

## Thin Layer Chromatography

Abstracted from Searle Separations Department Procedure SEP-120

### I. Introduction

General Procedure for the preparation and application for the following TLC visualization reagents

1. Bromocresol Green  
Specific detection method for carboxylic acids.
2. 2,4-Dinitrophenylhydrazine  
General detection method for aldehydes and ketones.
3. Dragendorff's reagent  
General detection method for alkaloids, quaternary bases, nitrogen-containing compounds, and lipids.
4. Fluorescamine  
Specific detection method for primary amines.
5. Iodine vapor  
General detection method for hydrocarbons.
6. Iodine/SWUV  
General detection method for hydrocarbons.
7. Malonic acid-Salicylaldehyde  
Specific detection method for nitrogen-containing heterocyclic compounds.
8. Ninhydrin  
Specific detection method for amino acids, Primary amines, secondary amines, and amino sugars.
9. Phosphomolybdic acid  
General detection method which acts as a reducing agent to reduce compounds such as sterols, steroids, and lipids.
10. Potassium Permanganate  
General detection method which acts as an oxidizing agent to oxidize compounds.
11. Rhodamine 6G

General detection method for lipids.

12. Sodium iodide-Cupric acetate  
General detection method for quaternary salts and organic compounds containing at least one double bond and/or hydroxyl group.
13. Sulfuric acid/LWUV  
General detection method for hydrocarbons.
14. Sulfuric acid/Methanol/LWUV  
General detection method specifically for use on reverse phase TLC plates.
15. Sulfuric acid-Phosphomolybdic acid  
General detection method which acts as a reducing agent to reduce compounds such as sterols, steroids, and fats.
16. t-Butylhypochlorite/Starch/KI  
General detection method for primary amines, secondary amines, tertiary amines and aromatic amines.
17. t-Butylhypochlorite/SWUV  
General detection method which acts as an oxidizing agent to enhance detection under short wavelength ultraviolet light (SWUV), or it acts to enhance detection with other visualization reagents.
18. Vanillin/Sulfuric acid  
General detection method for steroids, higher alcohols, phenols, and essential oils.

## II. Experimental

### A. Reagents

All reagents are to be of reagent grade quality or higher.

### B. Visualization methods: Preparation and Use

#### 1. Bromocresol Green

Accurately weigh 40 mg bromocresol green and dissolve in 100 mL absolute ethanol. Add 0.1N NaOH dropwise until a blue color just appears. Store reagent at 4 °C and wear gloves when handling and spraying.

Evenly spray the dried plate with bromocresol green solution in an operating hood. Allow the plate to stand in a ventilated hood for one to two minutes. A positive reaction for acids yields yellow spots on a green background when viewed under white light. Basic compounds appear as blue spots.

Note: For any TLC plate developed in a solvent system containing acid, it is necessary to dry the TLC plate, after development, in a nitrogen blanket for 2 to 3 hours to remove any remaining acid.

## 2. 2, 4-Dinitrophenylhydrazine

Accurately weigh 100 mg 2, 4-dinitrophenylhydrazine and dissolve in 100 mL absolute ethanol. Add 1 mL concentrated HCl. Store reagent at 4 °C. Wear gloves when handling and spraying.

Evenly spray the dry TLC plate with 2, 4-dinitrophenylhydrazine solution in an operating hood. Allow to dry, and view under long wave UV. Ketones will appear as yellow spots, aldehydes as bright orange spots.

## 3. Dragendorff's Reagent

a) Solution A: Accurately weigh 0.8 g bismuth nitrate and dissolve in 10 mL glacial acetic acid. Dilute mixture to 50 mL by slowly adding distilled water, as the reaction may be exothermic. Store at 4 °C, and wear gloves when handling.

b) Solution B: Accurately weigh 20 grams potassium iodide and dissolve in 50 mL distilled water. Store at 4 °C, and wear gloves when handling.

c) To a 50 mL volumetric flask, pipet 5 mL Solution A and 5 mL solution B, while wearing gloves. Bring to volume with 10% (v/v) aqueous sulfuric acid solution. Prepare fresh daily.

Note: Reaction is potentially exothermic, so exercise caution when handling the sulfuric acid solution

Carefully mix the combined solutions. Add 0.2 mL 30% hydrogen peroxide, again mixing carefully. Store at 4 °C, and wear gloves when handling and spraying.

Evenly spray the dried TLC plate with Dragendorff's Reagent in an operating hood. Orange-brown spots on a white background viewed under white light indicate a positive reaction. Document the test results immediately after color testing, as the spots fade with time.

