260°C, gradient or 200°C, 5 min	Production of fluorescence by temperature gradients (10°C/30 s) to determine the optimum heating temperature for the individual substances. Oligosaccharides require higher temperatures than monosaccharides. Detection limit: 1 nMol. The fluorescence colours are characteristic particularly for the methylated sugars
Lipids, e.g. β-sitosterol, geraniol, dolichol, squalene, cholesterol	200°C, 15 min	Induced fluorescence; detection limits: <1 μg cholesterol
C-Nucleosides	Moderate heating on a hot plate	No details of whether fluorescence or carbonization was produced
Nomifensine and metabolites	70°C, 2 h + UV_{254}	Heating and simultaneous UV irradiation produced intense yellow fluorescence ($\lambda_{fl} > 460$ nm, cut off filter)

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Table 1.3 Examples of fluorimetric detection after thermal treatment of layer after chromatography

Substances	*Temperature/time*	*Remarks*
Sugars, e.g. lactose, glucose, fructose	120°C, 15 min	Violet fluorescence on a dark blue background
Sugars, e.g. lactose, glucose, fructose	120°C, 15 min	Induced fluorescence; detection limits in nanogram range
Glucose, fructose	Infrared lamp or 170°C each for 3 min	Heating produced stable bluish-white fluorescence ($\lambda_{exe} = 365$ nm and $\lambda_{fl} > 400$ nm, cut off filter K 400), detection limits; 5–10 ng
Sugars, e.g. glucose, rhamnose, xylose etc.	160°C, 3–4 min or infrared lamp	Induction of brilliant stable fluorescence $\lambda_{exe} = 365$ nm and $\lambda_{fl} > 400$ nm (cut off filter K 400), sugar alcohols do not fluoresce; detection limits; 5–10 ng
Creatine, creatinine, uric acid in urine and serum	150°C, 3–4 min	Stable fluorescence $\lambda_{exe} = 365$ nm and $\lambda_{fl} >$ 400 nm (cut off filter K 400)
Sugars, e.g. sucrose, ribose, xylose	150°C, 3–4 min	Induced fluorescence $\lambda_{exe} = 365$ nm and $\lambda_{fl} >$ 400 nm (cut off filter K 400)

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Table 2 Some substances that produce intense fluorescence when treated with ionized nitrogen after they have been chromatographed

Substance	*Exposure time [s]*	*Substance*	*Exposure time [s]*
Cholesterol	60	Oleic acid	180
Cholesteryl pelargonate	60	Morphine	180
Progesterone	60	Codeine	180
Testosterone	60	Cocaine	180
Dieldrin	60	Dimerol	180
Tetrahydrocannabinol	60	Phenobarbital	180
Inositol	60	Chlorpromazine	180
Lauryl alcohol	180	d-Amphetamine sulfate	180
n-$C_{22}H_{46}$	180	Methadone	180
Phenol	180		

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Table 3 Reagents suitable for the recognition of functional groups

Functional group	*Reagent*	*Remarks*
Acetylene compounds	Dicobaltoctacarbonyl	Formation of coloured complexes. After the reagent excess has been washed out, reaction with bromine vapour yields cobalt bromide, which reacts with α-nitroso-β-naphthol to yield red chromatogram zones on an almost colourless background
Aldehydes	4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald reagent)	Aldehydes yield violet chromatogram zones on a whitish-yellow background. Some alcohols form yellow to orange-coloured chromatogram zones
Aldehydes	2,4-Dinitrophenylhydrazine	Formation of coloured hydrazones or osazones. It is possible to distinguish between saturated and unsaturated hydrazones using potassium hexacyanoferrate (III)
Aldehydes	Hydrazine sulfate + hydrochloric acid	Aromatic aldehydes yield coloured hydrazones
Alcohols	4-(4-Nitrobenzyl)pyridine	Amino compounds, esters and ethers do not interfere, but phenols and acids as well as epoxides, olefins and substances containing labile halogen probably do
Alcohols (diols, polyols, sugars)	Lead(IV) acetate – dichlorofluorescein	Diol cleavage of vicinal diols, e.g. sugars, sugar alcohols. The lead tetraacetate consumed is no longer available to decompose the fluorescent dichlorofluorescein
Amines (primary)	Ninhydrin	Reddish or bluish chromatogram zones are produced, amino sugars and amino acids also react. Unexpectedly ascorbic acid also reacts
Amines (primary aliphatic and aromatic)	Diphenylboric anhydride + salicylaldehyde (DOOB)	Fluorescent reaction products are produced
Amines (primary)	*o*-Phthalaldehyde (OPA)	In the presence of mercaptoethanol *o*-phthalaldehyde reacts with primary amines and amino acids to yield fluorescent isoindole derivatives

