Name of Procedure:

Thin-Layer Chromatography
10:1 Developing Solvent (METHANOL)

Suggested Uses:

The 10:1 Thin-Layer Chromatography Developing Solvent is used to separate and identify organic compounds. Suggested uses of this TLC solvent are for benzodiazepines and similar compounds.

Apparatus Needed to Perform Procedure Including Preparation of Reagent:

Thin-Layer Chromatography Plates
    Silica gel GF - fluorescent indicator
    Thickness: 250 microns
Developing tank
Micro pipets
UV light (long and short wave)
Fume hood
Gloves
Eye protection
Laboratory coat
Graduated cylinders
Reagent bottle
Funnel
Porcelain spot plate
Spatula
Methanol
Concentrated ammonium hydroxide
Potassium chloride

Formula for Preparing Reagent:

1. Measure out 10 milliliters of methanol and 1 milliliter of concentrated ammonium hydroxide.

Formula for Preparing Reagent (continued):

2. Pour measured solvents into reagent bottle and mix well.

3. Properly label reagent bottle.

Quality Control Check:

A quality control check of this reagent will be performed using a known standard of diazepam and following the application procedure listed below.

Expiration Date of Chemical Reagent:

The solvent can be used until depletion provided it is stored in an airtight reagent bottle.

Application of Procedure on Evidence:

1. A sample of the unknown (approximately 1-2 milligrams) is placed in the well of a porcelain spot plate and several drops of a suitable solvent are added to dissolve the sample.

2. A known standard (approximately 1-2 milligrams) is also dissolved in the well of a spot plate with several drops of a suitable solvent. (Known standard is selected after visual observation and/or a series of preliminary tests).

3. With a capillary pipet, several microliters of the unknown and standards solutions are placed side by side approximately 5 millimeters from the bottom of the TLC plate.

4. Place approximately 1 gram of potassium chloride in the developing tank.

5. Add 10:1 solvent to the developing tank to a depth of approximately 2 millimeters and allow several minutes for atmosphere to equilibrate.

Application of Procedure on Evidence (continued):

6. Allow all the spotting solvent to evaporate from the TLC plate.

7. Place the TLC plate in the developing tank and close the lid.

8. Allow the TLC plate to develop to the top of the plate.

9. Remove the TLC plate from the developing tank and allow the solvent to dry.

10. Visualize with UV light or an appropriate visualizing reagent.

11. Record results.

Safety Concerns:

Always wear eye protection, gloves and a laboratory coat when preparing this TLC solvent.

Literature References:

Randerath, Kurt, **Thin-Layer Chromatography,** New York, Academic Press, 1968

Moffat, A.C., **Clarke's Isolation and Identification of Drugs**, 2nd Ed., The Pharmaceutical Press, 1986, pp. 166-177.

Suzuki, Edward M. and Graham, William, "Isolation and Identification of Clorazepate," **Microgram**, Vol. XVII, No. 4, 1984.

This procedure has been used in the Drug Chemistry Section since 1985.