## 4. Fluorescamine

Accurately weigh 30 mg of fluorescamine and dissolve in 100 mL acetonitrile. Store at room temperature. Wear gloves when handling.

When acidic solvent systems are used to develop the plate, prepare fluorescamine solution in the following manner: Accurately weigh 30 mg fluorescamine and

dissolve in 100 mL of 50/50/1 acetonitrile/water/0.1N sodium hydroxide solution (v/v/v). Store at room temperature. Wear gloves when handling and spraying.

Evenly spray the dried TLC plate with the appropriate fluorescamine solution in an operating hood. Reaction will occur at room temperature under white light in approximately 5-10 min. The reaction may develop faster under long wave UV light or if the plate is heated.

Fluorescent spots on a dark background will be visible under long wave UV.

5. Iodine vapor (This method differs from that indicated by Searle; it is faster and uses much less iodine.)

Obtain a glass jar with tight-fitting lid of sufficient size to accommodate the TLC plates used. Add enough iodine crystals to just cover the bottom, then add silica gel to a depth of about ½". Cap and allow the mixture to stand for a few hours. Place the dried TLC plate in the bottle, replace the cap, and shake gently for about one minute. Remove the plate in an operating hood. Brown spots on a white background should be visible under white light. Document the test immediately after color testing, as spots fade with time.

6. Iodine vapor/SWUV

Prepare the iodine bottle and treat the plate as in 5. Be sure to leave to closed bottle in an operating hood. Dark spots on a fluoresescent background are visible under short wave UV. Document the test results immediately after visualizing, as spots will fade with time. Wear gloves when handling, as iodine is atoxic substance when absorbed through the skin.

7. Malonic acid-Salicylaldehyde

Accurately weigh 0.2 g malonic acid and 0.1 g salicylaldehyde in 100 mL absolute ethanol. Store the reagent at 4 °C, and wear gloves when handling and spraying.

Evenly spray the dried TLC plate with malonic acid-salicylaldehyde solution in an operating hood. Heat the plate (approximately 30 min. at ~120 °C). Yellow fluorescent spots on a dark background should be visible under long wave UV (366 nm).

8. Ninhydrin

Accurately weigh 0.2 g ninhydrin and dissolve in 100 mL absolute alcohol. Store the reagent at 4 °C, and wear gloves when handling and spraying.

Evenly spray the dried TLC plate with ninhydrin solution in an operating hood. Heat the plate at ~120 °C for approximately 3-5 min. Pink spots on a white background should be visible under white light.

9. Phosphomolybdic acid

Accurately weigh 10 g phosphomolybdic acid and dissolve in 100 mL absolute ethanol. Vacuum-filter the above solution with Whatman #1 filter paper. Transfer the filtered solution to an amber bottle. Store the reagent at 4 °C, and wear gloves when handling and spraying. Discard reagent if solution appears green.

Evenly spray the dried TLC plate with PMA solution in an operating hood until the plate is bright yellow. Heat the plate at ~120 °C for approximately 2-3 min. Compounds reacting positively will produce shades of blue, purple, brown, or green spots on a yellow background under white light.

10. Potassium Permanganate

Accurately weigh 1.5 g potassium permanganate and dissolve in 100 mL 1H NaOH. Store the reagent at 4 °C, and wear gloves when handling and spraying. Discard reagent if solution appears green.

Evenly spray the dried TLC plates with potassium permanganate solution in an operating hood. Allow the plate to stand in an operating hood for 10-15 min. Yellowish spots on a dark purple background should be visible under white light.

11. Rhodamine 6G

Accurately weigh 100 mg Rhodamine 6G and dissolve in 100 mL absolute ethanol. Store the reagent at 4 °C, and wear gloves when handling and spraying.

Evenly spray the dried TLC plates with Rodamine 6G solution in an operating hood. Reaction is properly viewed under long wave UV. However, Short wave UV may provide better contrast of spots to the TLC plate.

12. Sodium Iodide-Cupric acetate

a) Sodium iodide. Accurately weigh 5.0 g sodium iodide and dissolve in 100 mL distilled water. Store the reagent at 4 °C, and wear gloves when handling and spraying.

b) Cupric acetate. Accurately weigh 3.0 g cupric acetate and dissolve in 100 mL aqueous acetic acid solution (20% v/v). Store the reagent at 4 °C, and wear gloves when handling and spraying.