Table 3 *Continued*

Functional group	*Reagent*	*Remarks*
Amines (primary)	Trinitrobenzenesulfonic acid (TNBS)	On heating primary amines react with TNBA to yield intensely coloured Meisenheimer complexes. Amino acids also react
Amines (primary)	Fluorescamine	Primary aliphatic and aromatic amines yield fluorescent derivatives. Primary aromatic amines yield stable yellow-coloured derivatives that can be eluted from the TLC layer
Amines (primary aromatic)	Sodium nitrite + α-naphthol or Bratton-Marshall reagent	Diazotization of the primary amine followed by coupling with α-naphthol or N-(L-naphthyl)ethylenediamine. Sulfonamides also react
Amines (primary aromatic)	4-(Dimethylamino)benzaldehyde + acid	Alkaloids and indole derivatives also react
Amines (capable of coupling)	Fast blue salt B, fast blue salt BB, fast black salt K, diazotized sulfanilic acid (Pauly's reagent), diazotized sulfanilamide or 4-nitroaniline	Intensely coloured azo dyes are produced. Catecholamines, imidazoles and phenols also react
Amines (primary and secondary)	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride)	Fluorescent 4-nitrobenzofurazan derivatives are produced. Phenols and thiols also react
Amines (primary and secondary aromatic)	*p*-Chloranil	The reaction depends on the catalytic effect of silica gel. Monochlorobenzene as solvent for the reagent, also contributes. There is no reaction on cellulose layers
Amines (secondary aliphatic and alicyclic)	Sodium nitroprusside + acetaldehyde	Secondary aliphatic and alicyclic amines yield blue-coloured chromatogram zones (e.g. morpholine, diethanol amine)
Amines (long-chain primary, secondary and tertiary plus quaternary ammonium salts)	Cobalt(II) thiocyanate	Long-chain primary, secondary and tertiary amines and long-chain quaternary ammonium salts yield blue chromatogram zones on a pink background
Carboxyl groups (carboxylic acids)	Indicators, e.g. bromocresol green, bromocresol green + bromophenol blue + potassium permanganate, bromocresol purple, methyl red + bromothymol blue	Detection depends on the colour change of the indicator in acid medium. Quaternary ammonium salts give a colour change in some cases
Carboxyl groups (carboxylic acids)	2,6-Dichlorophenol-indophenol (Tillmann's reagent)	Organic acids release the red undissociated acid from the blue mesomerically stabilized phenolate anion. Reductones reduce the reagent to a colourless compound
Carboxyl groups (carboxylic acids)	Aniline + aldose (e.g. glucose)	The action of acid causes glucose to be converted to furfural which reacts with aniline to yield a coloured product
Halogen derivatives	Silver nitrate, ammoniacal (Dedonder's, Tollens' or Zaffaroni's reagent)	Halogen compounds yield black chromatogram zones on a pale grey background
Ketones	2,4-Dinitrophenylhydrazine	Formation of coloured hydrazones or osazones. It is possible to distinguish between saturated and unsaturated hydrazones using potassium hexacyanoferrate (III)

Table 3 *Continued*

Functional group	*Reagent*	*Remarks*
Nitro derivatives	Benzylcyanide + benzyl-trimethylammonium hydroxide	Nitro compounds, e.g. explosives, or pesticides containing nitro groups yield gray to bluish-green chromatogram zones on a brownish background
Peroxides	1-Naphthol + N^4-ethyl-N^{4-} (2-methanesulfonamidoethyl)-2-methyl-1,4-phenylenediamine (peroxide reagent)	A quinonimine dyestuff is produced on reaction with peroxides
Peroxides	Iron(II) sulfate + ammonium thiocyanate	Peroxides rapidly oxidize iron(II) to iron(III) ions which react to yield brown-red iron(III) thiocyanate complexes
Peroxides	Potassium iodide + starch	Peroxides release free iodine which forms a blue complex with the starch
Peroxides	N,N-Dimethyl-1,4-phenylenediamine (N,N-DPDD), N,N,N′,N′-tetra-methyl-1,4-phenylene-diamine (TPDD)	Peroxides, e.g. alkyl hydroperoxides, oxidize N,N-DPDD to Wurster's red and TPDD to Wurster's blue
Phenols	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride)	Fluorescent 4-nitrobenzofuran derivatives are produced. Primary and secondary aromatic amines and thiols also react
Phenols (capable of coupling)	Fast blue salt B, fast blue salt BB, fast black salt K, diazotized sulfanilic acid (Pauly's reagent) diazotized sulfanilamide or 4-nitroaniline	Intensely coloured azo dyes are formed. Catecholamines, imidazoles and amines capable of coupling also react
Thiols, thioethers, disulfides	Sodium metaperiodate + benzidine	Substances with divalent sulfur yield white chromatogram zones on a blue background
Thiols	7-Chloro-4-nitobenzo-2-oxa-1,3-diazole (NBD chloride)	Fluorescent 4-nitrobenzofuran derivatives are formed. Primary and secondary aromatic amines and phenols also react

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See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Spray Reagents.