The Instruction of Thin-Layer Chromatography Silica Gel Plate

Thin-layer chromatography (TLC) is an analytical method to monitor the progress of a reaction, to analyze mixtures or to establish conditions for a preparative separation of compounds using column chromatography. TLC is one of the easiest and most versatile methods of separating a mixture into its chemical components because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility. It has good separation effect for some tracer complex compounds, and is very suitable for qualitative and semi-quantitative determination. TLC is widely used in the research of medicine, chemistry, biochemistry, environmental science, etc.

Protocols

1. Spotting

(1) Choose a solvent with good solubility, appropriate viscosity and boiling point when preparing the sample. Methanol is a commonly used solvent.

(2) Spot a dot as small a diameter as possible (1-2 mm). The distance from the dot to the bottom of the silica gel plate is approximately 0.5-1 cm.

(3) Be careful not to break the thin-layer surface when spotting.

(4) Let the solvent evaporate completely. Avoid long time and high temperature heating.

2. Mobile Phase

(1) The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value. Rf values and reproducibility can be affected by a number of different factors such as layer thickness, moisture on the TLC plate, vessel saturation, temperature, and solvent parameters.

During the TLC analytical trials, the solvent system will be seek that moves the desired product to Rf=0.2-0.8 and keeps the other undesired products to distance of at least △R=0.2.

Many organic solvents are available, Table 1 lists commonly used solvent.

Characteristics of commonly used solvents in liquid chromatography

Solvent	Polarity	Viscosity (cp 20°)	Boiling point (°C)	UV cutoff (nm)
Hexane	0.06	0.33	69	210
n-Heptane	0.20	0.41	98	200
Toluene	2.40	0.59	111	285
Methylene chloride	3.40	0.44	40	245
Tetrahydrofuran	4.20	0.55	66	220
Ethanol	4.30	1.20	79	210
Ethyl acetate	4.30	0.45	77	260
i-Propanol	4.30	2.37	82	210
Acetonitrile	6.20	0.37	82	210
Methanol	6.60	0.60	65	210
Water	10.20	1.00	100	—

(2) Typically, the solvent system is a binary mixture of a higher and a lower polarity solvent. The ratio of solvents varies according to experiments. The solvent system of hexane/ethyl acetate or dichloromethane/methanol is commonly used for normal phase silica gel stationary phase.

(3) Acidic and basic organic compounds interact with residual surface silanol groups on a chromatographic support and cause peak tailing. Triethylamine, ammonium hydroxide, acetic acid, and trifluoroacetic acid are common mobile phase modifiers. The addition of a mobile phase modifier (typically one percent or less) reduces peak tailing and sharpens peaks, improving the resolution in separations of basic or acidic compounds.

3. Commonly used chromogenic agents and preparation methods

Chromogenic agent	Detector	Preparation method	Chromogenic method
Iodine	Unsaturated or aromatic compounds	In a 100 mL jar, added 10 g iodine, 30 g silica gel	Put the thin layer plate into the iodine jar. Spraying water on the plate can increase the sensitivity of color development. Yellowish brown
Phosphomolybdic acid	Widely used	10 g phosphomolybdic acid + 100 mL ethanol	Heat to 110℃ using a heat gun. Blue
Ninhydrin	Amino compounds (primary, secondary)	1.5 g ninhydrin + 100 mL n-butanol + 3 mL acetic acid	Heat to 110℃ using a heat gun. Blue violet
Potassium permanganate	Reducing group-containing compounds, such as hydroxyl, amino, aldehyde	1.5 g $KMnO_4$+10 g K_2CO_3+1.25 mL 10%NaOH+ 200 mL water Validity period:3 months	Yellow spots
Dinitrophenylhydrazine	Aldehydes or ketones	12 g dinitrophenylhydrazine + 60 mL concentrated sulfuric acid + 80 mL water + 200 mL ethanol	The saturated ketone develops blue immediately; the saturated aldehyde develops olive-green slowly; unsaturated carbonyl compounds do not develop color.
Bromocresol green	Carboxylic acids, pKa≤5.0	In 100 mL of ethanol, add 0.04 g of bromocresol green. Then add 0.1 M NaOH aqueous solution dropwise until the solution is blue.	Heat to 110℃ using a heat gun.
Ferric chloride	Phenol compounds	1% $FeCl_3$ + 50% ethanol solution	Phenols- blue or green
p-Anisaldehyde	widely used	135 mL ethanol + 5 mL concentrated sulfuric acid + 1.5 mL glacial acetic acid + 3.7 mL *p*-anisaldehyde	Heat to 110℃ using a heat gun. Color may vary.
Barium sulfate	Alkaloids	10% aqueous solution of cerium (IV) sulfate + 15% sulfuric acid	Heat to 110℃ using a heat gun for 8 min. Color may vary.
Vanillin (vanillin)	widely used	15 g vanillin + 250 mL ethanol + 2.5 mL concentrated sulfuric acid	Heat to 110℃ using a heat gun. Color may vary.

4. Observation of thin layer chromatography

(1) Remove the TLC from the development chamber with tweezers and let the solvent evaporate with a hair dryer;

(2) Observe the dark spots or fluorescent dots under UV light;

(3) Use chromogenic agent for non-fluorescent products

5. Storage and activation

The TLC silica gel plates are highly hygroscopic so that they should be stored in sealed and dry environment and kept away from volatile substances. Once the packaging is opened, unused plates should be sealed immediately to avoid moisture absorption or adsorption of other chemicals. If TLC silica gel plates have absorbed moisture, they can be reactivated in an oven at 110 ℃ for 30 minutes.

6. Security

Wear protective gloves and masks. Avoid breathing dusts. If injured, please get medical advice.