- c) While wearing gloves, evenly spray the dried plate with 50% (v/v) aqueous sulfuric acid solution in an operating hood. Heat the plate at ~120 °C for 3-5 min.
- d) After heating, evenly spray the TLC plate with the sodium iodide solution in an operating hood. Wear gloves while spraying.
- e) After spraying with sodium iodide solution, evenly spray the TLC plate with the cupric acetate solution in an operating hood. Wear gloves while spraying. Brown spots on a white background should be visible under white light. Document the test results immediately after color testing, as the spots fade with time.

13. Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>/LWUV)

50% (v/v) aqueous sulfuric acid solution: Slowly add 100 mL concentrated sulfuric acid to 100 mL distilled water cooled in a small container of ice.

NOTE: REACTION IS EXTREMELY EXOTHERMIC. Store solution at room temperature. Store the reagent at 4 °C, and wear gloves when handling and spraying.

Evenly spray the dried TLC plate with 50% (v/v) aqueous sulfuric acid in an operating hood. Heat the plate at ~120 °C for approximately 3-5 min. Fluorescent spots on a dark background should be visible under long wave UV (366 nm).

14. Sulfuric acid/Methanol/LWUV

10% sulfuric acid in methanol solution: Slowly add 10 mL concentrated sulfuric acid to 90 mL methanol. Store the reagent at 4 °C, and wear gloves when handling and spraying.

Evenly spray the dried TLC plate with 10% (v/v) sulfuric acid in methanol solution in an operating hood. Heat at ~120 °C for approximately one minute and allow to cool. View under long wave UV. Positively reacting spots will be luminescent against a dark TLC plate background.

15. Sulfuric acid-Phosphomolybdic acid

- a) While wearing gloves, evenly spray the dried TLC plate with 50% (v/v) sulfuric acid solution in an operating hood. Heat the plate at 120 °C for at least 3-5 min.
- b) After heating, evenly spray the TLC plate with PMA solution in an operating hood. Until the plate is bright yellow. Wear gloves while spraying.

Heat the plate at 120 °C for 3-5 min. Blue and brown spots on a yellow background should be visible under white light.

16. t-Butylhypochlorite/Starch/KI

a) In an operating hood, carefully pipette at least 1 mL t-butyl hypochlorite into a small beaker (5-10 mL). Place the beaker in an enclosed TLC chamber. Allow the chamber to stand approximately 30 min. prior to use. Store the hypochlorite reagent at 4 °C, and wear gloves when handling. Store the TLC chamber in an operating hood. Prepare the chamber daily, discarding the used t-butyl hypochlorite and replacing with fresh t-butyl hypochlorite in a new beaker.

b) Starch/KI: Boil 200 mL distilled water. Accurately weigh 1.0 g starch and 1.0 g potassium iodide and dissolve in boiling water. Allow the solution to cool and vacuum filter, using Whatman #1 filter paper. Transfer the filtered solution to an amber bottle. Store the filtered solution at 0 °C and wear gloves when handling.

Note: The solution will degrade over time and should be periodically discarded.

c) Place the dried TLC plate in the closed TLC chamber saturated with t-butyl hypochlorite vapors for approximately 5 min. Be sure to leave the closed chamber in an operating hood. Remove the plate from the chamber and let stand in a ventilated hood for 30 min.

d) Evenly spray the plate with starch/KI solution. Purple spots on a white background should be visible under white light. Document the test results immediately after color testing, as spots fade with time.

Note: Conditions of this procedure may be modified to achieve the desired reaction.

Residual  $\text{NH}_4\text{OH}$  or triethylamine on the TLC plate will also respond positively, thereby interfering with this visualization technique. It is necessary to remove these reagents after development, using a nitrogen blanket, operating hood, or hot plate/oven set at low temperature (when compound is heat-stable).

17. t-Butyl hypochlorite/SWUV

Prepare a t-butyl hypochlorite chamber as in 16. Place the dried TLC plate in the TLC chamber saturated with t-butyl hypochlorite vapors for approximately 5 min. Be sure the closed chamber is in an operating hood. Remove the plate from the chamber, and let it stand in an operating hood for at least 15 min. View under short wave UV.

18. Vanillin/Sulfuric acid

Accurately weigh 0.5 g vanillin and dissolve in 100 mL of 80/20 (v/v) sulfuric acid/absolute ethanol. Store at room temperature. Wear gloves when handling and spraying.

Evenly spray the dried TLC plate with vanillin/sulfuric acid in an operating hood. Heat the plate at  $\sim 120^{\circ}\text{C}$  for 3-5 min. View under white light. Positive reaction will yield variously colored spots.